Clinical Equine Oncology

Clinical Equine Oncology

Derek C. Knottenbelt OBE BVM&S DVM&S Dip ECEIM MRCVS

Consultant Professor in Equine Medicine
University of Glasgow, UK
Emeritus Professor in Equine Internal Medicine
University of Liverpool, UK

Janet C. Patterson-Kane BVSc PhD Dip ACVP MRCVS

Veterinary Pathologist
IDEXX Laboratories, Inc.
West Sacramento, CA, USA

Katie L. Snalune BSc MA VetMB Cert EM (Int.Med.) Cert ES (Soft Tissue) MRCVS

Clinical Head of Hospital
Newnham Court Equine Clinic
Kent, UK

ELSEVIER Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto 2015

ELSEVIER

ISBN 978-0-7020-4266-9

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging in Publication Data
A catalog record for this book is available from the Library of Congress

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

With respect to any drug or pharmaceutical products identified, readers are advised to check the most current information provided (i) on procedures featured or (ii) by the manufacturer of each product to be administered, to verify the recommended dose or formula, the method and duration of administration, and contraindications. It is the responsibility of practitioners, relying on their own experience and knowledge of their patients, to make diagnoses, to determine dosages and the best treatment for each individual patient, and to take all appropriate safety precautions.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Printed in China

For Elsevier
Content Strategist: Robert Edwards
Content Development Specialists: Wendy Lee/Carole McMurray/Veronika Watkins
Project Manager: Andrew Riley
Designer/Design Direction: Miles Hitchen
Illustration Manager: Jennifer Rose, Karen Giacomucci
Illustrator: Antbits

Preface

Writing and constructing a major textbook is not to be undertaken lightly! Before a project such as this can even be proposed, there has to be a reason for it. 'Why do we need a book on equine cancer medicine?' 'Will it help practitioners in particular to improve the quality of their practice and will it be of benefit to the horse itself as a species?' Those were just a couple of the questions we asked before we set out on the project. It did not take long to realize that there is no other similar textbook on the subject. There are, of course, many excellent textbooks on general medicine and surgery relating to the horse and many of these have significant oncology content. There are those books that are focussed on one particular anatomical system or syndrome. Lameness, skin diseases and dentistry are just three of them. There are dedicated textbooks dealing with infectious disease, reproductive medicine and surgery, and surgical and medical tomes that are comprehensive sources of information. The increasing specialization of equine practice is a worldwide phenomenon that has resulted in the development of dedicated training requirements in defined aspects of practice and this is both a laudable and a positive contribution to the improving standard of medical and surgical care that we provide to our patients and their owners. However, in spite of cancer medicine being a major human and small animal specialism, cancer medicine in the horse clearly lags a long way behind. A quick search on cancer on any literature database will confirm the exponential rise in publications at all levels that deal with cancer in humans, small animals and experimental conditions. Furthermore, single-case reports on equine oncological topics abound in the modern scientific literature. There is, however, no dedicated equine oncology textbook and indeed, whilst the literature is littered with 'case reports' and small case series, there has been no concerted or constructive effort to expand this important area of medicine.

The demographics of the equine population are changing dramatically. In the developed world, horses are increasingly being kept into old age and so with greater numbers of older horses, we have to expect greater numbers of cases of cancer in the population. Even in the developing world, significant improvements in management and care mean that horses, mules and donkeys are living for longer, and again this will inevitably mean increasing numbers of cancer cases. There is a clear need for a focussed comprehensive text on equine cancer medicine and surgery. That is our main reason for setting out to produce this book on equine oncology.

The horse remains an important aspect of human life. In places where horses are 'pleasure and leisure' animals, they provide enormous enjoyment and also bring financial returns to nations and to individuals. The equine industry employs vast numbers of people and equine 'sport' provides physical exercise for people between 2 and 90 years of age. No other sport does that!

In the developing world, the Equidae are genuinely life-saving or, at the least, life-changing. The family donkey or pony empowers women in particular and relieves human burden. The loss of a donkey or pony, for a simple rural farmer, for example, can be catastrophic, and has implications for livelihood, lifestyle and even for life itself. We have a duty to support the concept that 'horses' are here to stay and that, realistically, there are no viable alternatives to the equine beast of burden in vast areas of the world. Of course, the concept of 'carbon'-guzzling machines as an alternative may be seen as a way of reducing the welfare compromises that are commonly publicized but simply fail to answer the real-life situation. It is only through improved animal welfare that we can enhance the lives of countless millions of people worldwide.

Whilst many advances have been made in almost every area of equine veterinary practice, cancer medicine has never attracted much in the way of research interest or even clinical specialization. It is a matter of considerable regret and not a little disgrace that the veterinary profession has failed to grasp this particular nettle and that equine cancer medicine attracts so little attention.

Over the last 100 years or so, there have been some significant surveys on equine tumour prevalence, which confirm that the horse suffers a wide range of neoplastic disorders but that the numerical majority of the tumours affect the skin. These tumour types are well recognized and some sort of consensus has evolved on the best treatments and management. Less has been done to try to understand the diseases themselves, even though the opportunities to do so are clearly enormous! The tumours can usually be seen, easily examined in detail and easily biopsied, and usually can be diagnosed definitively.

Modern information systems allow owners and veterinarians alike to access vast amounts of material, though much of the internet-delivered information is of little overall value. A lot of it is blatantly misleading and often positively harmful. As owners become more aware of the availability of treatments and as their access to comments and facts improves, they become more demanding. That is no bad thing – an enquiring and challenging client serves to ensure that the best possible approach is taken and means that the veterinarian must keep up to date and be aware of the latest developments in diagnostic approaches, particularly in treatment. It drives evidence acquisition, and that in turn, drives progress.

This book is arranged in three sections. *Section I* sets out to provide a platform for understanding the cellular basis of cancer and its aetiopathogenesis, along with the broad principles of diagnosis, treatment and management of cancer cases.

Section II sets out the finer details of the main groups of cancer in horses and provides an in-depth description of the literature and what is generally known about the various conditions. It is an attempt to bring a degree of evidence-based information into the various tumour conditions. In-depth understanding of a particular tumour type from a pathological perspective provides interest and hopefully stimulates research and the acquisition of clinical evidence. There are, of course, varying amounts of information available relating to the various tumour types; the 'big five'

in equine cancer are: sarcoid, melanoma, (squamous cell) carcinoma, lymphoma and mastocytoma, and inevitably they are emphasized. However, here we have tried to consider even the rarest of reported tumours on the basis that something is rare simply because it is seldom encountered; and when it is encountered, we need some information about it that might help! Rare tumours offer very different challenges to the clinician, since the evidence may be sparse, to say the least. And yet evidence is only gained through critical scientific and clinical investigations. Gradually, the bricks will be laid on the walls and evidence will build up, to the benefit of the patients themselves.

We hope the *Section III* will provide busy practitioners with a ready reckoner in respect of the tumours that occur in each of the 10 body systems. We have tried to make this reasonably comprehensive and we hope that it will enable a clinician to diagnose at least the majority of tumours and to have rapid access to clinically useful information when a diagnosis is provided clinically or by the pathologist.

To aid this process, disease summary boxes have been included for each of the major tumour types in this final section, condensing the main points under 'Notes', 'Clinical features', 'Diagnostic confirmation', 'Management' and 'Prognosis' headings. Incidence is graded by colour:

common rare very rare

Colour-coding is also used to indicate prognosis:

hopeless poor guarded good

Clinicians might benefit a great deal from the pathological input, while pathologists might similarly gain from seeing the cases that they are consulted upon. The relationship between pathologists and clinicians is a vital part of the developing sphere of equine oncological medicine. We hope this will be an expanding resource to readers over the years as more cases are added to it.

Inevitably, with a broad project such as this, there is a degree of overlap and repetition between the sections but we make no apology for this, simply because the three main sections will, we believe, be read and consulted at different times under different conditions. We have set out to make it as comprehensive as we can but we have, of course, been constrained in the amount of text and the number of images we could include. We hope that the book will provide a basis for the recognition of equine oncology as a specialism and as an important and challenging clinical topic.

Cancer medicine is a serious challenge to us all and we hope this book will help to widen clinical and research interest in aspects of cancer medicine in horses for the future good of the horse. We have set out to redress the imbalance as best we can, recognizing that there is more unknown than known in equine oncology. We extrapolate at our peril, of course, but that is an inevitable result of 'being behind', so we call for more effort both in the clinical circumstances and in research.

We commend this book to you – there are inevitably some aspects that we have had to reduce and even exclude but we feel sure this is a constructive contribution to equine medicine that will bring clinical and welfare benefits to the horse and donkey.

Even a cursory glance at the index pages of most equine journals will attest to the importance of cancer medicine; more and more publications on isolated cases and small case series are being published. We welcome this and we are excited by the prospect of increasing interest and increasing awareness of equine oncological disease amongst owners and veterinarians alike!

Derek C. Knottenbelt

Acknowledgements

A project of this size necessarily involves moral and physical/professional support from a lot of people!

We are grateful to the founders of modern clinical oncology and in particular to Professor Alain Theon, who has almost single-handedly driven the development of radiation therapy and chemotherapy in horses. He has been an inspiration to many people (including ourselves) for many years. In addition to his immense achievements in the science and practice of equine oncology, he has made considerable contributions to this book through his pictures, advice and support.

We would very much like to thank all those who have so willingly contributed pictures and information to the book. They are far too numerous to list here. The reality is that countless owners and veterinarians have helped us – be assured we are deeply indebted to you all. We would particularly like to thank the following for their help: Tim Mair, Garance Christen, Ophelie Clottu, Victoria Coppas, Olly King, Alexandra Imhasly, Fred Pauwels, Susan Jones, Maarten Oosterlinck, Dylan Gorvy, Eugenio Gillan-Garcia, Luiz Mendes, Katherina Pieber, David Donaldson, Jennifer Stuart, Veronica Roberts, Marisa Rodrigues, Santiago Gutierrez-Nibeyro, Richard Blundell, Susanna Monteiro, Sidney Rickets, Alistair Foote, Andy Durham, Gail Leeming, Cynric Temple Camp, Christoph Koch, Gabor Bodo, Fernando Malalana, Neil Townsend, Barbora Bezděková, Petr Jahn, Carney Jackson, Dennis Brooks, David Wilkie, Paddy Dixon and Guy Grinwis. Marianne Sloet has remained an amazing supporter of this project, offering her help at every opportunity, and we thank her also for being such a great friend.

We are also especially grateful to Jeremy Kemp-Symonds for his help with the Laser surgery and Photodynamic therapy sections (Ch. 9). He has extensive experience of both, and his contribution is therefore invaluable. Professor Youssef Tamzali has contributed to the section on Electrochemotherapy (also in Ch. 9) and, again, his experience of its use in a wide range of skin tumours in horses provides useful evidence for efficacy. Youssef has remained enthusiastic about the success of this project and his support is greatly appreciated.

Ceri Adams-Stevens has been a Trojan during the preparation of this project. She has contributed a great deal in the engine room of text production, and to the management of the references for each section in particular. She has been a true stalwart. We are deeply grateful to her.

Robert Edwards, Veronika Watkins, Andrew Riley and Clive Hewat of Elsevier have been stalwart supporters who have tolerated our literary and time-keeping inadequacies with good humour. Thank you also to the back-room art team – great work! The picture quality is terrific and a credit to you all, just as it is to us. Without the enthusiasm, support and help of Wendy Lee, we would surely have never completed this project – thank you, Wendy, you are amazing! The innovative nature of this book and its unusual presentation is a real credit to the enthusiasm and drive of the whole publishing team!

Dedication

This book is dedicated to the HORSE, the DONKEY and the MULE. Without the permissive and submissive domestication of the horse as a species, mankind would probably still be breaking stones and rubbing sticks to make fire. We are where we are in evolution because of the horse. It's payback time now – the horse needs us and we still need the horse.

Don't walk in front of me,
I may not follow.
Don't walk behind me,
I may not lead.
Just walk beside me and be my friend.
(Albert Camus, 1913–1960)

1 Introduction

A neoplasm is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stimulus which evoked the change.[1]

The term 'cancer' is derived from the Greek word *karkinos*, or crab, which is thought to relate to the 'crab-like' appearance of blood vessels on tumours, the vessels resembling a crab's claws reaching out (Fig. 1.1). Cancer is the general name for a large and complex group of diseases in which cells in a part of the body begin to grow out of control. Although there are many kinds of cancer, they all start because abnormal cells acquire an abnormal and potentially harmful growth pattern. Untreated cancers can cause serious illness and even death; the term 'cancer' is widely feared, since the perception is widely held that it is almost synonymous with death. This has imparted a rather negative attitude to the broad range of cancers that affect animals but it has also provided impetus for research. The understanding of the origins of cancer has advanced dramatically over the last 3–4 decades as a result of the revolution in molecular biology and the dramatic advances in molecular technology. Powerful experimental tools have been placed in the hands of cancer scientists and physicians and these have made it possible to explore the most minute aspects of the complex molecular machinery that govern the replicative and functional processes of a cell. Comparative examination of normal and tumour cells has been possible to establish the specific defects that cause cancer cells to replicate and behave abnormally.

One of the first written descriptions of cancer and its treatment is in an Egyptian papyrus dating from around 3000 BC, the Edwin Smith Papyrus.[2] This recorded eight cases of human breast cancer, but commented then that there was 'no treatment for the condition'. In 400 BC, as part of his *Humoral Theory of Disease*, Hippocrates, the accepted father of modern medicine, attributed cancer to an excess of 'black bile'. He used the words 'carcinos' and 'carcinoma' to describe tumours and hence the term 'cancer' was coined. Hippocrates is credited with being the first to recognize the difference between benign and malignant tumours. He believed that it was best to leave cancer alone because those who received 'treatment' had a poorer survival outcome than those who did not; he already recognized the benefit of not interfering if no positive outcome could be guaranteed or at least expected.

Galen (AD 130–200), a Greek physician believed cancer was curable in its early stages and proposed that advanced tumours should be operated upon either by 'cutting around the affected area' or by cauterization. He already had an awareness of the basic requirements of cancer therapy – to treat the condition early when the tumour was small and to ensure that the whole tumour was removed. He also made the first suggestions on the aetiology – proposing that it was the result of unhealthy diets and bad climates!

Moses ben Maimon, known in English as Maimonides and in Hebrew as Rambam (1138–1204), is widely regarded as the greatest Jewish philosopher of the medieval period. He also achieved fame as a physician.[3] In his fifth medical treatise he recognized that large tumours required wide surgical excision, 'such that the roots of the tumour and its surroundings up to the point of healthy tissue [should be] removed'. He also recognized the limits of surgery when the tumours involved were close to major organs. He was aware that when they could either not be totally removed or affected adjacent organs and tissues that were intolerant of removal, the outlook was much worse and the 'removal' process was more complicated. The same challenges face the oncologist today in spite of the sophistication of modern medicine.

It was only in the early eighteenth century that interest in the problems and challenges of cancer were revisited. In 1713, an Italian physician Bernardino Ramazzini, the acknowledged father of occupational medicine, noticed the high incidence of breast cancer and the virtual absence of cervical cancer among nuns.[4] This observation identified the basis of the importance of hormonal factors in cancer and environmental exposure/challenge. His work is a very early example of an elegant and meticulous epidemiological study that led to a new approach to cancer medicine.

In 1775, the London physician Dr Percival Pott identified that chimney sweeps had an occupation-related cancer risk. Soot that collected under their scrotum was associated with scrotal cancer. These discoveries led to additional studies, which identified other occupational cancer risks. The identification of these risks forced health and safety measures to be taken, including the need to 'wash thoroughly'. As

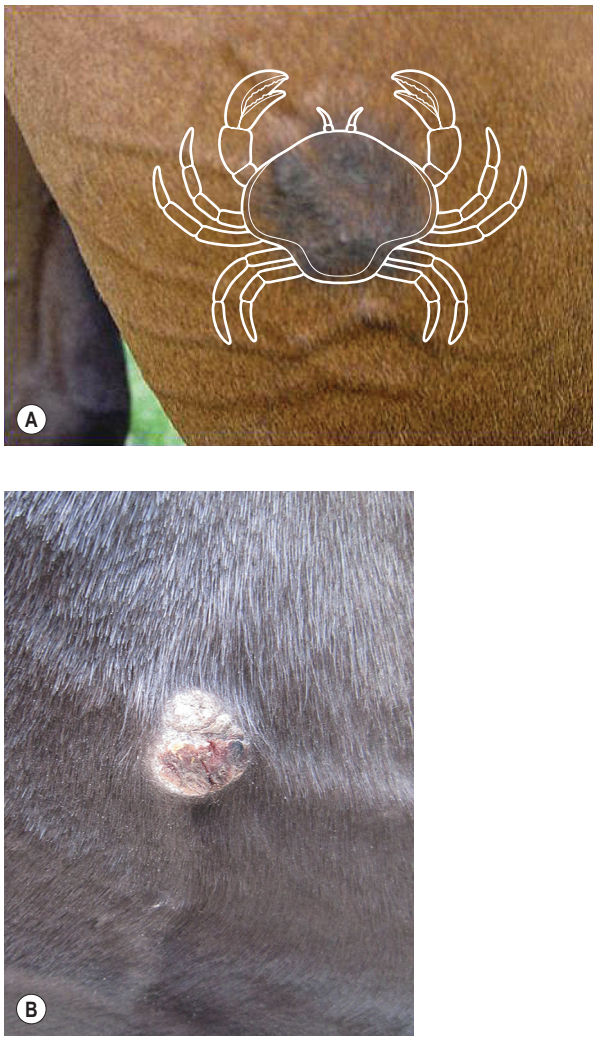


Figure 1.1 The crab-like appearance of tumours. The term 'cancer' was used to account for the crab-like appearance of a visible tumour with its radiating blood vessels and tumour extensions into adjacent tissues. **(A)** A verrucose haemangioma. **(B)** An equine sarcoid. Both have radiating blood vessels that give the tumours a 'crab-like' appearance. The similar 'crab-like' gross appearance of the dissected tumours is also thought to have been an origin of the term 'cancer' but of course the histological invasion of tissues was not recognized until the modern medical era.

soon as suitable measures were taken, the incidence of that particular cancer fell dramatically. It was the first genuine record of a preventive measure that eliminated a particular and highly dangerous cancerous disease.

The causes of cancer remained uncertain until 1910, when Francis Rous provided scientific backing to the 'viral theory of cancer' by inducing tumours through injecting cell fluid extracts obtained from the 'Rous sarcoma' in chickens. Possibly the greatest advance into the role of 'chromosomal abnormalities' in cancer was, however, made by Theodor Boveri, in 1914. Boveri proposed the 'somatic mutation theory of cancer'; this theory suggested that cancer was caused by abnormal chromosomes.

In 1939, Charles Huggins and his group, started studying androgen levels and the appearance of prostate cancer in dogs. Huggins was later awarded the 1966 Nobel Prize for Medicine in recognition of this work. The research succeeded in showing the relationship between hormones and certain cancers. This laid the groundwork for hormone therapy for certain cancers. This important research proved that administration of the female hormone oestrogen slowed the growth of prostate cancer in males. Hormone therapy, also called 'androgen ablation', is now a common treatment for prostate cancer and he also recommended 'the Huggins operation' – castration – for particularly advanced cases. In separate research, he showed that some breast cancers can be slowed by removing the sources of endogenous oestrogen – the ovaries and adrenal glands. Medications that block the natural production of oestrogen are now part of the standard treatment of breast cancer. Prior to Huggins' research, cancer had generally only been treated with surgery and painkilling drugs.

While investigating chemical warfare agents during the Second World War, in 1942, Louis Goodman noted that the blood of soldiers exposed to nitrogen mustard had abnormally low levels of white cells. He reasoned that nitrogen mustard might open new approaches other than surgery and radiation in the management of some forms of lymphoma, then the only effective anti-cancer therapies available. Goodman found that the substance significantly retarded the progression of lymphoma by causing serious tumour cell damage. His rather paradoxical conclusion, that this biologically 'harmful' effect might be harnessed in the treatment of cancer, led to the first paper reporting the use of nitrogen mustards as chemotherapeutic agents against various human lymphoid tumours.[5]

The 'DNA provirus' hypothesis of cancer was proposed in 1960 by Howard Temin. In this, he proposed that certain RNA viruses were capable of inserting their genetic material into the DNA of host cells and that at least some of the genetic components of these inactive 'proviruses' could eventually be expressed and thereby could contribute to the formation of cancer. In 1976, Varmus discovered the first cellular oncogene[6] and this research was rewarded with a Nobel Prize in recognition of its fundamental importance to cancer medicine.[6,7] The role of papilloma viruses in equine cancers (and in particular sarcoid) was identified in the latter half of the twentieth century.

The first recognition of cancer metastasis was made by Joseph Recamier. In his 1829 treatise *Recherches sur le traitement du cancer*, he used the term 'metastasis' as a definition for the spread of cancer to remote sites in the body. A major development followed in 1838, when Johannes Muller, a German pathologist, began to establish cancer histopathology as an independent branch of medical science. Over the remainder of the eighteenth century, individual pathologists and surgeons developed the science of 'oncological medicine' and this led to the publication of Steven Paget's 'seed and soil' theory of cancer, in 1889. This landmark study involved the careful analysis of over 1000 autopsy records of women who had breast cancer and he reported that the patterns of metastasis were not random. He proposed that tumour cells (the seeds) have a specific affinity for specific organs (the soil) and metastasis would only result if the seed and soil 'were compatible' (p. 53). Although there are some well-recognized difficulties with this hypothesis, it still stands the test of time in modern cancer medicine pathophysiology.

Following the discovery of X-rays by Wilhelm Conrad Röntgen in 1895, and the pioneering work of Marie and Pierre Curie, the diagnosis of cancer was significantly

improved – radiography proved to be a non-invasive and, in some cases, accurate method of establishing the presence of serious cancer conditions. However, during all the enthusiasm for the diagnostic capabilities of ionizing radiation (and X-rays in particular), their harmful and cancer-inducing properties were not appreciated during the early development of radiology. Marie Curie died in 1934 of aplastic anaemia, probably brought about by her years of exposure to radiation.

The beneficial (therapeutic) effects of radiation on cancer were probably discovered serendipitously; scientists and physicians were unable to explain why radiation regressed or slowed the progression of some tumours. The question as to who discovered the curative or anti-cancer effects of radiation should go to two general practitioners in Stockholm, Tor Stenbeck and Tage Sjögren, who in 1899, identified the benefit of radiotherapy. They cured a patient with malignant basal cell carcinoma using 99 radiation treatments over a 3-month period.[8] As with the Swedish case, in the early days, cures or treatments were largely restricted to superficial cancers and there was a high rate of recurrence of tumours in treated patients; this was probably simply due to the crude nature of the treatment and the massive and rather empirical doses of radiation that were used. Through the late 1930s, linear accelerators were developed to provide a more rational, controlled and successful treatment regimen and since then, radiation has become the 'gold standard' treatment for many cancers in humans and animals (admittedly to a lesser extent in the veterinary species).

The question, 'Why does cancer not occur more frequently?' became the focus of the role of genetics in the natural suppression of cancer cells within the body. The importance of genetics in cancer was first identified in 1986 by Friend and colleagues, in their studies with the retinoblastoma gene (Rb), which was also one of the first genes identified as being associated with an inherited (familial) form of cancer.[9] The importance and awareness of mutations in the aetiology and progression of cancer have increased dramatically with the development of new rapid genomic technology.

Since the mid-1990s, the knowledge base for cancer pathogenesis and treatment has developed exponentially. This simply reflects the perceived importance of cancer as a whole and it has also been magnified by the plethora of tumour types that have been identified even within a single broad classical disease entity. It is almost impossible to keep up with the published science in oncology! In 1995, the first DNA microarray chip was constructed and used to measure gene expression levels in plants.[10] This technology has been advanced dramatically and is now used to study many human and veterinary cancers. Currently, 'gene chips' are being investigated as tools in the development of individualized treatment plans.[11] The first successful creation of tumour cells was reported in 1999; human epithelial and fibroblast cells were transformed into tumour cells in a laboratory by the co-expression of telomerase (hTERT), the simian virus 40 large-T oncoprotein and an oncogenic allele of HRAS.[12] Our ability to 'make' an experimental cancer cell in the laboratory is clearly reflective of the detailed understanding of oncogenesis and provides an ideal basis for the development of focussed therapeutic approaches with a greater specificity for tumour-transformed cells.

The emphasis on early detection of tumours and the development of more and more sophisticated therapy has led to an 'expectation of success' in patients and owners that is not always realistic. However, enormous progress has been made in our understanding of the disease processes involved in the transition from normal cells to those that have a life-threatening behaviour. The causes of cancer have challenged research scientists and medical and veterinary practitioners since cancer was first identified. Cancer has been recognized in even the earliest writings of man and its complexity means that it is no surprise that it still challenges both the veterinary and medical professions.

Historically, three main hypotheses have been proposed for the origins of cancer, with each having a strong following as well as strong antagonists. One of these models proposed the concept that cancer was a disease of abnormal cell differentiation in which abnormal or errant cells behaviour resulted in progressive and repeated abnormal 'choices' of pathways in cell differentiation. Considering that the process of differentiation of the phenotype of the cells occurs without genetic changes, this model suggested that the development of cancer was an epigenetic process: the alteration of cell behaviour/phenotype in the absence of a genetic alteration. This theory had, therefore, important implications in recognizing that there were genetic origins in cancer.

An alternative hypothesis involved the effects of viruses, since a number of tumours were known to be associated with virus infections in both human and animal species. Examples that supported the theory included the Rous sarcoma virus of chickens, which was shown in 1911 to be involved in tumour development; this retrovirus was the first oncovirus to be described. The concept of the viral aetiology of cancer suggested that the virus, or more commonly a part of the virus, was able to insinuate itself into the genetic structure of the host cells, thus making it able to transform the cells from a normal behaviour into a malignant state.

It is now accepted that cancer probably results from mutations that promote autonomy rather than dependency. In cancer, there is disturbance of the delicate balance between the cell replication cycle and the controlling influences from within the cell and from outside it. These changes can result in the cell continuing to replicate, even in the presence of strong cellular signals that would normally inhibit or destroy the cell. Many of these controlling signals are cell and tissue-type specific, so that the genetic alterations that provide for a growth advantage may be different in different tissues. To complicate matters still further, the inherent genetic instability of cells with a liability to cancerous transformation also results in changes that have redundancy and so that, even in the same tissue, a tumour can develop from different genetic changes; this makes the use of genetic mechanisms in diagnosis more complicated and encourages the holistic approach when all aspects are combined together to reach a positive conclusion on the true nature of the tumour.

Cancer can, with a high degree of confidence, be viewed as a genetic disease that results in the abnormal proliferation of a single lineage of cells derived originally from a single cell that has undergone some form of genetic mutation. The resulting clonal expansion and increasing genetic instability result in the variations in the clinical behaviour of the cells. The completion of the sequencing of the equine genome gives us a much better opportunity to define the various

forms of cancer in horses. The completed map should permit the abnormal genes to be identified and so, while this may not yet provide any significant improvement of our management of the tumours, it will at least categorically define the type of tumour and the definitive mutations that occur and hopefully that will lead to an increased awareness of the prognosis for many cases. The concept that cancer growth and progression is the result of a single clonal expansion of a small proportion of the tumour cells arises from the observation that in many malignancies, no matter what method was used to assay the proliferative potential, only a small proportion of cancer cells are in fact able to proliferate extensively and continuously. This is indeed a fortunate aspect of cancer pathology.

The challenge of cancer to the medical and veterinary professions is increasing significantly. It is certainly true that the prevalence of tumours in horses is increasing. This is largely due to changes in demographics, with increasing numbers of horses being kept into older age and to the improving recognition of tumours by both owners and veterinarians. Over 80% of reported equine tumours involve the skin or the subcutaneous tissues; this may be somewhat misleading, since these tumours are the obvious ones to an observer and they are also those that have had most research and therapeutic developments. Given the superficial and obvious nature of most tumours in horses, it is easy to conclude therefore that the majority of tumours should be curable, provided that suitable early access to the case can be obtained. However, there are major problems that arise as a result of mismanagement and/or irresponsible neglect of the tumours and so the overall prognosis is significantly worse than perhaps it should be.

From a veterinary perspective, it is important to realize that much of the experimental work that is carried out on the causes and treatments of cancer are developed and 'tested' in laboratory animals and the reality is that the 'return' to the animal kingdom for this sacrifice has been paltry to say the least. Cancer is no less important in veterinary species than in mankind and many of the lessons that have been learned have involved animal sciences. As the demographics of the equine population changes with the increasing number of older animals in the population and as animal welfare has become increasingly more important to owners and veterinarians alike, more cases are being presented and so veterinary (equine) cancer medicine has at last started to be viewed as a specialist subject worthy of specialist research input. Cases are being detected earlier and given their true clinical importance. Diagnostic and therapeutic advances are slower in equine practice than other arms of medical and veterinary medicine. Although this is probably understandable, given the size of the patient and therefore the logistics, and the cost of any diagnostic processes or therapeutic interventions, it does mean that much of the clinical advancement is driven by extrapolation or by university teaching hospital case materials.

It is often stated that cancer is many different diseases but probably this is simply because cancer in different organs and tissues has different implications and manifestations. The common overall feature to all cancers is that it is a direct result of the failure of cell regulation with inappropriate and ultimately uncontrolled cell proliferation. There are clearly significant clinical and pathological differences between a skin carcinoma and an adenocarcinoma of the large colon, but it is also important to recognize that there are also major differences in the way in which cancer manifests within one organ in different patients. This latter fact is possibly related to differences in phenotypic heterogeneity among the cells of a particular cancer type. It is this heterogeneity that contributes so much to the difficulty of diagnosis of the various cancers – it has been said that, 'cancer has as many manifestations as there are cases of cancer', thus implying that no two cases will be the same. Not only does this concept add to the difficulty of diagnosis but possibly, even more importantly, it might explain the singular differences that are encountered in the treatment responses. A particular treatment might be very effective in one case and have far less effect in an ostensibly identical tumour in another patient. In the horse, this applies as much as in any other species, and this in turn leads to the 'unexplained failures' and amazing successes that individual clinicians might achieve. Cancer medicine tends then to be a rollercoaster of elation and depression. Fortunately, in the equine species, there are a few well-recognized common tumour states and most of these have visible differences and so treatment choices remain fairly narrow. A good example is the equine sarcoid (pp. 203 and 545), which although it is primary neoplasm of fibroblasts, has at least six morphological forms that can be recognized – each of these has some form of therapeutic limitations.[13]

References

1. Willis R. The spread of tumours in the human body. London: Butterworth; 1952: 46–55.
2. Morton TT, Moore RJ. A chronology of medicine and related sciences. Aldershot: Scholar Press; 1997.
3. Frank JB. Moses Maimonides: Rabbi of medicine. Yale J Biol Med 1981; 54:79–88.
4. The American Cancer Society Inc. The history of cancer. Atlanta: The American Cancer Society Inc.; 2002: 13–17.
5. Goodman LS, Wintrobe MM, Dameshek W, et al. Nitrogen mustard therapy; use of methyl-bis(beta-chloroethyl)amine hydrochloride and tris(beta-chloroethyl)amine hydrochloride for Hodgkin's disease, lymphosarcoma, leukemia and certain allied and miscellaneous disorders. J Am Med Assoc 1946; 132:126–132.
6. Varmus HE, Shank PR. Unintegrated viral DNA is synthesized in the cytoplasm of avian sarcoma virus-transformed duck cells by viral DNA polymerase. J Virol 1976; 18:567–573.
7. Marx JL. Cancer gene research wins medicine Nobel. Science 1989; 246: 326–327.
8. Berven E. The development and organization of therapeutic radiology in Sweden. Proceedings of the 47th Annual Meeting of the Radiological Society of North America, 1961: 19–23.
9. Friend SH, Bernards R, Rogelj S, et al. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 1986; 323:643–646.
10. Schena M, Shalon, D, Davis RW, et al. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 1995; 270:467–470.
11. Wulfkuhle J, Espina V, Liotta L, et al. Genomic and proteomic technologies for individualization and improvement of cancer treatment. Eur J Cancer 2004; 40:2623–2632.
12. Hahn WC, Counter CM, Lundberg AS, et al. Creation of tumour cells with defined genetic elements. Nature 1999; 400:464–468.
13. Knottenbelt DC. A clinical classification of the equine sarcoid. Clin Tech Equine Pract 2005; 4:278–295.

2

The challenges and problems of equine oncological practice

While cancer is probably not regarded as the most important overall clinical condition of the horse, it is a significant cause of morbidity and mortality. It is unfortunate that in the absence of proper central recording systems, the true incidence rate (often erroneously referred to as the 'incidence') for cancer is not established to any significant degree of certainty either internationally or by country or region. (The annual incidence rate is defined as the number of new cases of cancer developing in the population in a defined time period – usually 1 year.) As most surveys published involve hospital submissions, often to referral/second-opinion centres (many of which are based in universities), there are inherent problems in using these figures to estimate prevalence or incidence in the equine populations as a whole. Animals referred to specialists are usually of a higher monetary or emotional value to the owner. These horses are also more likely to be presented with conditions that are more complex or more difficult to diagnose and treat; this is in contrast to conditions easily treated in the field by the practitioner or not referred for whatever reason, that will be excluded from such analyses. Additionally, it is often impossible to know what the total size of the equine population is.[1] Trends in incidence rates often provide very significant information that can help establish possible aetiologies or factors that predispose to cancer development. The temporal incidence rate measures the rate of new case development in a defined period of time (e.g. week, month, year, decade, etc.). The sex and breed incidence rate defines the rate at which new cancers develop in defined sexes or breeds.

Neoplastic disease has nevertheless been reported in every body system of the horse, although the general consensus, based on university teaching hospital caseloads and a few slaughter house and necropsy studies, is that the commonest overall system involved in tumour development is the integument.[1] This predominance of skin tumours may bias any general conclusions about neoplastic disease in the horse.

Cancer in its various forms in the horse has particular difficulties and challenges. Recognition of the significance is often much less than an equivalent domestic pet or of course a human patient. This has meant that over many years, in spite of the horse having significant morbidity, there has been little effort to establish an evidence-based approach to many of the cancer problems that challenge the practicing veterinarian and the specialist alike. It is probably fortunate for the horse that the majority of tumours that are reported in the species are cutaneous or at least superficial and this makes their clinical recognition much easier. Skin tumours are more likely to be noticed, sampled or excised and submitted to diagnostic laboratories, and therefore noted in surveys. Many cancers that are not directly visible are commonly overlooked and, therefore, many cases do not get diagnosed. This does indicate, however, that even when only considering skin cancer, for a veterinary pathologist dealing with a significant equine caseload, it is an important consideration that requires knowledge and expertise.

It is also unfortunate that the mysteries of cancer are often used to 'smooth' out a diagnosis in a difficult case, whether it has the condition or not. This is clearly counterproductive in terms of equine welfare and is also a major block to advances in our understanding of tumours and their effects in horses. Many specialist centres will recognize that cases are referred for investigation of clinically significant symptoms without thought of the possibility of neoplastic disease or are diagnosed with 'cancer' when the symptoms of non-cancerous disease cannot be explained by simple and recognized diseases.

The early diagnosis of cancer is seen in most species as a primary clinical aim and, notwithstanding the problems associated with the diagnosis and management in horses, this should surely be the objective also of the equine clinician. The main priority for a clinician dealing with horses (regardless of whether the case is one of neoplastic disease or not) is the quality of life for the animal. This takes absolute precedence over any other consideration, but of course the quality of life of a patient may be compromised during treatment in the expectation of a complete cure or at least a palliation of the signs so that there is an overall improvement in quality of life. The most fundamental problem with assessing quality of life for a horse is the difficulty in assessing how much pain and/or discomfort a horse suffers, either through the disease itself or through the treatment of the disease or both. There is a reasonable tendency to anthropomorphize the situation that the animal faces and whilst this is an acceptable way to assess it, it is probably far more complex than this. One horse can be highly sensitive to pain but another may be far more stoical and donkeys are probably one of the most stoical creatures known – in spite of obvious and severe disease they usually maintain a stoic and implacable outward appearance. The donkey is therefore often treated as a 'stupid, non-feeling' creature but this is very far from the case; they suffer as do all creatures – they just do not like showing it. We are left with our own

individual professional ideas of where the acceptable boundaries lie and what we are individually prepared to allow a horse to suffer by way of pain and discomfort. Cancer medicine will inevitably involve profoundly difficult decisions that may conflict with the opinions of others, both professional and lay.

The main aims of any clinical oncology facility dealing with equine cancer cases can be summarized in four points:

1. To save lives
2. To relieve suffering and improve the welfare of equine patients
3. To recognize when the case is beyond medical or surgical help and welfare issues dictate that euthanasia is required in a timely fashion; this does not always mean immediate euthanasia is required
4. To improve the knowledge base in cancer medicine with robust evidence-based studies to establish better diagnostic facilities, best practice in therapy and to provide a better indication of the prognosis.

The objectives in the longer term must surely be:

- To prevent cases as far as possible by giving sensible advice and instituting management processes, based on a sound understanding of the aetiopathogenesis of the various conditions
- To detect tumours earlier and to institute appropriate therapy to minimize the clinical effects and maximize the prognosis
- To improve awareness in owners regarding the detection and treatment of tumours in horses
- To provide faster and better-quality treatment that is within the inevitable financial constraints of horse ownership
- To develop centres of excellence in equine oncology, so that equipment can be used sensibly and so that evidence can be gained on best practice
- To encourage research efforts in the general area of equine oncology.

All these objectives are laudable of course, but each one brings significant problems that are more challenging in the horse than in any other domestic species. There are difficulties with almost every aspect of cancer medicine in horses. The horse is a large animal with an inherent behaviour pattern that is not usually tolerant of pain. Horses usually have a finite value and owners may be unwilling or unable to undertake the best treatments because of financial constraints. On the other hand, the horse is not usually treated as a 'commodity' like a farm animal and in contrast to domestic pets, they usually also have major commercial value – they cost a lot to buy. Increasingly, emotional value is also involved; many owners have a deep affection for their horses and this means that both financial and emotional value are involved in any decisions that have to be taken in regard to treatment or other diagnostic procedures. Fortunately, there is still a sympathetic sentiment, which means that owners do appreciate that a horse that is in unremitting suffering to no end requires euthanasia. The human is the only 'animal' civilized society that will watch and accept incurable suffering. The horse deserves to be given the best possible support, but equine cancer medicine unfortunately lags far behind that in most other species.

The analysis of many successes and failures in the management of cancer reveals the important role of the clinician who deals with the case.[2] The speed of diagnosis relative to the onset of the tumour and the timely intervention at a stage when the chances of success are greatest are key factors in the process of successful outcomes.

> *Where guesswork, an amateurish approach and defeatist attitude may fail, an intelligent and compassionate understanding and prompt skilful treatment may succeed.*

There has been very little attempt to investigate equine cancer diseases – most of the literature reports involving large numbers are surveys of tumour incidence in university teaching hospitals,[3–7] abattoir studies or are case descriptions of a single or few cases.[3–6,8] This is entirely understandable, since the diagnosis of cancer in horses is largely restricted to those tumours that affect the skin and are therefore belittled and regarded as an incidental nuisance or those that are life-threatening internal tumours, for which there is a perception that 'nothing can be done anyway'. The latter horses have most often been simply destroyed upon diagnosis, whether the animal really needs to be destroyed or not.

In the USA in 1940, it was estimated that the cancerous (malignant) and benign tumour rate in the normal working horse population was 40 and 389, respectively, per 100 000 horses and that of those, 11 and 6 per 100 000 horses, respectively, died or were destroyed as result of the tumour state.[9] Thus, at that time, it was concluded that there is a marked predominance of non-life-threatening benign neoplasms in the horse.[1] Published figures from veterinary schools indicate that tumours account for about 1–3% of surgical cases.[4] The most prominent are invariably the equine sarcoid, squamous cell carcinoma (of the eye region and of the penis in particular), granulosa cell tumour and melanoma. In a retrospective study of equine neoplastic disease presented to a US university pathology department over a 4-year period, only 21 neoplasms were diagnosed from 687 equine necropsies (3.1%) and 215 from 635 submitted biopsy specimens (33.9%); a total of 236 neoplasms were therefore identified in 1322 cases (17.9%).[5] Again, the most common neoplasms were sarcoid (43.6%) and squamous cell carcinoma (24.6%). Papilloma (5.5%), nerve sheath tumour (4.2%), melanoma (3.8%), lipoma (3.0%), granulosa cell tumour (2.5%), fibroma (2.1%), cholesteatoma (1.3%) and lymphosarcoma (1.3%), were less commonly identified (Table 2.1). In an abattoir survey of 1308 presumably healthy horses destined for the food chain in the UK, 139 horses (11%) had a total of 151 neoplasms; 71 horses (5.4%) had thyroid gland neoplasia (adenoma), 24 (1.8%) had adrenal gland masses (phaeochromocytoma), 20 (1.5%) had mesenteric lipomas. Melanoma (14 cases; 1.1%), myoma (four cases; 0.3%), disseminated sarcoma (four cases; 0.3%), granulosa cell tumour of the ovary (three cases; 0.2%) and two cases (0.15%) had sarcoid (see Table 2.1). This study was remarkably different from the previous university and other studies, in that skin tumours were by no means the most prevalent tumour type. The major difference between this latter study and others is that the population of horses was clearly different, being destined for meat use as opposed to being subjected to necropsy in a post-mortem facility, i.e. these were an ostensibly healthy population as opposed to being an inherently unhealthy population.

Table 2.1 The overall prevalence of tumours encountered in a histological/necropsy survey of cases submitted specifically for diagnosis and a study in ostensibly normal horses presented for slaughter

Tumour type	Percentage of neoplastic cases submitted for histology/necropsy (n = 1322)[9]	Percentage of healthy horses presented for slaughter found to have tumours (n = 1308)[8]
Skin tumours		
Sarcoid	43.6	0.15
Melanoma	3.8	1.1
Papilloma	5.5	
Nerve sheath tumour (neurofibroma)	4.2	
Organ tumours		
Granulosa cell tumour	2.5	
Lymphoma (~sarcoma)	1.3	
Lipoma	3.0	1.5
Fibroma	2.1	
Cholesteatoma	1.3	
Thyroid neoplasia		5.4
Adrenal gland tumour		1.8

It is hard to compare the two studies but they do have some interesting differences possibly associated with the different class of animal involved.

In terms of the various organs involved, there have been several studies of the prevalence and incidence of cutaneous and mucocutaneous tumours in horses.[10] Most identify that the commonest tumours of the horse affect the skin; around 30–50% of pathology submissions (necropsy and biopsy) involve skin tumours. Among the skin tumours reported in this survey, sarcoid, squamous cell carcinoma, dermal melanocytic tumours of various types, papilloma and mast cell tumour were the most prevalent, accounting in total for around 88% of the skin tumour group. The majority of the skin tumours were, as in most surveys, sarcoid and these were identified in Paint, Quarter Horse and Arabian horses, and was the only common tumour in donkeys and mules. Squamous cell carcinoma constituted 18.3% of all neoplasms, with ocular (periorbital, palpebral and conjunctival forms) being the most common. Penile carcinomas also occurred with some frequency. The results of this study would probably be no surprise to experienced clinicians in primary practice or referral clinics because the same broad results have been identified in several other significant studies of tumour prevalence. The variations in breed numbers in different geographical areas and population demographics may have some influence on the incidence of particular tumour types but, for the most part, the findings would probably be more or less consistent in all horse, donkey and mule populations. Apart from the sarcoid, little has been published about the prevalence of tumours in wild Equidae.

Apart from skin tumours, the breed prevalence of various tumour types has not been explored fully in horses but, anecdotally at least, there are some breeds that seem more liable to certain types of tumour. For example, it is widely accepted that the Appaloosa, Shire and Clydesdale appear to be more liable to ocular and periocular carcinomas. In one study, Appaloosa, Arabian and Quarter Horses were over-represented in the sarcoid affected population when compared with Thoroughbred horses.[11,12] A familial relationship to the development of a recognized tumour type is also a strong indicator of genetic susceptibility, although of course there may be common management and exposure factors when families of horses are kept under the same conditions. A familial tendency to sarcoid has been identified and similar tendencies have also been identified in other conditions and are well recognized by horse owners and equine veterinarians.[13,14] Again, there may be confounding factors that might predispose to cancer development, such as a pale, non-pigmented skin colour – in itself that may have no extra liability to cancer from a genetic perspective – but the colour is still a genetically controlled factor.

It is generally accepted that equine melanomas occur most commonly in grey horses over the age of 5 years and that, at least in the early stages, the tumours are benign. Since grey is a dominant colour in horses and since a very high proportion of grey horses will be affected to some degree, at least some aspects of susceptibility to neoplastic disease must be genetically based. Within breeds, familial lines of horses are known to have a predisposition to certain tumours. This has been most obvious in sarcoid tumours, where sequential generations of horses in a family have been severely affected.[15] There are plentiful non-equine examples of genetic predispositions to cancer development and the fact that some breeds and some families of horse are more liable to one form of tumour or another should probably not be surprising.

It is clear that specific tumour types have a tendency to occur in defined sites; this applies in particular to sarcoid (p. 545), melanoma (p. 239) and squamous cell carcinoma (p. 220). A clinical study of 296 Lipizzaner horses, found that dermal melanomas were present in 50% of them, with most being found in older horses.[16] In 75.6% of cases, melanotic tumours were detected on the ventral skin surface of the tail or in the perianal skin. A significant proportion of them ultimately become malignant, so this is probably an indicator that age is related, at least on balance, to developing malignancy. However, there are reports of isolated cases of aggressive tumour development in both melanoma, sarcoid and internal tumours, such as lymphoma, in very young horses (even neonates). In contrast to melanomas in solid-coloured horses, which are characterized by early metastasis, this study found that melanomas in grey horses showed less malignancy but a relatively high heritability. The heritability estimate of 0.36 with a standard error of 0.11 indicates a strong genetic impact on the development of melanoma in ageing grey horses, but other aspects and epigenetic factors may also be involved.

In most species, there is a general acceptance that neoplastic disease increases in prevalence with age. Historically, horses seldom survived into their old age and there was a widely held perception that cancer was commoner in middle-aged/young adult horses. For example, lymphosarcoma is said to affect horses between 5

and 10 years of age. The demographics of the equine population are changing, as more and more horses live to old age. Age-related incidence of tumours can be expected in any population of animals simply through time-related cell mutations and exposure to carcinogens. Clinically, it may be of more value to consider the population most prone to neoplasia, i.e. geriatric horses. In one necropsy survey conducted at the University of Kentucky Livestock Disease Diagnostic Center (n = 817 horses ≥15 years), there was an incidence of neoplasia of 8% in 15–19-year-old animals, rising to 17% in horses >30 years.[17] However, although the results of North American surveys have indicated a clear age-dependence for malignant tumours, that is not the case for benign lesions; in fact one such study showed an age-related decrease in benign neoplasia involving the skin in contrast with other veterinary species.[9] The reason for this bias is probably that many younger horses are affected by benign cutaneous papilloma and sarcoid. Notwithstanding the controversy regarding the true nature of pituitary enlargement in older horses (equine Cushing's disease/pituitary adenoma), there is clearly an age-related increase in the incidence of pituitary 'adenoma'. The same can surely be said of the melanoma – older grey horses are invariably affected to some extent and it could possibly be said that 'every grey horse will develop a melanoma if it lives long enough!' Age also has an implication in respect of the malignancy of some of the neoplastic diseases. This applies perhaps more definitely to penile squamous cell carcinoma, where the condition appears to have a more aggressive course when it occurs in geldings under 8 years of age and a more benign behaviour when it occurs in horses over the age of 15 years (p. 630).

The sex of the animal will have obvious implications for cancer development. Mares may have ovarian tumours, such as granulosa (thecal) cell tumour, while stallions may have testicular tumours (such as seminoma or interstitial carcinoma). Mares are the only sex that is likely to get primary mammary tumours, although their very low incidence makes it almost impossible to identify potential trigger factors – 'does pregnancy and lactation increase or decrease the likelihood of their development?' Geldings are more liable to penile carcinoma but 'does early castration increase or decrease the liability to carcinoma?' In Equidae the neoplasm with the strongest sex association is squamous cell carcinoma; genital forms of carcinoma are one of the commonest tumours encountered in horses. Interestingly, gastric carcinoma consistently occurs more frequently in male horses than in female horses.[18]

Management and geographical location may also influence the development of tumours, particularly of the skin. Eyelid, conjunctival and facial carcinomas are far more common in hot, sunny, tropical regions, even to the extent that they are almost expected in suitably coloured animals. The connection between two or more predisposing factors is important. Clearly the more 'force' that is applied to the process of carcinogenesis, the more likely a horse is to have a tumour. For example, an Appaloosa horse probably has a genetic predisposition to carcinoma of the third eyelid (membrana nictitans) but when the conjunctiva is non-pigmented that tendency might be greater and when the horse is then subjected to an environment where it would be exposed to high levels of ultraviolet radiation (such as in tropical climates), the tendency is even greater. Similarly, carcinoma of the penis is far commoner in geldings than stallions, so management measures, such as castration, may influence the tendency for the development of the tumour and when that is coupled with non-pigmented penile skin and failure to carry out routine hygiene checks in geldings, the likelihood of carcinoma probably increases significantly. Castration of many male horses at an early age may at least partially explain why testicular tumours are considered to be rare in equines in comparison with other domestic/companion animal species, including dogs.[19] Again, disappointingly, there is little evidence-based information on the role of environment, feeding and other management, such as the use of chemical anthelmintics, rugs and UV protectors, in carcinogenesis. This means it is very hard to provide good guidance to owners of horses that may or may not be predisposed to cancer development.

References

1. Reid SW, Howie F. Factors associated with neoplastic disease in the horse. Equine Vet Educ 1992; 4:66–68.
2. Ackerman HJ, del Regato JA. Cancer diagnosis, treatment, and prognosis. In: del Regato JA, Spjut HJ, eds. Ackerman and del Regato's Cancer: diagnosis, treatment, and prognosis, 5th edn. St Louis, MO: CV Mosby; 1977.
3. Baker JR, Leyland A. Histological survey of tumours of the horse, with particular reference to those of the skin. Vet Rec 1975; 96:419–422.
4. Cotchin E. A general survey of tumours in the horse. Equine Vet J 1977; 9:16–21.
5. Sundberg JP, Burnstein T, Page EH, et al. Neoplasms of Equidae. J Am Vet Med Assoc 1977; 170:150–152.
6. Pascoe RR, Summers PM. Clinical survey of tumours and tumour-like lesions in horses in south east Queensland. Equine Vet J 1981; 13:235–239.
7. Johnson PJ. Dermatologic tumours (excluding sarcoids). Vet Clin N Am Equine Pract 1998; 14:625–658.
8. Cotchin E, Baker-Smith J. Tumours in horses encountered in an abattoir study. Vet Rec 1975; 96:339.
9. Priester WA. Skin tumours in domestic animals. Data from 12 United States and Canadian Colleges of Veterinary Medicine. J Natl Cancer Inst 1973; 47:1333–1344.
10. Valentine BA. Survey of equine cutaneous neoplasia in the Pacific Northwest. J Vet Diagn Investig 2006; 18:123–126.
11. Angelos J, Oppenheim Y, Rebhun W, et al. Evaluation of breed as a risk factor for sarcoid and uveitis in horses. Anim Genet 1988; 19:417–425.
12. Mohammed H, Rebhun W, Antczak DF. Factors associated with the risk of developing sarcoid tumours in horses. Equine Vet J 1992; 24:165–168.
13. James VS. A family tendency to equine sarcoids. S.W. Veterinarian 1968; 21:235–236.
14. Gerber H. Sir Frederick Hobday Memorial Lecture. The genetic basis of some equine diseases. Equine Vet J 1989; 21:244–248.
15. Martens A, de Moor A, Ducatelle R. PCR detection of bovine papilloma virus DNA in superficial swabs and scrapings from equine sarcoids. Vet J 2001; 161:280–286.
16. Seltenhammer MH, Simhofer H, Scherzer S, et al. Equine melanoma in a population of 296 grey Lipizzaner horses. Equine Vet J 2003; 35:153–157.
17. Williams N. Disease conditions in geriatric horses. Equine Disease Quarterly 2000; 8(2). http://www.ca.uky.edu/Gluck/q/2000/jan00/Q_jan00.htm#geriatric.
18. Moore JN, Kinter LD. Recurrent oesophageal obstruction due to squamous cell carcinoma in a horse. Cornell Vet 1976; 66:590–597.
19. Brinsko SP. Neoplasia of the male reproductive tract. Vet Clin North Am: Equine Pract 1998; 14:517–533.

3 Tumour nomenclature

Introduction

The science of oncology has its own vocabulary and, while this may seem to be an unnecessary complication, its development has provided clinicians with very useful terms that have simplified the understanding of oncology and facilitated communication between medical colleagues and scientists. Tumour nomenclature also provides a uniform and universal means of identifying tumours and contributes meaningfully to the management and prognosis.

A neoplasm is termed 'benign' if the mutations that have taken place allow the tumour an advantage over local tissues only – the tumour remains confined at its original site, but this does not mean that its effects will not be clinically serious, factors such as size, location, susceptibility to trauma and/or functionality of the tumour cells (e.g. endocrine activity) have a strong bearing on the tumour's significance.

A tumour is termed 'malignant' if a series of mutations occurs that enables the tumour cells to destructively invade local and surrounding tissues and then to metastasize to remote sites; not all do so successfully. The term 'cancer' is usually applied to malignant neoplasms.

The genetic/clonal origin of tumours means, of course, that it is reasonable to assume that tumours comprise one single cell type; of course this does not mean that a tumour mass comprises only cells of that type since the bulk of the tumour is made up of abnormal (cancer) cells, stroma, supporting and inflammatory cells, blood vessels and endothelial cells, as well as other tissue types that might become 'innocently' incorporated into the expanding tumour mass. Additionally, as part of the process of increasing malignancy, there is a generation of multiple genotypic variants (subclones) of these genetically unstable cells, such that by the time the neoplasm is clinically recognized, the cells are heterogeneous and subject to selection pressures (e.g. immune cell attack) that favour cellular subclones more able to survive, invade and/or metastasize. Some tumours are composed of more than one cell type, arising from germ cells/stem cells/progenitor cells (see p. 399) or potentially (and rarely) from two cell types (e.g. carcinosarcoma).

For the most part, tumours are classified according to their cellular origin (p. 201) as:

- Epithelial (endodermal, mesodermal [e.g. renal tubule epithelium] and ectodermal)
- Mesenchymal (mesodermal)
- Neurogenic, neuroectodermal or germ cell (or stem cell) tumours
- Haematopoietic (mesodermal).

The terms 'malignant' and 'benign' form the basis of the nomenclature of tumours in all species (Table 3.1). They, however, represent just the broadest of categorization. As will be seen later, this simple classification is not always clear and as such, it may have less clinical value than the further sub-classifications. Therefore, a more detailed and useful classification has evolved, which uses the site of origin of the tumour (i.e. the cell or tissue from which a tumour arises) and the particular (and often unique) microscopic appearance and characteristics. Although even this classification has some difficulties, it is logical and provides a degree of consistency that allows tumours to be categorized and so define at least some aspects of prognosis and therapy. In some instances in human oncology, neoplasms are named after the physician who first described them. For example, the malignant lymphoma called Hodgkin's disease was described in 1832 by the English physician Thomas Hodgkin. In a few circumstances, such as for example Paget's disease, the name could be grossly misleading, since there are several diseases that have the same name, only one of which is a cancer. Furthermore, whilst the name conjures up an immediate 'mental picture' of the disease and a certain element of 'romance' in people who know about its origin, it is meaningless to those who do not. The name of the discoverer actually adds nothing extra to the understanding of the condition but it does retain some recognition of the 'discoverer'. Fortunately, this system has not been applied to any equine tumours, although that may reflect the fact that no-one has been interested enough in the horse to discover new tumours!

A complication arises when tumour-like masses are identified and their differentiation is a basic first principle of tumour classification. Fundamental issues that will need to be addressed are shown in Figure 3.1 and include:

1. *Is this a tumour or not?* There are many circumstances when tumour-like states are encountered as a result of inflammatory or developmental disorders. A tumour, by definition, is a tissue swelling, although the term tumour has, become synonymous with 'cancer'. It is important to differentiate inflammatory swellings from cancerous or neoplastic swellings and conditions. Potential tumour-like conditions that will necessarily fall into the differential diagnosis of tumours in the

Table 3.1 The basic differentiating features of benign and malignant tumours

	Benign	Malignant
Extent of cell differentiation/anaplasia	Limited/absent Well-differentiated cells (recognizably similar to normal tissue type)	Marked Lack of differentiation that is variable in extent, with the most undifferentiated cells being referred to as anaplastic; atypical cell structure/form. Well-differentiated malignant tumours may be difficult to distinguish histologically from benign tumours
Local invasion	Limited Usually cohesive with well-demarcated margins that do not invade local normal tissues. This does not necessarily equate with encapsulation, but such lesions are frequently encapsulated	Extensive Locally invasive with ill-defined margins. Some are more structured and expand locally, requiring close histological examination for detection. The presence of a capsule does not rule out malignancy
Rate of growth/ expansion	Slow progression Periods of rapid/slow expansion or static and may have some periods of apparent regression/remission	Rapid (or variable/slow) Erratic growth patterns
Mitotic index	Low Few mitotic figures and these are normal in appearance	High Usually cohesive with well-demarcated margins that do not invade local normal tissues. This does not necessarily equate with encapsulation, but such lesions are frequently encapsulated
Blood supply	Variable	Variable (usually high)
Metastasis	No	Yes (frequently present) The more undifferentiated the tumour, the more likely are metastases

It is important to realize that not every case will fit precisely into categories and the transition to malignancy is probably best regarded as a continuum. Also, different tumour types have different rates of progression and clinical threat; even within the same tumour type, there is enormous variation. It is probably true to say that the only predictable thing about cancer is that it is unpredictable.

practical situation include inflammatory nodules, which may resemble proliferative tumours, and destructive disorders, which may resemble invasive destructive tumours, as well as non-inflammatory, non-neoplastic conditions. Differentiation of the true neoplastic states is a basic clinical objective requiring skill and patience. An error of diagnosis made as result of a casual or inadequate clinical assessment may have serious consequences.

2. *Is this mass a neoplasm/cancer?* The differentiation between non-neoplastic conditions is a fundamental responsibility of the clinician and pathologist and often samples are required to differentiate them (Fig. 3.2). Not all cancerous conditions consistently/persistently produce a swelling – in some cases, there is a destructive component to the condition and then there may be a separate set of differential diagnoses that need to be considered.
3. *Is this neoplasm benign or malignant?* Differentiation between benign and malignant tumours is possibly the single most important aspect of the investigation after the basic tumour type has been established. By definition, benign tumours will usually have less prognostic severity than malignant ones, but it is important to realize also that benign tumours can be very serious in other ways, such as space-occupying effects or may cause functional limitations.
4. *Is this tumour treatable?* The client and clinician will wish to establish an answer to this quickly and if possible, with certainty. This is the point at which evidence-based medicine (see p. 416) becomes important. The owner needs to understand what is known about the condition, in terms of its clinical behaviour and its responses to the various treatment options that are available and practicable. Only when all aspects are considered together can an informed decision be made.
5. *What is the prognosis (with or without treatment)?* The long-term outlook is possibly the biggest factor for horse owners and veterinarians. Simply knowing what the cancer might do and how it might affect both the horses' state of health and its usefulness makes decisions on the management easier and more rational. Of course some cancers will have no or limited treatment options and others may have well established treatments that will define the prognosis to a lesser or greater extent. The development of new treatments means that assessment of prognosis changes and so the clinician needs to keep up to date with published reports and scientific papers. There is little worse than a clinician basing life and death decisions on old, outdated information. Tumour size and location are significant factors in determining the prognosis for a cancerous mass (Fig. 3.3). For the most part, tumours are progressive in their expansion and growth so as might be expected the prognosis will inevitably be influenced by the duration of the tumour.

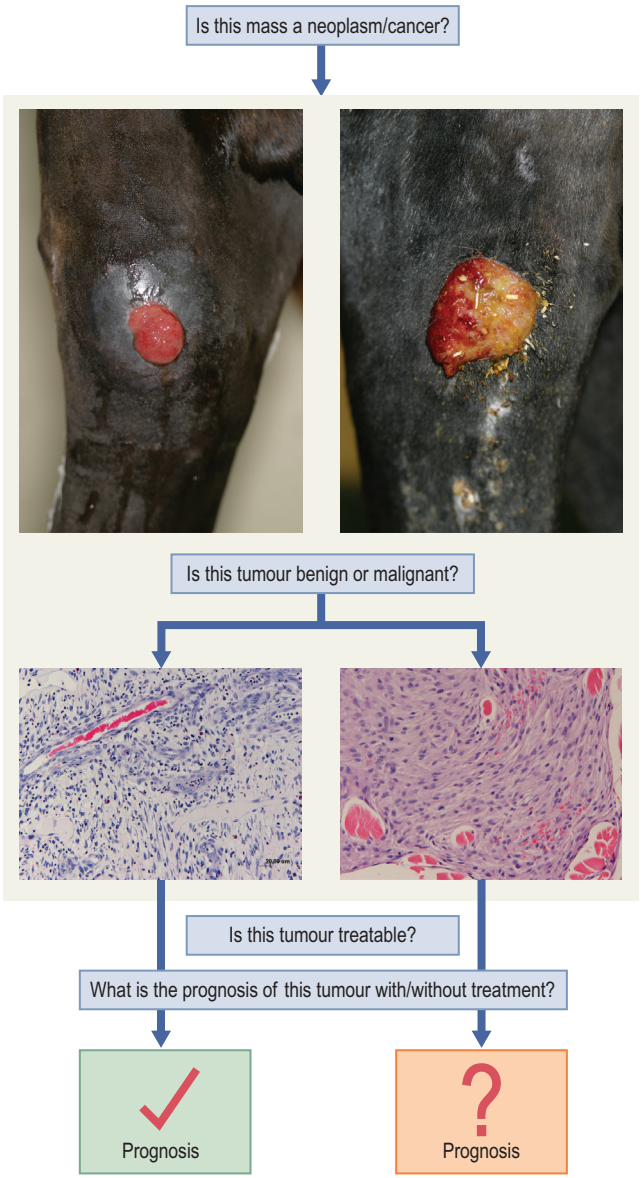


Figure 3.1 The similarity in appearance of a non-cancerous mass and a cancerous growth. These two masses on the lateral tarsus of two horses illustrate the similarity of appearance between a non-cancerous mass (granulation tissue, left) and a cancerous growth (sarcoid, right). The diagnosis is clearly vital, since the prognosis and treatment for the two conditions are very different.

Benign and malignant tumours

Cancerous tumours are classically defined as benign or malignant, based upon a set of clinical and pathological criteria (Fig. 3.4; see also p. 17). Those that are benign do not spread or cause cancer, while malignant tumours are cancerous and may invade other parts of the body. The division between the two categories of neoplastic tumours is not always clear and in many tumour states (but not invariably), there is natural progression through stages of cancer development from a benign mass to a highly dangerous malignant state. That progression varies temporally in individual tumours and in individual animals with that tumour type. The reasons for these variations are

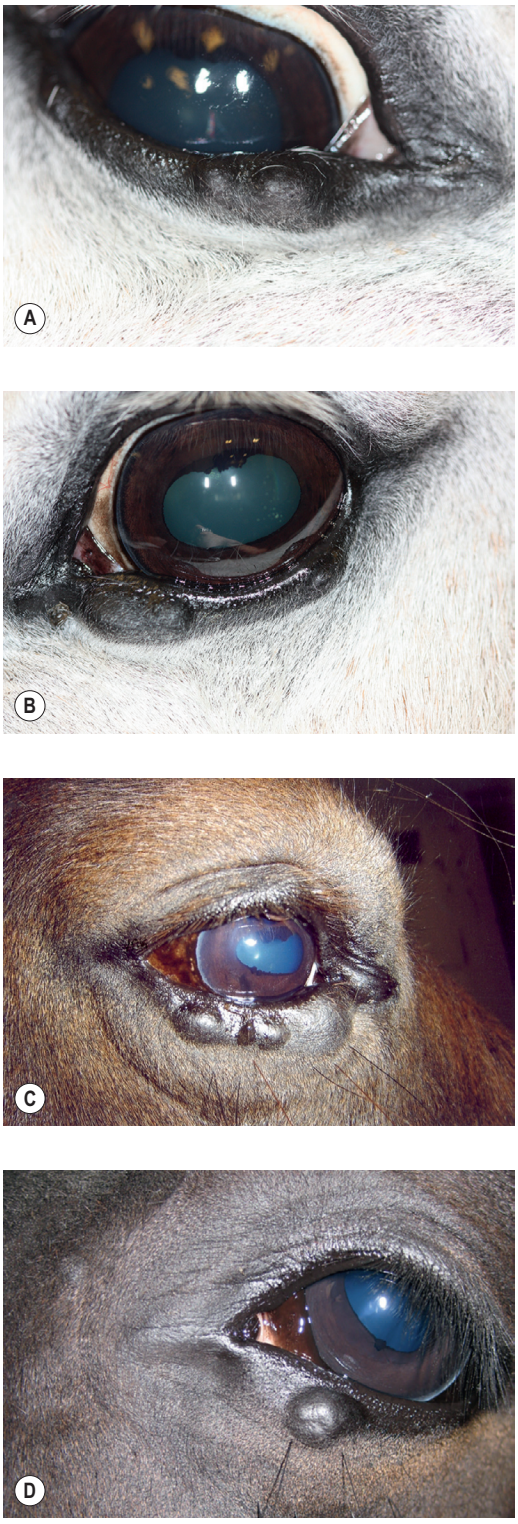


Figure 3.2 Palpebral nodules. These palpebral nodules look very much the same and it would clearly be easy to mistake them for each other on clinical examination alone. **(A,B)** Two images from the same horse. While the lesion in the right eyelid **(A)** proved to be an eosinophilic nodule (collagenolytic granuloma), that in the lower left eyelid **(B)** was a focal melanocytoma. **(C)** The nodules in the lower and upper eyelids of the bay 12-year-old pony were focal lymphomas and were part of a wider, more generalized neoplastic problem. **(D)** A focal sarcoid in the eyelid margin with significantly different implications from all the other similar looking nodules shown in this panel. No assumptions should be made about any condition without a full investigation, which might include biopsy, ultrasound, radiography or other diagnostic procedures.

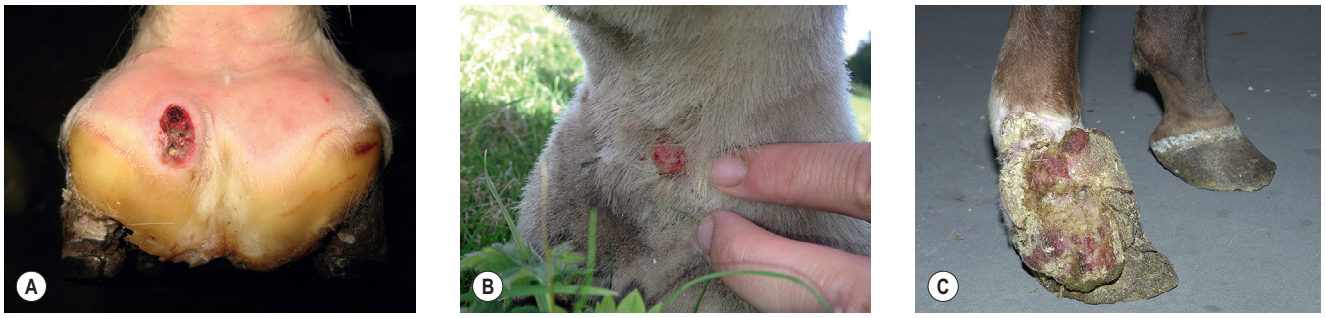


Figure 3.3 Three different tumour types illustrating the variability in prognosis. From a prognostic perspective, the first question that is asked is, 'Is this tumour treatable?' – if not, then, 'Can it be managed and will it/does it have an adverse effect on the patient?' Tumour **(A)** is a haemangiosarcoma with a high (malignancy) grade – removal of the tumour might be 'in time' to preclude metastatic spread but this clearly has a very different outlook to the sebaceous adenocarcinoma shown in **(B)**. Although there is little evidence base for them, surgical resection is likely to be curative. The sarcoid tumour **(C)** has no metastatic capacity but is clearly limiting on function and untreatable and so has a very poor prognosis.

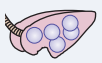
						
	Normal	Transformed	Benign	Malignant	Micro-metastatic	Metastatic
Differentiation	+	–	–	–	–	–
Contact inhibition	+	–	–	–	–	–
Anchorage dependence	+	–	–	–	–	–
Genetically stable	+	–	–	–	–	–
Proper host response	+	+/–	–	–	–	–
Tumorigenic	–	+/–	+	+	+	+
Invasive	–	–	–	+	+	+
Able to disseminate	–	–	–	–	+	+
'Normal' morphology	+	–	+/–	–	–	–
'Normal' histology	+	n/a	+/–	–	–	–
Growth at ectopic site(s)	–	–	–	–	–	+

Figure 3.4 Main features that can be used to differentiate between benign and malignant tumours. Some of the main features that can be used clinically and physiologically/pathologically to differentiate between benign and malignant tumours are shown here. It is easy to view each of these features as black or white features but tumours are best viewed as entirely unpredictable. Therefore, careful examination of all the features of a tumour will help to build up a 'consensus' opinion as to whether a particular lesion is best fitted to one or other category. In addition, malignancy itself carries a graded scale ranging from mild to extreme. *(Figure reprinted from Matrisian LM, Welch DR. Invasion and metastasis: In: Mendelsohn J, Howley PM, Israel MA, et al. (eds) The molecular basis of cancer, 3rd edn. Philadelphia: WB Saunders; 2008: 254. (Fig. 19.3); copyright 2008, with permission from Elsevier.)*

not always clear but the clinical interpretation of the pathological diagnosis of the particular tumour must take account of the known or suggested variability. In fact, benign tumours can still be very dangerous if they occupy vital space or alter local function sufficiently to compromise the animal (Fig. 3.5). For example, a very benign tumour within an eye or in the brain would inevitably have consequences. A benign functional endocrine tumour, e.g. a pituitary adenoma, may be a genuine threat to the animal and cause significant morbidity (see p. 376); in contrast a benign thyroid adenoma can be quite alarming but usually has no clinical implication, even when relatively large (Fig. 3.6).

The owner of a horse whose tumour has been confirmed as 'benign' may be very relieved by that knowledge and may not fully understand that it might still result in considerable threat to the horse's life. Nevertheless, a diagnosis of 'malignancy' will invariably and quite justifiably cause greater concern. However, not all malignant tumours are immediately life-threatening and the patient may well have some useful life ahead. A good example is the equine melanoma, where many tumours start out as pathologically benign and end-up malignant – that transformation in any particular tumour may be very slow and in any case, there may be no material disadvantage to the horse, provided that the dissemination of the tumour is limited and the rate of growth of the metastatic tumours is low. Additionally, 'benign' is not always synonymous with 'treatable' and 'malignant' does not always mean 'untreatable'. For example, a benign melanoma in the ciliary body is probably untreatable, but a malignant tumour of a similar type on the perianal skin may be easily removed (with the caveat that micro-metastasis may have already taken place).

Benign tumours

A benign tumour is a tumour that lacks the ability to metastasize or spread. The term 'benign' implies a mild and

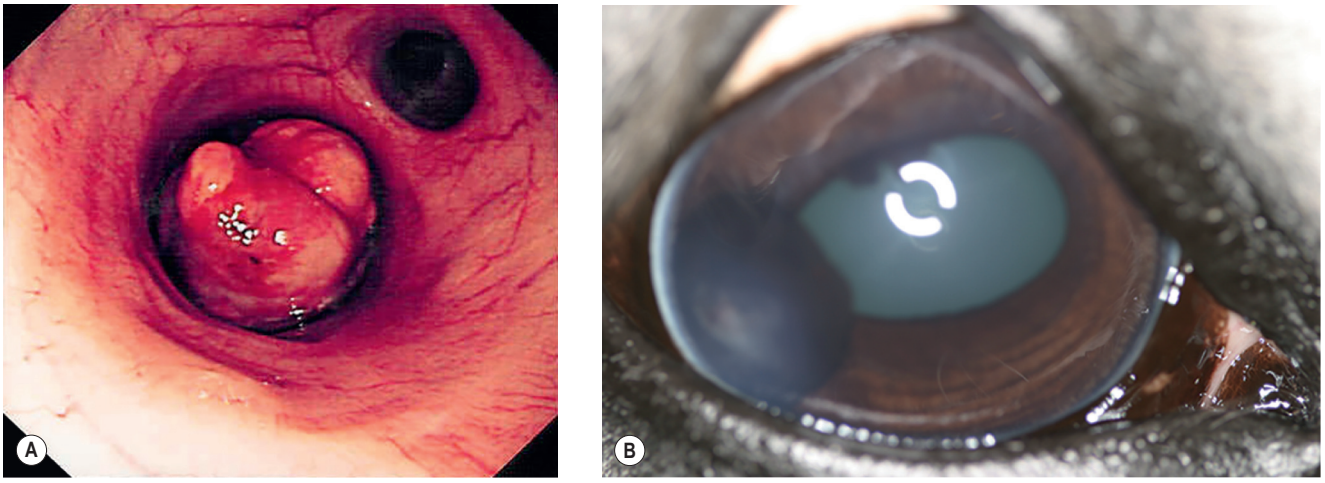


Figure 3.5 Pathological behaviour is not always the most important parameter of a tumour. Small tumours in vital places may have severe, even life-threatening effects, whilst large tumours may be clinically irrelevant. Nevertheless, confirmation of malignancy will usually have profound implications over an indeterminate period. Some benign and malignant tumours progress slowly, whilst others are much more rapid and their clinical and pathological effects will be correspondingly faster or slower. **(A)** Bronchial granular cell tumour is the commonest primary lung tumour of horses. These tumours are usually benign and localized but they may have a profound clinical consequence. **(B)** This iridal melanoma was confirmed as malignant histologically but remained slow growing and localized for 8 years before becoming a significant problem.

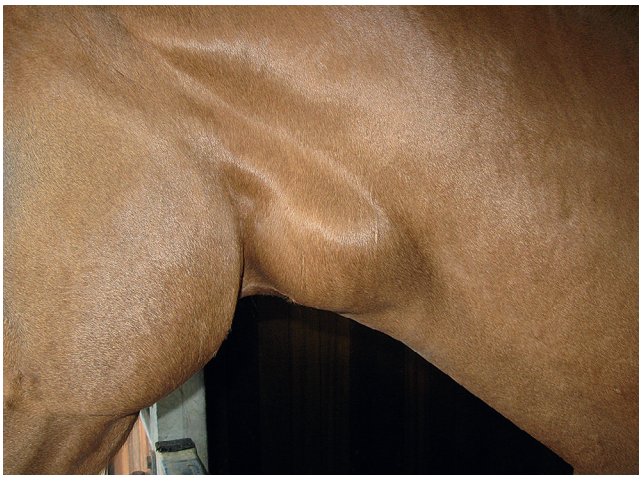


Figure 3.6 A unilateral enlargement of the thyroid gland in a 14-year-old mare. Endocrinological tests revealed no abnormality and the tumour continued to expand slowly without causing any space-occupying effects. It was removed on the basis of potential transformation to malignancy and on cosmetic grounds. The tumour was soft and of a more or less uniform consistency that bulged when sectioned. There were no deleterious effects following the surgery.

non-progressive disease. Benign tumours are considered to be 'non-cancerous' and by definition, are not locally invasive or malignant. Benign tumours are typically (but not always) surrounded by an outer surface (fibrous sheath or capsule) that probably inhibits their ability to behave in a malignant manner (see Fig. 3.6); this aspect probably hinges on the lack of the tumour cells to 'break out' of the confining/restricting basement membranes. There are conflicting opinions regarding capsule formation; first that it is a passive phenomenon caused by pressure exerted by the expanding mass on surrounding, pre-existing collagenous tissue. The second opinion is that it is essentially a foreign-body response instigated to try to 'wall off' the neoplasm.

Both the appearance of the individual cells and the histological architecture in benign tumours are similar to the normal tissue. Many benign tumours are harmless but by virtue of their size and location, they can have significant clinical effects and definable morbidity. Examples of this include tumours which produce a 'mass effect' (compression of vital organs, e.g. blood vessels), such as the benign mesenteric lipoma (Fig. 3.7), or productive tumours of endocrine organs (which may overproduce certain hormones). Examples include granulosa/thecal cell tumour of the ovary and the common pituitary enlargement in older horses that was historically regarded as an adenoma (p. 641).

Many types of benign tumours have at least the potential to develop into malignant cancers; this state probably arises from additional genetic changes in a subpopulation of the tumour's neoplastic cells (p. 47). The cells in the lesions, as in most tumours which frequently progress to cancer, show defined abnormalities of cell maturation and appearance, collectively known as 'dysplasia'. These cellular abnormalities are not seen in benign tumours that rarely or never turn cancerous, but are seen in other pre-cancerous tissue abnormalities, which do not form discrete masses. A prominent example of this phenomenon is the squamous papilloma or squamous dysplasia that occurs on the penile skin of geldings in particular (p. 630). In some circumstances, these may become transformed to (aggressive) malignant carcinoma, but in most, they remain very benign. Some authorities prefer to refer to dysplastic tumours as 'pre-malignant' and reserve the term 'benign' for tumours which rarely or never give rise to cancer (Fig. 3.8).

Most benign tumours are named by attaching the suffix *-oma* to the name of the tissue or cell from which the cancer arose. For example, a tumour that is composed of cells related to bone cells is called an 'osteoma', a benign tumour that derives from dental cementum is termed a 'cementoma', and when the cell type is fat it is termed 'lipoma'. This rule is followed, with a few exceptions, for tumours that arise from mesenchymal cells (the precursors of bone and muscle).

The term *adenoma*, for instance, designates a benign epithelial tumour that either arises in endocrine or other glands (e.g. sebaceous or mammary glands) or forms a

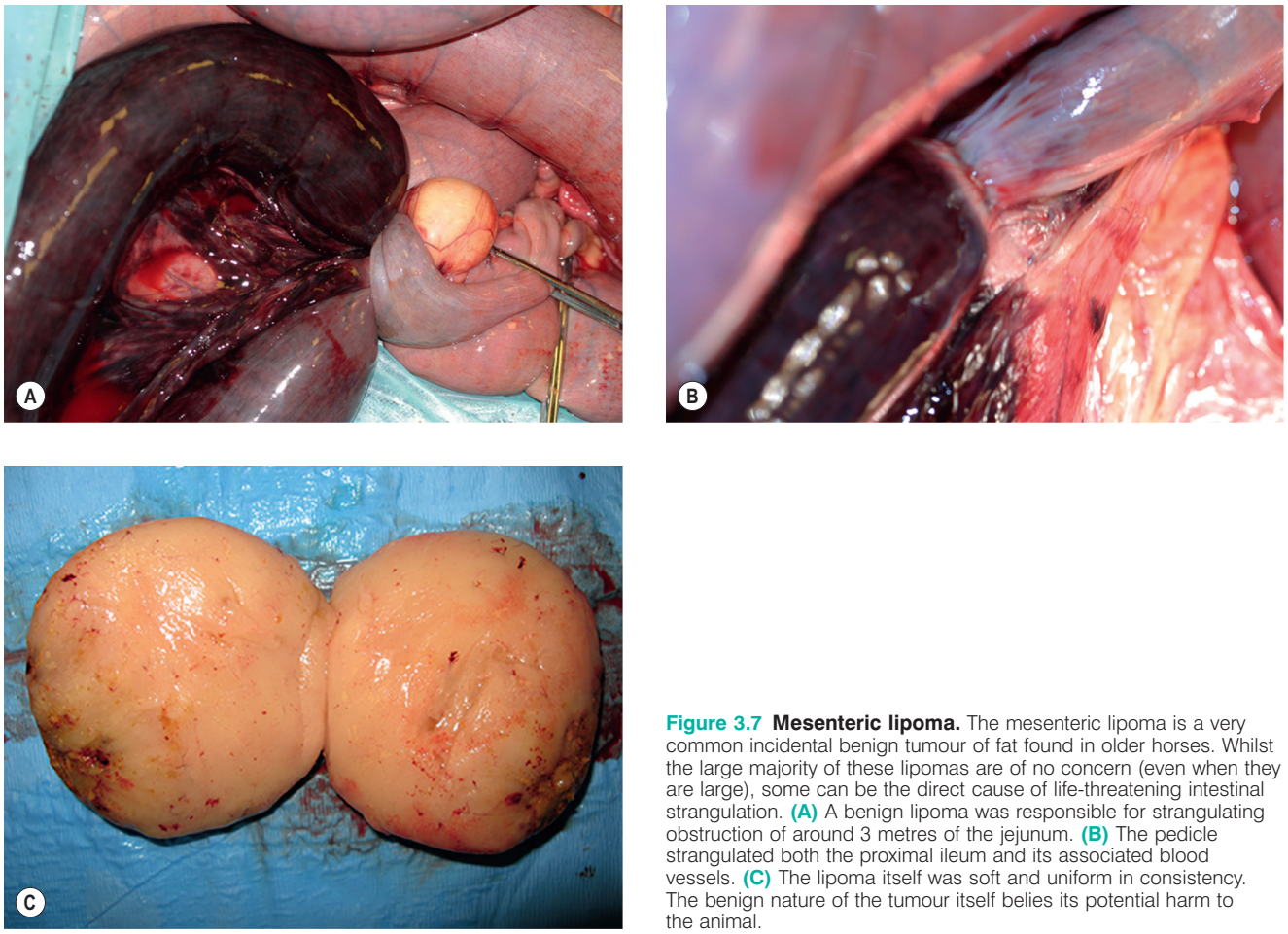


Figure 3.7 Mesenteric lipoma. The mesenteric lipoma is a very common incidental benign tumour of fat found in older horses. Whilst the large majority of these lipomas are of no concern (even when they are large), some can be the direct cause of life-threatening intestinal strangulation. **(A)** A benign lipoma was responsible for strangulating obstruction of around 3 metres of the jejunum. **(B)** The pedicle strangulated both the proximal ileum and its associated blood vessels. **(C)** The lipoma itself was soft and uniform in consistency. The benign nature of the tumour itself belies its potential harm to the animal.

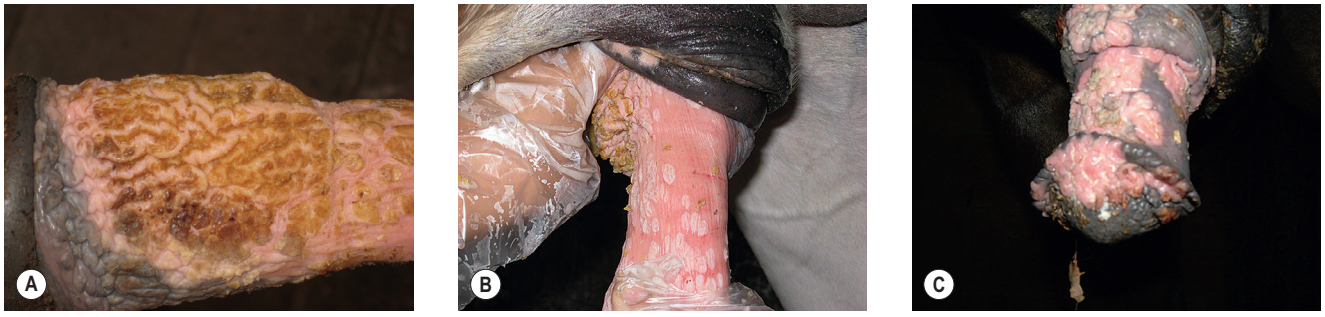


Figure 3.8 A pre-carcinomatous/dysplastic tumour state. (A) This rather mild surface pre-carcinomatous/dysplastic tumour state was encountered in a 15-year-old gelding. The yellow adherent material over the sites (revealed by washing and removal of the material) was largely inflammatory and fibrinous material admixed with keratin **(B)**. The keratin production is consistent with well-differentiated cells. Treatment of the sites resulted in a complete resolution apart from in one lesion, where a more aggressive transformation has taken place. **(B,C)** The small pallid circular areas of leukoplakia are consistent with early dysplastic changes in the keratinocytes. They are characteristically well-differentiated but are pre-carcinomatous changes. At this stage, they are usually treatable and even curable.

glandular structure. A tumour of that contains large cysts is called a 'cystadenoma', e.g. ovarian cystadenoma.

Benign tumours arising from epithelial cells (those cells that form sheets that line the skin and internal organs) are classified in several ways and therefore have a variety of names. Sometimes, classification is based on the cell of origin, whereas in other cases it is based on the tumour's microscopic or gross appearance. The mesenteric lipoma is a very common incidental benign tumour of fat found in older horses (see Fig. 3.7). Whilst the large majority of them are of no concern (even when they are large), some can be the direct cause of life-threatening intestinal strangulation. In this case (Fig. 3.7), a benign lipoma was responsible for strangulating obstruction of around 3 metres of jejunum. The benign nature of the tumour itself belies its potential harm to the animal.

When a tumour gives rise to a mass that projects into a lumen, it is called a *polyp* (Fig. 3.9). Most polyps are epithelial in origin. Strictly speaking, the term 'polyp' should be restricted to benign growths; a malignant polyp should be referred to as a 'polypoid cancer' in order to avoid confusion.

A *papilloma* is a benign, exophytic epithelial tumour, built up of finger-like projections from the skin or mucous membranes. When used without context, it is usually used to describe 'warts', which are reactive 'responses', usually to a papilloma virus infection (Fig. 3.10). There are, however, a number of other conditions characterized by papillomatous changes, including the sarcoid (p. 203) and the squamous papilloma precursor of carcinoma (see Fig. 3.10C).

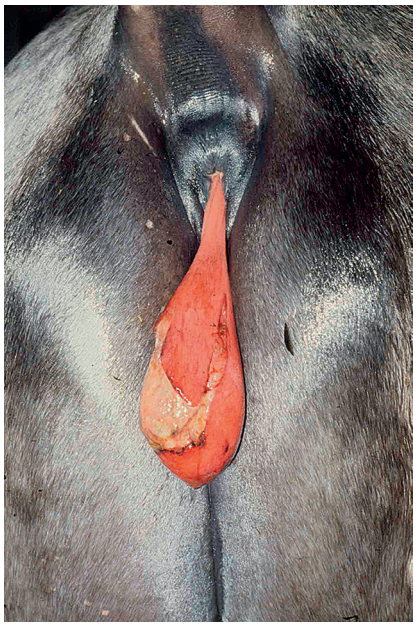


Figure 3.9 A rectal polyp with its classical polypoid appearance. This tumour is benign and was attached to the dorsal rectal wall some 20 cm from the anus. It was very easily treated and did not recur. A 'polyp' is the general descriptive term used to describe any mass of tissue that projects outward from the normal surface level of a mucous membrane or the skin, being a structure growing from a relatively narrow base or a slender stalk. In horses, polyps occur mostly in the gut and nasal cavities.

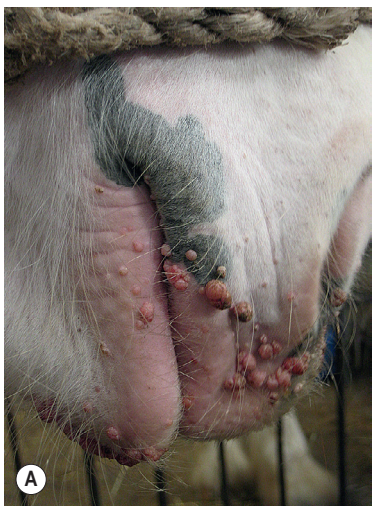
Malignant tumours

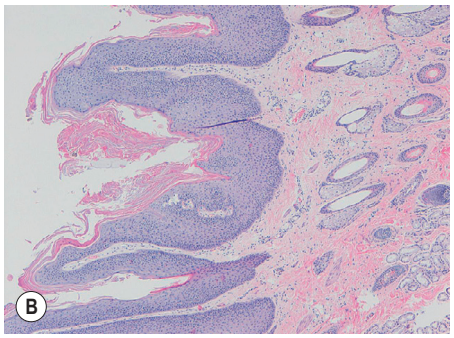
'Malignant' is the term applied to tumours that behave in an aggressive and invasive manner.

The method for classification and description of malignant tumours follows similar rules in using prefixes and suffixes. The tissue of origin again provides the basis of the name. The suffix *-sarcoma* indicates neoplasms that arise in mesenchymal tissues and the name of the cell of origin is added; for example, a malignant tumour derived from fibroblasts would be termed a 'fibrosarcoma' and a tumour of blood vessels would be called a 'haemangiosarcoma'. Tumours deriving from epithelial tissues use the suffix *-carcinoma*. Most cancers arising from the ectoderm and endoderm are carcinomas, since these cells primarily become surface epithelium and gland parenchyma. Thus, a tumour of squamous epithelium is termed a 'squamous cell carcinoma' and one that affected the basal cells of the skin would be called a 'basal cell carcinoma'.

Just as *-adenoma* designates a benign tumour of epithelial origin that takes on a gland-like structure, so *-adenocarcinoma* designates a malignant epithelial tumour with a similar growth pattern. Usually the term is followed or preceded by the organ of origin, e.g. sebaceous adenocarcinoma of the skin and adenocarcinoma of the colon.

Malignant tumours of the blood-forming tissue are designated by the suffix *-emia* (Greek: 'blood'). Thus, *leukaemia* refers to a cancerous proliferation of white blood cells (leucocytes). Cancerous tumours that arise in lymphoid organs (such as the spleen, thymus or lymph glands) are termed 'malignant lymphomas'. The term *lymphoma* is often used without the qualifier *malignant* to denote cancerous lymphoid tumours; however, this usage is





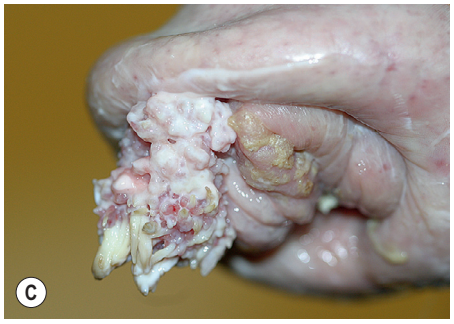


Figure 3.10 Papillomatous lesions. **(A)** A typical viral papilloma on the muzzle of a young gelding. **(B)** This is a benign virally induced tumour which is very recognizable histologically when the characteristic frond-like epidermis with a fibrovascular 'core' is present with marked epidermal hyperplasia. **(C)** Not all papillomatous lesions are insignificant. This squamous papilloma on the penile skin is a pre-carcinomatous state and that again can be easily recognizable histologically.

confusing, since the suffix *-oma*, as mentioned above, more properly designates a benign neoplasm.

The suffix *-oma* is also used to designate other malignancies, such as 'seminoma', which is a malignant tumour that arises from the germ cells of the testis. In a similar manner, malignant tumours of melanocytes (the skin cells that produce the pigment melanin) should be called 'melanosarcoma', but for historical reasons the term *melanoma* persists.

Most cancers arising from the ectoderm and endoderm are *carcinomas*, since these cells primarily become surface epithelium and gland parenchyma. Of course, the ectoderm is also the source of nervous tissue, but tumours arising here are less common than carcinomas.

Cancers arising from the mesoderm can be carcinomas (e.g. adrenal, genitourinary), sarcomas (solid connective tissue and all muscle types) or cells of haematopoietic origin (leukaemias, lymphomas).

The cells and the histological architecture in malignant cancers are bizarre. The grade of a cancer, depending on the tumour type and system used, may be a function of one or more factors, including how bizarre the cells look (see Figure 3.14) the mitotic rate; their architectural arrangement or density of packing; how deeply or extensively the tumour cells invade underlying or surrounding tissues; or the presence or extent of tumour cell necrosis. The higher the grade, the more likely the cancer is to behave aggressively. It should be noted that grading is not appropriate for all types of cancer; some endocrine neoplasms, for example, can have a 'benign' appearance and yet have metastasized throughout the body. The stage of a cancer is how far the clinician knows it has spread (p. 19).

Staging and grading of tumours are concepts that are widely used in human, and to a lesser extent, in small animal veterinary practice. They are not usually applied to equine cancers for a multitude of rather weak reasons! The lack of a significant bank of cases makes it difficult to predict what is going to happen and the absence of effective diagnostic methods adds to the complications. Some form of clinically useable staging that predicts, with some degree of accuracy, what the tumour will most likely result in, would be a really helpful development for horses.

All tumours, whether benign or malignant have two basic components:

1. The proliferating abnormal, tumour cells that constitute the *parenchyma*; they determine the overall behaviour and the pathological consequences of the tumour.
2. A supportive *stroma* comprising variable connective tissue components, extracellular matrix and blood vessels. The growth and evolution of the tumour is strongly dependent on the nature of the stroma, and this is clinically recognizable and pathologically useful.

An adequate stromal blood supply is a critical factor in the natural history of a tumour (p. 48).

Assessing tumour activity and behaviour in vitro and in vivo

The classification of the severity and clinical nature of a neoplasm can be made through the process of grading/staging. This expresses the level of differentiation (*grade*) and the extent of spread of the tumour (*stage*). However, attempts to establish this system in horses has commonly been frustrated by the lack of correlation between the clinical and histological features. Grading is therefore not yet widely employed and, instead, a morphological description is more commonly used.

Grading

Knowing what the tumour is, is a fraction of what needs to be known about its pathological behaviour.

The American Joint Commission on Cancer provides guidelines for grading tumours using a simple system that classifies cancer cells in terms of how abnormal they appear compared with normal tissue cells, when examined under a microscope.[1]

The objective of a grading system is to provide useful guidance about the probable growth rate of the tumour and its tendency to spread. This is achieved through attempting to quantify the degree of cell differentiation and the number of mitoses within the tumour; this is expressed as a proportion of the cells present and so correlates quite well with the extent of tumour aggression.

Based on the microscopic appearance of cancer cells, pathologists may describe tumour grade in 4 degrees of severity (G1–G4) (Fig. 3.11). The term *GX* is used when the grade cannot be assessed ('undetermined grade'):

- The cells of *Grade 1* tumours resemble normal cells and may have a relatively normal function. Thus, a Grade 1 carcinoma (Fig. 3.11) might produce keratin and may be histologically very similar to normal cells. It would tend to grow and multiply slowly. Grade 1 tumours are generally considered the least aggressive in behaviour. There is recognizable differentiation of the cell type involved and low numbers of mitotic cells.
- Cells of *Grade 2* tumours have noticeable abnormalities, but are still recognizable as the parent tissue. They may have less proliferative ability and are much less physiologically normal (see Fig. 3.11).
- The cells of *Grade 3* (*or Grade 4*) tumours are more difficult to relate to normal cells of the same type. Grade 3 tumours tend to grow more rapidly and spread faster than tumours with a lower grade (see Fig. 3.11).
- *Grade 4* is the most undifferentiated and rapidly dividing tumour behaviour and these tumours are more usually termed 'anaplastic', on the basis that totally undifferentiated cells are seldom encountered (see Fig. 3.11). Given that they have an almost infinite variety of functional and behavioural changes it is usually very hard to differentiate and identify these tumours without specific immunohistochemical and specialized procedures which being invariably required to pathologically identify the tumour type. Often, the tumour type itself can be deduced clinically from intuitive supposition but this is a minor part of the process of tumour investigation and management.

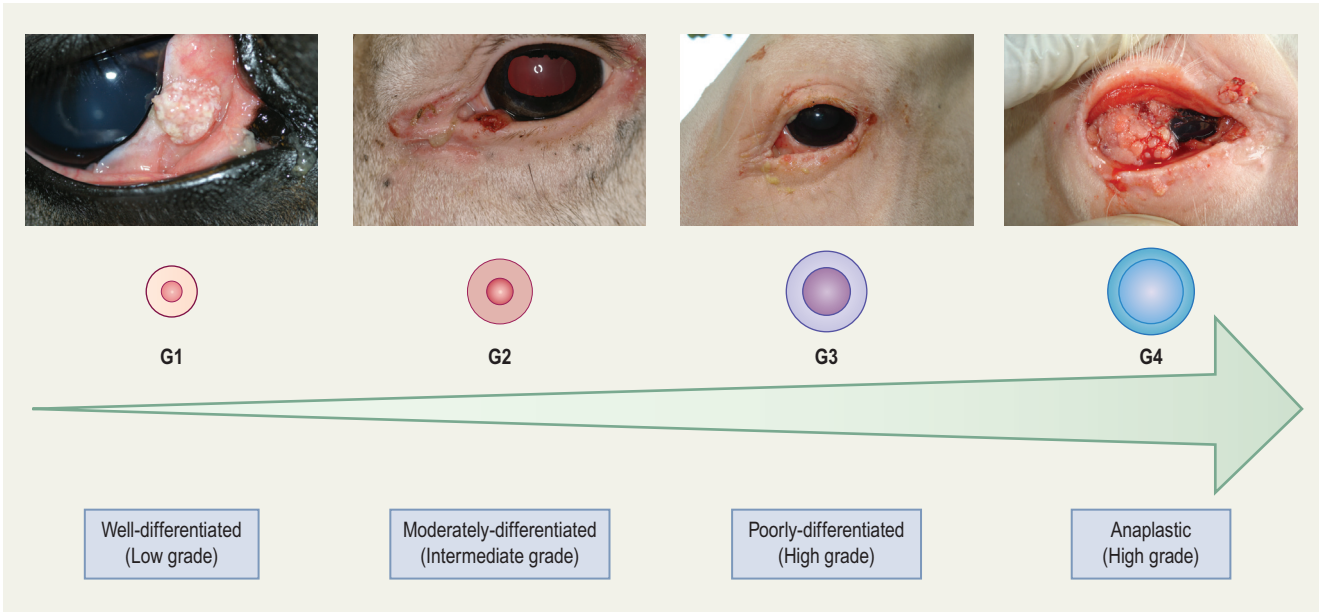


Figure 3.11 Tumour grading. The grade of a tumour gives a strong guide to the likely clinical and pathological behaviour of the tumour. The range is from well-differentiated cells that closely resemble the parent cells (G1) to the most undifferentiated cells that often bear little morphological similarity to the parent cells (G4). In the latter case, specific stains are often required to identify the parent cell type. A series of palpebral/conjunctival carcinomas ranging from the benign well-differentiated squamous papilloma/squamous dysplasia through to the most severe, poorly-differentiated and infiltrated carcinoma is illustrated. The G4 case had metastatic local lymph node involvement. Tumour grading is a system that is widely adopted in equine oncology because of its simplicity and its applicability to the common tumours of the horse. There are constraints on the other systems of classifying the clinical behaviour of tumours in horses.

Since the grading system is not a strict division, subtle variations in the grading system that take account of more sophisticated tissue staining methods are used to grade each tumour type, but the general principle is to attempt to quantify the extent to which the tumour cells differ from cells of the appropriate normal/parent tissue. Whilst histological grade, also called 'differentiation', refers to how much the tumour cells resemble normal cells of the same tissue type, nuclear grade refers to the size and shape of the nucleus in tumour cells and the percentage of tumour cells that are dividing (mitotic rate). The latter is usually expressed as a number of mitotic figures per high power (×400) microscopic field or per 10 high power fields. Grading may play a role in treatment decisions where appropriate information is available. Cells of Grade 3 or Grade 4 tumours are more difficult to quantify.

Although the grade is a useful pathological parameter, it is not necessarily an accurate index of the pathological behaviour because the histological appearance and clinical behaviour may not be closely correlated. In an attempt to avoid spurious 'opinion', tumours are also graded histologically, according to the degree of differentiation from normal, using terms such as well-differentiated/moderately-differentiated/highly-undifferentiated, etc. (Fig. 3.12).

To some extent, the classification of a certain tumour may vary between different pathologists, as this is, in reality, a spectrum of change.

Note

Grading is probably less useful than staging in most circumstances, but since staging is seldom used in horses, the grade is a more commonly employed description and is probably better understood.

Staging

Applying a stage to the spread of the tumour relies on performing examinations and tests to establish the extent of the cancer within the body and it particularly refers to whether the disease has spread from the original site to other parts of the body (i.e. whether it has metastasized); it is therefore an index of the degree of cancer progression. It is, potentially at least, a more useful measure of the tumour's nature than the grading system. The size of the primary lesion, the extent of spread into the adjacent tissues and local lymph node(s), and the presence of remote metastatic dissemination are incorporated into the system. It is important to know the stage of the tumour in order to provide a likely prognosis, to plan the best treatment and to monitor the effects of treatment procedures.

Although there are two recognized systems in human medicine (the 'TNM' system and the 'Ann Arbor' system), neither one is ideal for the equine species, and somewhat regrettably, neither is widely employed in equine practice. This is probably understandable, since two of the major assessed parameters are not easily incorporated into equine oncology, but there has been no real effort to develop systems of tumour classification that apply to the particular difficulties of equine oncology.

TNM tumour staging

The preferred UICC (Union for International Control Cancer) staging system is the TNM system. It uses the tumour size (T), the involvement of regional lymph nodes

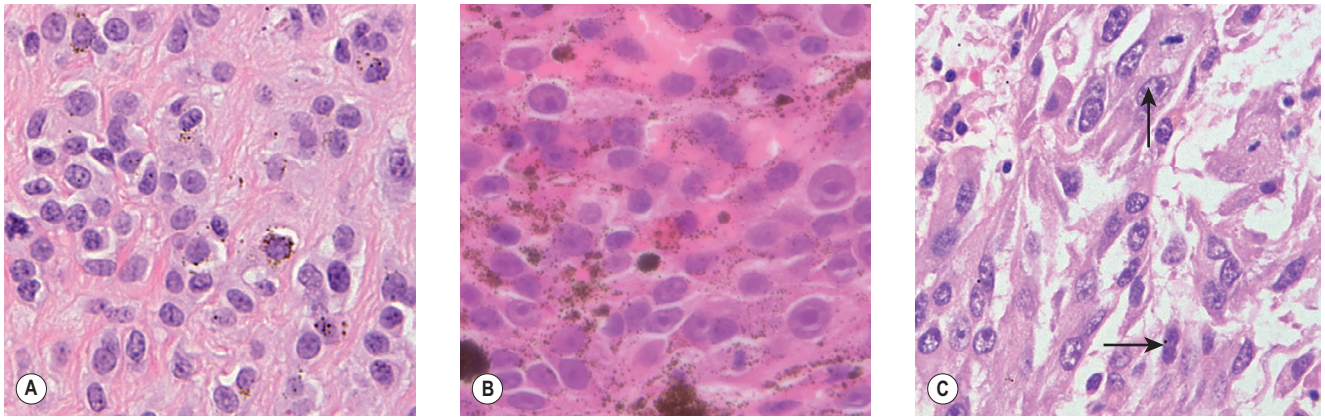


Figure 3.12 The equine melanoma. **(A)** Well-differentiated tumour cells in a melanocytic naevus that are polygonal or round with small amounts of melanin pigment and minimal nuclear or cytoplasmic size variation. The cells are subtly different in morphology and cell function. Well-differentiated melanoma cells are the earliest changes that will be seen and, often, special stains have to be used to identify them. **(B)** A moderately-differentiated melanoma, in which the polygonal tumour cells show significant variation in nuclear size and the nuclei contain prominent, central nucleoli (some melanin pigment is still observed). The loss of cellular differentiation occurs in stages and cells that are moderately-differentiated will retain some of the normal function (i.e. accumulation of melanin) but will have more obvious cellular changes with a higher replication (mitotic) rate. **(C)** A poorly-differentiated melanoma, in which the tumour cells are spindle-shaped, contain minimal pigment and significant numbers of mitotic figures, and are difficult to recognize as being of melanocytic origin. The undifferentiated tumour shows little resemblance to the parent cell type in both morphology and function. The equine melanoma affords an excellent example of the stages of cellular differentiation. From a histological perspective, it can be hard to separate early well-differentiated melanoma from normal melanocytes. The further down the path of loss of differentiation, the more difficult it becomes to identify the parent cell type. Mitotic figures (arrows) and abnormal cell structures are commonly found in advanced poorly-differentiated tumours.

(N) and the extent of metastases (M) to numerically express some concept of the severity of the tumour.

Measured/assessed parameters

T: This describes the size or the direct extent of the primary tumour. The size is dictated by the numerals 1–4; small tumours are termed '1' and very large ones '4'. Where the tumour is restricted to the epithelium, it is termed in situ (or intraepithelial) and the suffix *-is* is added to the T as in T-*is* signifying that the tumour is still restricted by the basement membrane. A good example of such a tumour is afforded by the in situ squamous cell carcinoma of the cornea or lateral limbal conjunctiva in horses (Fig. 3.13).

N: The degree of spread to regional lymph nodes is classified as 1–3; '1' being that the first local drainage node is involved and '3' means that the most distant available node in the precise pathway of the lymphatic drainage is involved:

- N0: Tumour cells absent from regional lymph nodes
- N1: Regional lymph node metastasis present (at some sites, tumour spread to the closest or a small number of regional lymph nodes)
- N2: Tumour spread to an extent between N1 and N3 (N2 is not applied at all sites)
- N3: Tumour spread to more distant or numerous regional lymph nodes (N3 is not used at all sites).

It is standard procedure in human medicine to biopsy or even remove lymph nodes when a primary mass is excised but most veterinarians do not do this! When they do, it is usually by accident, but it is obviously very useful in terms of staging and the initial diagnosis. Biopsies must be wedge biopsies at least, rather than punch or needle biopsies (see p. 108).

M: The letter M is simply whether distant metastasis is absent (M0) or present (M1).

In order to ensure consistency of expression, the letter X is used where the parameter was not assessed or investigated.

Further letters are commonly appended to the TNM system to try to provide more useful information, particularly about the pathological/histological investigations. These include:

G (1–4): This describes the pathological *grade* or extent of differentiation of the cancer cells. 'Low-grade' tumours are those that are well-differentiated and the cells therefore appear similar to normal cells, whereas 'high-grade' tumours appear poorly-differentiated, often to the extent that the parent cell type cannot immediately be recognized (Fig. 3.14).

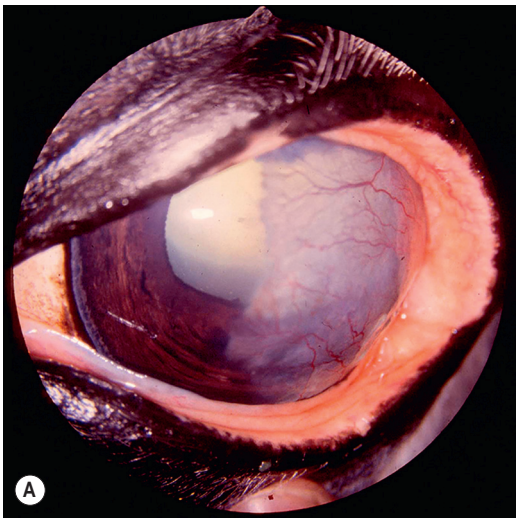
R (0/1/2): Where tumours are removed surgically, the completeness of the *tumour excision* is described; whether the boundaries are free of cancer cells (R0) or not (R1 – marginal boundaries or R2 – no boundaries and the tumour extends beyond the boundary). In the latter case, recurrence can be expected and further excision may be indicated if feasible.

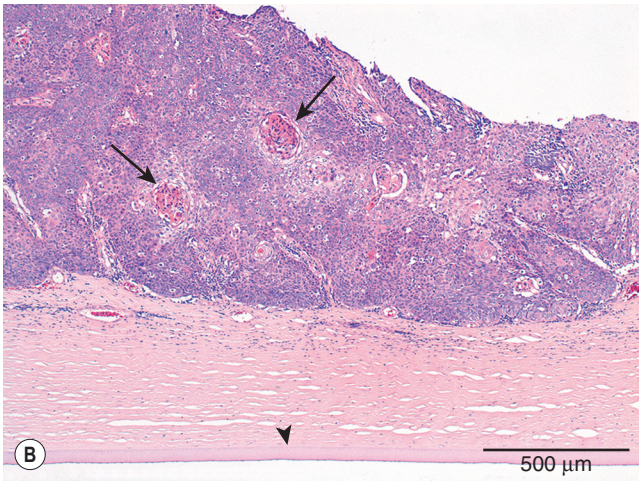
L (0/1): *Invasion into lymphatic vessels* is classified as absent (L0) or present (L1).

V (0/1/2): *Invasion into veins* is described as absent (V0), present microscopically (V1) or macroscopically (V2).

C (1–5): This is a modifier of the *certainty* (quality) of the last-mentioned parameter and can be used to assist a third party in making decisions as to whether a classification is reliable or less certain.

Thus, a large aggressive tumour might be described as T4/L3/M2, while a benign local tumour might be shown as T1/N0/M0. The system is more flexible than the other classifications and allows a clearer description to be made.





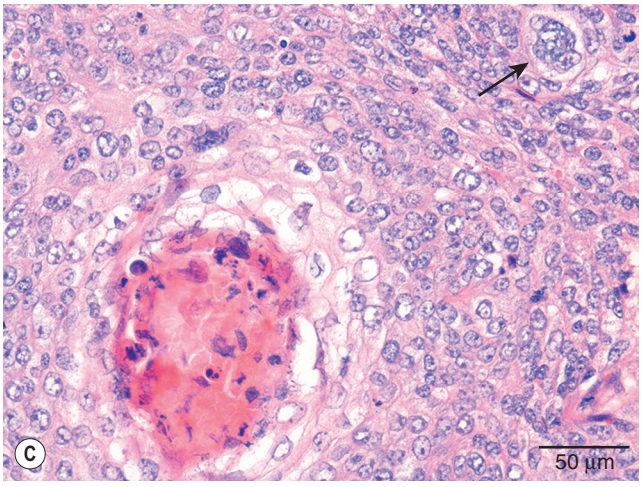


Figure 3.13 An in situ intraepithelial carcinoma. (A) A good example of an in situ (intraepithelial) carcinoma of the cornea of a Dutch Warmblood carriage horse gelding, aged 16 years. Grossly, it is possible to examine this with a slit lamp and get some idea of the depth of the tumour. It is well vascularized and highly active. **(B)** Histologically, there is marked irregular proliferation of the surface epithelium with multifocal areas of apoptosis and dyskeratotic cells (arrows). Note the intact Descemet's membrane (arrowhead) (H&E). **(C)** Magnified image of the cancer showing accumulation of necrotic and partly keratinized cells surrounded by a layer of pale, swollen keratinocytes. Occasional cells with marked nuclear atypia can be seen (arrow) (H&E). *(Figures B and C courtesy of Dr Guy Grinwis, Veterinary Pathology, Utrecht.)*

The additional letters are used to provide significant extra information and the G classification is a critical addition, since it attempts to establish the severity of the pathological behaviour of the cells involved and, in that way, it retains elements of the grading system as well.

Ann Arbor tumour staging

The Ann Arbor staging system is used primarily for human lymphoma. In this system, the principal stage is determined by location of the tumour:

Stage I indicates that the cancer is located in a single region, usually one lymph node and the surrounding area. Stage I often will not have outward symptoms. This might usefully apply, for example, to the isolated lymphoma that occurs in the conjunctiva in horses (p. 620).

Stage II indicates that the cancer is located in two separate regions, an affected lymph node or organ and a second affected area, and that both affected areas are confined to one side of the diaphragm – that is, both are above the diaphragm, or both are below the diaphragm. This is seldom applicable in horses, in any case, but there are some lymphosarcoma cases that have restricted distributions within the thorax or the abdominal cavity (e.g. hepatosplenic lymphosarcoma, p. 535).

Stage III indicates that the cancer has spread to both sides of the diaphragm, including one organ or area near the lymph nodes or the spleen. This stage usually merges with stage IV in horses, since most lymphosarcoma cases that have multiple foci are classified as diffuse or generalized.

Stage IV indicates diffuse or disseminated involvement of one or more extralymphatic organs, including any involvement of the liver, bone marrow or nodular involvement of the lungs. Most equine lymphosarcomas ultimately fall into this category.

Modifiers are used to add to the stage in some particular circumstances and these are expressed as letters:

A: Signifies the *absence* of systemic implications.

B: The *presence* of systemic signs. This is used if the disease is '*extranodal*' (i.e. not in the lymph nodes) or has spread from lymph nodes to adjacent tissue.

S: Applied to show that the tumour has *spread* to, or involves, the spleen.

X: If the largest detectable tumour is over 10 cm in size.

Additionally, there are other aspects that are used in human medicine; the relative width of the mediastinum and the findings at laparotomy are used, but these aspects are much less applicable to the horse, since imaging the required detail is, at present at least, impossible and the logistics of laparotomy is a significant barrier.

The Ann Arbor staging system takes no account of the pathological behaviour of the tumour and so is probably less useful as a prognostic indicator without the subtle modifications that have been applied to the system (the 'Cotswold modifications'). Nevertheless, this system might easily be applied to the lymphoma group of diseases in the horse.

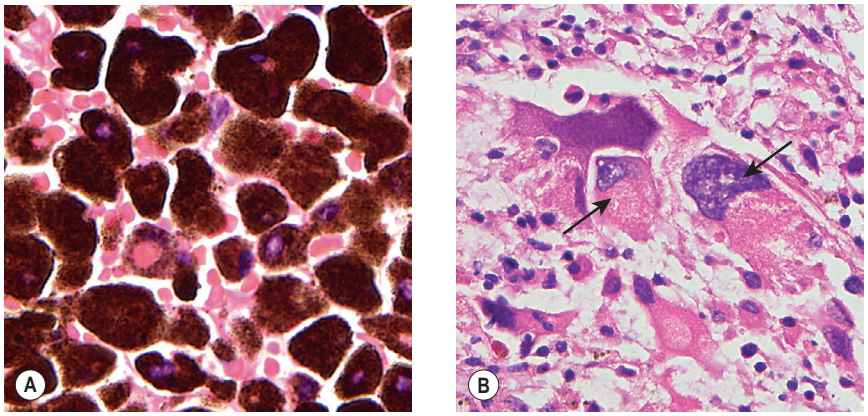


Figure 3.14 Low-grade and high-grade melanoma. **(A)** A low-grade melanoma. There is plentiful accumulation of melanin and the cells (after bleaching) will be more or less polygonal and uniform, with few mitotic figures. **(B)** A high-grade melanoma. An anaplastic melanoma shows large, irregularly-shaped giant tumour cells, some with giant nuclei (arrows). These are difficult to relate to either the normal cell type or better-differentiated forms of melanocytic neoplasia. The transition between the two grades from a diagnostic perspective is gradual, but the changes within a tumour itself can be (and usually are) abrupt and correspond to clonal expansion of abnormal cells following mutations.

These staging systems are not used widely at present in horses, possibly because the overall prevalence of tumours is low (or at least because they are poorly described in the literature) and because few are fully understood and completely characterized. The system does, however, enable better comparisons between treatments and prognosis and so there is merit in the development of a suitable dedicated system in equine oncology.

Reference

1. American Joint Committee on Cancer. AJCC cancer staging manual, 6th edn. New York: Springer; 2002.

4

Biology of tumour growth

The molecular and genetic (cellular) basis of cancer

For tissues to function normally, the cells must replicate, repair and renew; this process is naturally a carefully controlled balance between the proliferation and differentiation of cells and their death. Therefore, tissues and organs need a predictable cell structure and physiology to function normally; the life-cycle of cells is controlled to ensure that their specific genetic blueprint is accurately expressed, so that each copy of the cell is a perfect replica and is able to function identically. Control of the cell-cycle is pivotal to achieving the required balance between formation and natural cell destruction; a fundamental feature of cancer is the dysregulation of the cell replication cycle.

The mechanisms and pathways that influence and regulate the cell-cycle are very complex but can be simplified. Within every living cell there is a cell-cycle 'clock' that determines whether or not a cell should divide. This 'clock' effectively controls, and regulates intracellular processes and integrates the regulatory signals received by the cell with the current health status of the individual cell. This dictates the progression through natural replication to ensure that there is an effective and useful generation of new identical and healthy daughter cells.

The cell-cycle can be conveniently, if simplistically, divided into four individual phases (Fig. 4.1): these are simply convenient divisions for understanding the way a cell replicates, but it is wrong to view this as a production line that has distinct halts or pauses. It is, however, regulated by distinct physiological events within the cell. The process is smooth and continuous and there are significant differences between organs that are undergoing growth (i.e. increasing the absolute numbers of cells) and those which have reached maximal size and which are then limited to maintenance of function. The ordered progression through the cell-cycle is an intricate process that is governed by positive and negative signalling molecules both externally and internally.

Broadly, the cycle can be divided into the 'business' phases, which include the S-phase (synthesis), during which DNA is replicated, and the divisional phase, the

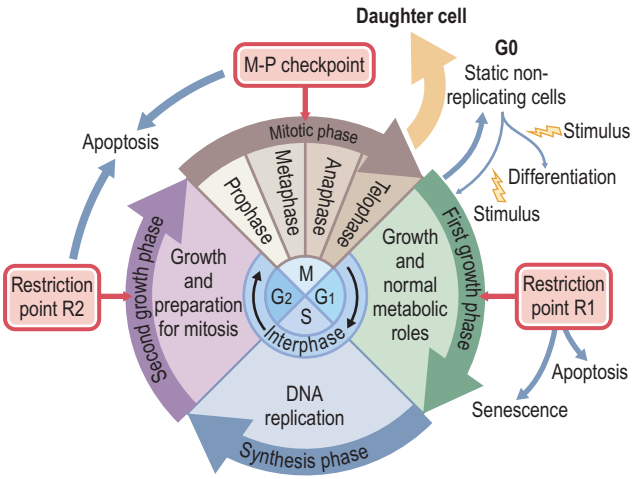


Figure 4.1 The cell-cycle. The cell-cycle is the fundamental method of cell replication. The completed cycle, which comprises four recognized stages, results in the production of a daughter cell identical in every way to the 'parent cell'. The first growth phase (G1) is the stage where intracellular metabolism and growth prepares the cell DNA for replication – the manufacture of the building materials required. This is followed by DNA synthesis stage (S) – DNA is prepared. The second growth phase (G2) is the stage that prepares the cell for mitosis. The mitotic phase (M) is the stage of replication during which the cell undergoes cellular division in the classical manner to produce two identical cells. At the end of cell division, the cell may be diverted back into the cell-cycle for further replication but the large majority of cells are 'shunted' into the G0 stage, in which most cells spend their functional lives. Some cells differentiate into specialized cells and may then remain static for life without any further replication. Nerve cells are good examples of this process. Other cells perform their functions in organs and structures but with suitable stimulus they can be redirected back into the cell-cycle to restore organ integrity of functionality. At various points in the cell-cycle, 'decisions' have to made on the stability and purpose of the process. There are three restriction points at which fundamental checks are made to ensure the integrity of the cycle. The R1 restriction point is governed by genes of the p53 family and this point checks that the genetic codes are correct. If not, the cell is either blocked for further replication and is 'passed' into a state of static non-functionality (senescence), or the pathways for apoptosis are triggered and the cell is dismantled in an orderly fashion. The R2 and the M-P restriction points serve similar purposes and can divert cells with 'irreparable' problems to an apoptotic pathway. In this way, the construction of the new cell is strictly monitored and controlled to ensure perfection. Any cellular alteration that either evades the restriction points or damages the restriction point function will be potentially dangerous in producing an abnormal cell clone.

M-phase, in which mitosis, cell (division), takes place. DNA is packaged, the cells divide and DNA is distributed appropriately to the daughter cells (see Fig. 4.1). The S- and M-phases are separated by G-phases (GAP), during which the genetic structure is 'proofread' and checked for perfection to ensure that the DNA replication is completed and parcelled correctly prior to the division of the cell in the M-phase. The first GAP-phase (G1) separates the M-phase from the S-phase, whilst the second gap phase (G2) separates the S-phase from the M-phase.

Additionally a G0-phase can exist when the cells are held in a static state or quiescent; in this circumstance the cells leave the cell-cycle by the absence of external signals that drive replication. When there are no external signals the cells are usually in the quiescent (G0) phase but when external, pro-differentiation, signals arrive either as result of the death of an adjacent cell, or through external endocrine and cytokine mediators, the cell then re-enters the cell-cycle. Organs naturally have to grow in addition to replicate and so this also explains how organs can expand in response to defined stimuli from other endocrinological or cytokine signals.

Progression through the four phases of the cell-cycle is exquisitely controlled by multiple components, which control transition between the stages at checkpoints; these are stages at which 'decisions' are made about whether the cell can progress or whether reparative processes need to be instigated or diversion of the cell towards a static non-dividing state or destruction are taken. This regulation allows DNA replication and cell division to be coordinated and also protects against DNA damage. The presence of any damage or error in the DNA results in prevention of progression through the main checkpoints of the cell-cycle and leads to cell-cycle arrest. Arrest of the cell-cycle does not mean that the cell is necessarily destroyed, since at these stages decisions are taken concerning whether the damage is repairable or, if the damage is severe, whether the cell needs to be diverted towards a planned and processed dismantling of the cell – apoptosis. It is this control of the cell-cycle progression and apoptosis that ensures cell integrity and identicalness and this is the fundamental basis also of cancer cell formation.

The cyclins are one important recognized group of proteins involved in the pathways of the cell-cycle; they regulate the transition from one stage to the next at the checkpoints through regulating cyclin-dependent kinases (CDKs), which regulate the process in the G1- and S-phases (Fig. 4.2). One of the most important checkpoint controls is the transition between the G1- and S-phases, which commits the cell to subsequent division. The process of control at this checkpoint can be inhibited by proteins, such as p53 and CDK inhibitors or can be enhanced by external mitogenic growth factors. The other transition is between the G2- and M-phases (see Fig. 4.1). Failure to control transition through the checkpoints of the cell-cycle leads to uncontrolled or excessive cell proliferation. This genomic instability also increases as damaged DNA is replicated through sequential generations of daughter cells.

The control of the cell-cycle and apoptosis (naturally planned or controlled cell death without local or remote harm) are central to the development of cancer. The process is complex and is being continually explored by researchers. The progression of a cell through the cell-cycle is positively

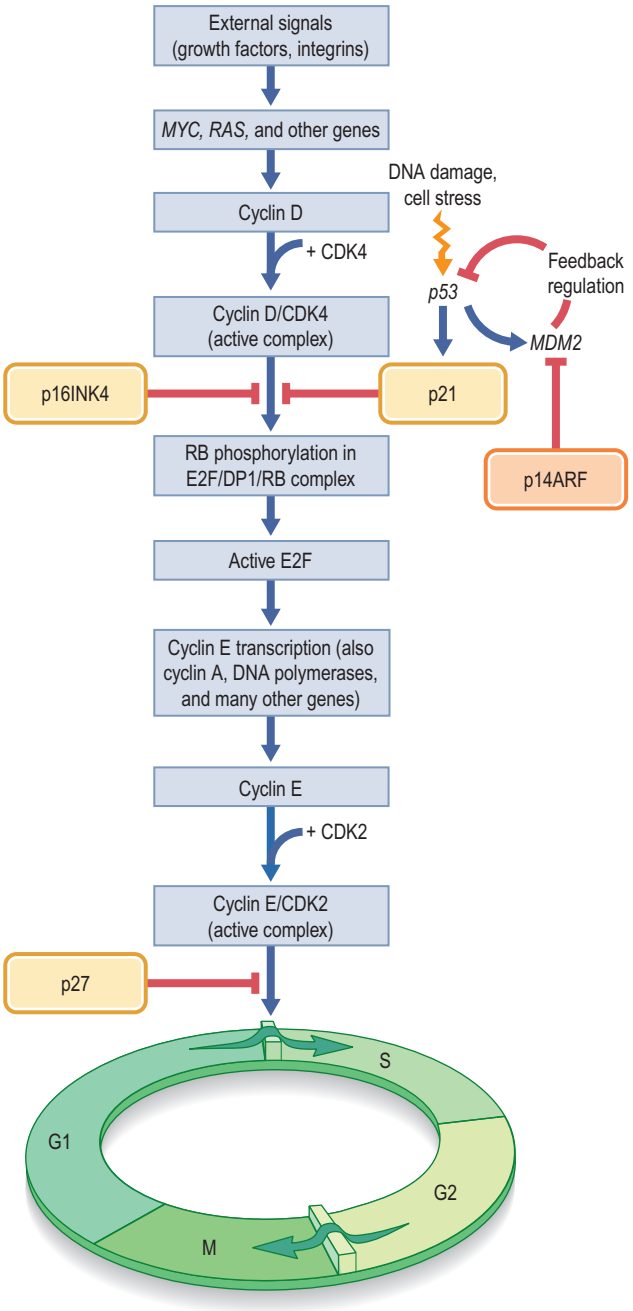


Figure 4.2 The role of cyclins, CDKs and cyclin-dependent kinase inhibitors in regulating the G1/S cell-cycle transition. Schematic illustration of the role of cyclins, CDKs and cyclin-dependent kinase inhibitors in regulating the G1/S cell-cycle transition. External signals activate multiple signal transduction pathways, including those involving the *MYC* and *RAS* genes, which lead to synthesis and stabilization of cyclin D (there are several D cyclins, but, for simplification, we refer to them as 'cyclin D'). Cyclin D binds to CDK4, forming a complex with enzymatic activity (cyclin D can also bind to CDK6, which appears to have a similar role as CDK4). The cyclin D–CDK4 complex phosphorylates RB, located in the E2F/DP1/RB complex in the nucleus, activating the transcriptional activity of E2F (E2F is a family of transcription factors, which we refer to as 'E2F'), which leads to transcription of cyclin E, cyclin A and other proteins needed for the cell to go through the late G1 restriction point. The cell-cycle can be blocked by the Cip/Kip inhibitors p21 and p27 and the INK4A/ARF inhibitors p16INK4A and p14ARF. Cell-cycle arrest in response to DNA damage and other cellular stresses is mediated through *p53*. The levels of *p53* are under negative regulation by *MDM2*, through a feedback loop that is inhibited by p14ARF. *(Figure reprinted from Kumar V, Abbas A, Fausto N. Robbins and Cotran pathologic basis of disease, 7th edn. Philadelphia: WB Saunders; 2004: 291 (Fig. 7.29); copyright 2004, with permission from Elsevier.)*

regulated by a family of cytokines (enzymes) known as 'cycline-dependent kinases' (CDKs).

A tumour is formed by the clonal expansion of a single cell that has incurred some genetic damage that imparts recognizably abnormal characteristics to it. The loss of cellular growth control is the result of a single, or more usually a series, of genetic changes in the affected cells and arises basically from an evolutionary selection of the cells that survive best – it is in fact a process that mimics evolution itself! Oncogenes, tumour suppressor genes and DNA repair genes are the most significant genetic influences in cancer pathogenesis; they are those genes that directly or indirectly affect the regulation of the basic cell processes (see later).

Non-lethal genetic damage is the most fundamental 'bottom line' of cancer biology. This genetic damage can be acquired through exposure to environmental challenges that can be exogenous (genuinely environmental) or from the endogenous products of cell metabolism. Examples of the former include chemicals, radiation or viruses. The damage may also be inherited, i.e. derived directly from one or other parent line. In addition, there are some spontaneous mutations that occur randomly without apparent cause. Additionally and importantly, cancer cells have an inherent genetic instability that increases the likelihood of genetic mutations or epigenetic changes in response to micro-environmental conditions surrounding the cell itself.

Single and/or sequential mutation of the relatively few important genes involved in cancer development lead to uncontrolled cell replication and proliferation with additional mutations that arise as a result of additional mutagenic occurrences acting on inherently unstable genetic components of the mutated cells, leading first to an ability to invade basement membranes and then to an ability to spread/metastasize. Although in many species the genes involved in major cancer processes have been identified, there is little specific information on the horse. However, the mutations can result in cancer through excessive function, malfunction or non-function and each of these properties will clearly influence the nature of the tumour itself.

There are four principal recognized genes that are the targets for the genetic damage that leads to the development of cancer. These are:

1. Growth promoting proto-oncogenes
2. Growth inhibiting tumour suppressor genes
3. Genes that regulate natural/organized cell death (apoptosis)
4. DNA repair genes.

Classically, the first two of these are discussed together.

Oncogenes: Oncogenes are hyperfunctional forms of normal genes that promote autonomous cell growth and replication. They have been identified as integral to the development of cancer and exert their influences on crucial stages of the cell-cycle involved in regulating the crucial stages of the cell-cycle and may involve growth factors, growth factor receptors, intracellular cell signalling proteins and nuclear transcription proteins. Their normal counterparts are called 'proto-oncogenes'; these are the physiological regulators for cell proliferation and differentiation and are effectively the drivers of normal cell replication and their expression is under the influence of normal mitogenic signals. In contrast, oncogenes are capable of promoting cell replication and growth independent of the normal mitogenic signals. Effectively, they 'don't listen' to the commands for their behaviour and produce oncoproteins, which resemble the normal products, but they are devoid of regulatory elements. Once this is triggered, the process becomes autonomous and remains independent of regulatory signals. Cancer cells have strategies to acquire self-sufficiency.

The mechanism for conversion from proto-oncogene to oncogene can arise from:

- Point mutations in which a single DNA base pair mutation may result in the production of a hyperfunctioning protein
- Chromosomal rearrangement in which a chromosomal translocation result in a proto-oncogene being relocated at another site where it may be able to overexpress itself. The translocated gene/genes may also fuse genes together and so express hyperfunctional proteins
- Gene amplifications may occur: multiple copies of a proto-oncogene may lead to excessive production of proteins and, although the protein may be normal, it is in excess and so will have profound effects on cell functionality
- Viral insertion mutagenesis occurs when a viral gene causes activation of proto-oncogenes.

The significant effects of the gene transformation from proto-oncogene to oncogene include overproduction, which in turn leads to dysregulation of normal cell growth, increased proliferation, loss of apoptosis and potential transformation to malignancy. Since the 1970s, dozens of oncogenes have been identified in human cancer but relatively few have been identified categorically in the horse. Many cancer drugs target these DNA sequences and their products.[1]

Tumour suppressor genes: These are genes that naturally prevent abnormal cellular proliferation and genetic instability: they can be viewed as the cellular policemen/repairmen! The protein products of these genes are intimately involved in a variety of cell functions including the cell-cycle control/checkpoints, cell signalling, triggering of apoptosis and repair of faulty DNA. Inactivation or loss of tumour suppressor genes by mutation or deletion will necessarily lead to loss of function, reducing the effectiveness of restriction at cell control points, thereby promoting growth and replication. Simplistically, these genes serve to recognize the potential of mutations and act to protect the cell or the body by repairing damage, triggering cell death to ensure that the changes are not taken a step further, and stabilizing the genetic structure so that cells are 'shunted into a siding' where they remain harmless.

Tumour suppressor genes can be inactivated by:

- Point mutations or small deletions/insertions of genetic material
- Chromosomal abnormalities, such as translocations and insertions
- Epigenetic changes, such as DNA methylation.

Inactivation or loss of these genes by mutation or deletion leads to loss of function and a reduction in the restrictions of cell division or growth. This results in an inherent and inevitable genetic instability and loss of apoptosis, which shifts the balance of the cells towards malignant behaviour (Table 4.1).

Table 4.1 The role of tumour-suppressor genes and their protein products in human examples

Tumour suppressor gene	Function of TSG expressed proteins	Clinical examples (in human medicine)
p53	Arrest of cell-cycle Triggering of apoptosis Repair of damaged DNA	The most commonly mutated gene in most cancers
Retinoblastoma	Cell-cycle regulator at restriction checkpoints	Retinoblastoma Lung cancer
BRCA1 & 2	Transcription factors DNA repair	Familial breast and ovarian cancers
APC	Inhibitors of cell signal transduction	Colon, stomach and pancreatic cancer
WT-1	Nuclear transcription	
P161NK 4	Inhibitors of cyclin-dependent kinase (CDK)	Pancreatic/ oesophageal carcinoma and melanoma

After Peedell C, Stark DPH, eds. Basic cancer biology. In: Concise clinical oncology. Oxford: Butterworth–Heinemann; 2005: 13; copyright 2005, with permission from Elsevier. A similar table for equine neoplastic disease would be very difficult to prepare, since so little is known about equine neoplastic disease.

Under normal circumstances, these genes act recessively; both copies of the gene (alleles) must be mutated to produce the loss of function – mutation of a single gene acquired from parent is not enough in itself to cause a problem, but where there is a single mutated gene the patient may have a predisposition to cancer development. A good example of such a state occurs when there is an inherited single mutation in the *p53* gene (p. 40), which greatly enhances the lifetime risks of cancer. Such a patient might easily develop multiple cancers rather than just a single type.

The guardian of the genome: (T)*p53* gene

The *p53* gene is the single most common gene to be mutated in human cancers and there is no reason to doubt that it is also an important aspect of equine/veterinary tumours. The *p53* gene is a transcription factor that can be activated by checkpoint kinases to cause cell-cycle arrest.[2] The major functional activities of the p53 protein are cell-cycle arrest and the initiation of apoptosis in response to DNA damage. The *p53* gene is actually a member of a multigene family with similar functions. *p73* is usually regarded as the 'big brother' of *p53* and is located on another locus; it encodes similar proteins that can also cause cell-cycle arrest, as well as apoptosis in appropriate conditions. *p63* is the newest member of the family of protective genes to be identified.

p53 is regarded as the 'molecular policeman' or the 'guardian of the genome'; it plays a major role in cell-cycle arrest and control of some DNA repair genes and is also intimately involved in apoptosis that follows the detection of significant DNA damage (Fig. 4.3); it is called in to apply the emergency brakes when DNA is damaged in particular

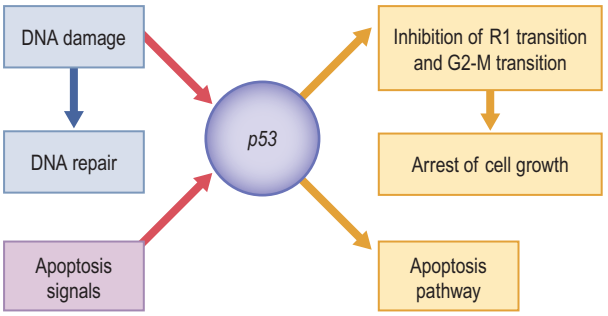


Figure 4.3 ***p53* and DNA repair genes.** The role of *p53* and DNA repair genes in cell-cycle arrest and apoptosis.

by gamma, X-ray and ultraviolet irradiation or mutagenic chemicals. It is also called in, in the absence of DNA damage, when oxidative injury, senescence and other cell stressors are present.[3] Cells may undergo *p53*-mediated apoptosis following chemotherapy or radiotherapy but loss of the *p53* gene function (loss of the gene or its critical damage) alone results in survival of cells that have been damaged by the treatment or by any other insult. The *p53*-deficient cells are therefore more resistant to chemotherapy and to radiotherapy as well as being much more sensitive to the adverse effects of other genetic mutations, however those are induced. In fact, *p53* is a pivotal player in the prevention of cancer development and its progression through the early stages of cancer transition to the most severe forms of tumour development! In normal circumstances, some cells have no ability to repair or replicate, e.g. nerve cells, and these cells usually lack the mechanisms that trigger cell-cycle progression.

The natural checkpoints in the cell-cycle function to limit inappropriate cell proliferation and cell survival and collectively block cell transformation. The *p53* tumour suppressor pathway that induces cell-cycle arrest or apoptosis has an important effect on the fate of a cell. This gene, which in the horse is located on the short arm of chromosome 17, is therefore a fundamental and vital one for the normal cell function and protection. (More exactly the *TP53* gene is located from base pair 7 571 719 to base pair 7 590 867 on chromosome 17 at position 13.1.) Remarkably, it is by far the most common single gene that is mutated in human cancer conditions and we have no reason to doubt that a similar situation occurs in veterinary species including the horse.[3]

The multiple families of protective genes (*p53*, *p63* and *p73*) can probably compensate for each other to some extent and that may explain why even when one of the genes is damaged, loss of cell-cycle control is not as severe as it otherwise might be.[4]

When normal cells are subjected to molecular stress (such as might occur with exposure to radiation), DNA damage or oxygen depletion, they are usually either efficiently arrested at one or more of the cell-cycle barriers or are directed towards apoptosis, or both. Checkpoint reparative mechanisms may be triggered to allow a cell to re-enter the cell-cycle and these processes are largely under the control of the *p53* gene family (*p53*, *p63* and *p73*). The gene plays a pivotal role in maintaining genomic stability and undertakes the genetic surveillance following exposure to damaging

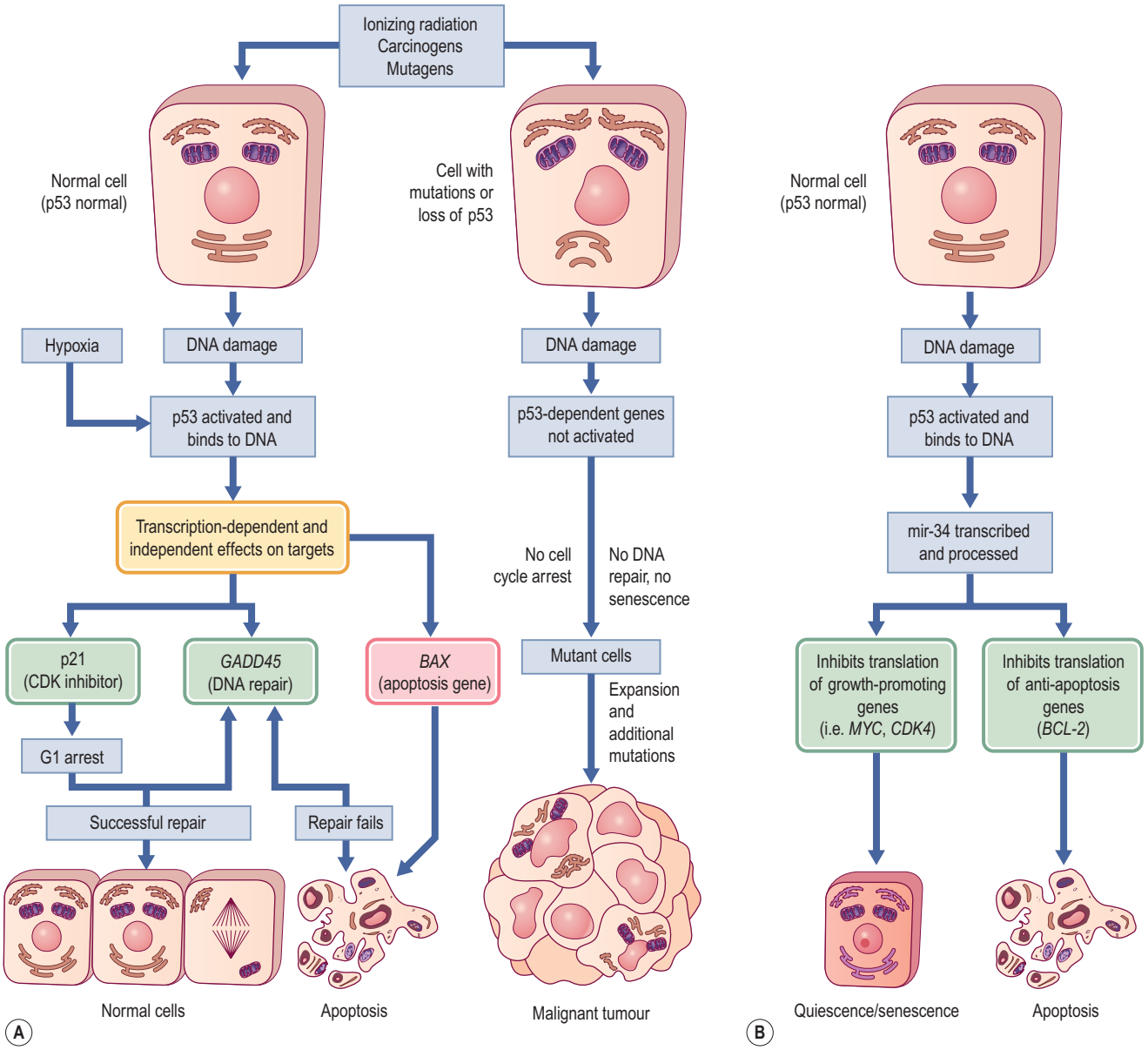


Figure 4.4 Repair of DNA. (A) The role of p53 in maintaining the integrity of the genome. Activation of normal p53 by DNA-damaging agents or by hypoxia leads to cell-cycle arrest in G1 and induction of DNA repair, by transcriptional upregulation of the cyclin-dependent kinase inhibitor *CDKN1A* (p21) and the *GADD45* genes. Successful repair of DNA allows cells to proceed with the cell-cycle; if DNA repair fails, p53 triggers either apoptosis or senescence. In cells with loss or mutations of p53, DNA damage does not induce cell-cycle arrest or DNA repair, and genetically damaged cells proliferate, giving rise eventually to malignant neoplasms. **(B)** p53 mediates gene repression by activating transcription of miRNAs. p53 activates transcription of the mir-34 family of miRNAs. Mir-34s repress translation of both proliferative genes, such as cyclins, and anti-apoptotic genes, such as *BCL-2*. Repression of these genes can promote either quiescence or senescence as well as apoptosis. *(Figure reprinted from Kumar V, Abbas A, Aster J. Robbins and Cotran pathologic basis of disease, 8th edn. Philadelphia: WB Saunders; 2010: 291 (Fig. 7.32); copyright 2010, with permission from Elsevier.)*

insults, such as gamma and UV radiation, chemicals and oxidative stress (Fig. 4.4).

Homozygous loss of the *p53* gene probably occurs in the large majority of cancers, in all species.[5] In most cases, both alleles are affected equally and there are (in contrast to the *Rb* gene) few occasions when there is a heterozygous inherited state. These changes are therefore most often acquired rather than inherited in the germ line of the cells. Only rarely will an individual inherit a single mutant *p53*, and in this case (as is the case with the *Rb* gene), this will result in an increased tendency to further mutation and so to cancer development.

The role of the *p53* gene is in fact more complex in that it has related genes that also have an influence. The *p63* and *p73* genes are members of the multi-gene family that controls many of the genetic functions that protect against mutation. *p53* deficits can most likely be compensated to some extent in some circumstances by the normal function of the other genes in the group. These genes link cell damage with DNA repair, cell-cycle arrest and apoptosis. *p53* plays a fundamental role in the process of DNA repair by arresting the cell-cycle at the G1-phase and triggering activity of DNA repair genes. The *p53* gene family directs a cell with irreparably damaged DNA to undergo apoptosis, thus

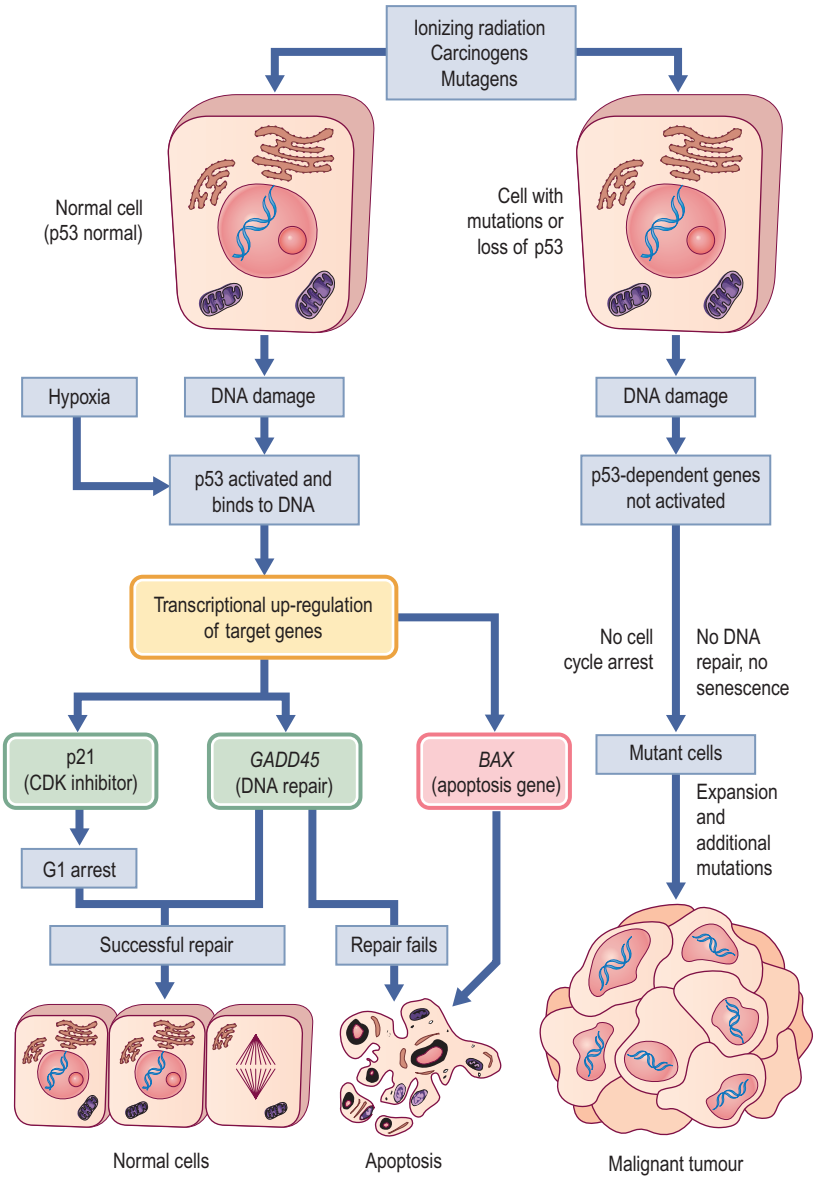


Figure 4.5 The *p53* gene. *p53* plays a pivotal role in maintaining the integrity of the genome in any cell. When the *p53* gene is activated by a cell insult, such as radiation, chemical toxins or hypoxic insult, the cell is arrested in G1-phase. DNA repair is triggered by transcriptional upregulation of the cyclin-dependent kinase inhibitor p21 and the *GADD45* gene complex. If the DNA is successfully repaired, the cell is 'allowed' to proceed through the cell-cycle. If however, DNA repair fails or the damage is too severe to 'contemplate' repair, p53-induced apoptosis occurs – the cell is shunted into the recycling depot. If *p53* itself is damaged or mutated, DNA damage does not induce arrest or repair and then genetically damaged and genomically unstable cells are left to proliferate uncontrollably. Every daughter cell will carry the same basic 'fault' and the lack of repair also means that mutational changes are more likely to occur and pass unrecognized – the cell becomes genomically unstable and can move progressively towards more abnormal cell behaviour, leading eventually to malignancy (see p. 39). *(Figure reprinted from Kumar V, Abbas A, Fausto N. Robbins and Cotran pathologic basis of disease, 7th edn. Philadelphia: WB Saunders; 2004: 303 (Fig. 7.37); copyright 2004, with permission from Elsevier.)*

removing the threat of an uncontrollable cell replication or indeed uncontrollable necrosis of the cell resulting in inflammatory mediator release.

Without the controlling function of the *p53* family of genes, DNA is not repaired and the mutations are fixed within the dividing cell in such a way that the cell develops a single and almost inevitable pathway to malignant transformation (Fig. 4.5).

The role of the *p53* in controlling apoptosis following DNA damage is also involved in some forms of chemotherapy and radiation therapy. Both of these common therapeutic approaches are effective because they induce DNA damage and subsequent apoptosis. Tumours that retain their p53 function are more likely to respond to such treatments than tumours that carry mutant alleles of the *p53* family of genes. In the human field, this is an important aspect of determining the likely effects of both chemotherapy and radiation therapy; some tumours have a fully functional *p53* and respond well, while others, which carry a *p53* mutation, are relatively resistant to chemotherapy or radiation or both. Cancer therapy with the ability to increase normal p53 activity in tumour cells that have retained the p53 activity is one approach that is being explored. A second potential option is to establish ways of killing cells that have a defective p53 function, since then the cell becomes irrelevant. The latter includes strategies that use modified viruses (usually adenovirus) that lyse cells that lack the p53 function.

It is important to realize that there are other genes that function as tumour suppressors but they are largely identified through detection of specific deletions in cancer cases, it is hard to extrapolate in these circumstances to the horse from the human situation. Significantly, the *p53* gene does not appear to be adversely affected in equine sarcoid tumours.

DNA repair genes

DNA repair genes are the intracellular 'repair workers'. They support and maintain genetic stability and are specifically involved in the repair of damaged DNA.

Additionally, they exert an indirect effect on cell proliferation or survival by influencing the ability of the organism to repair any non-lethal damage in other genes, including proto-oncogenes, oncogenes, genes that regulate and control apoptosis and tumour suppressor genes. Additionally and importantly, compromise in the function of the DNA repair genes can predispose to mutations and so predispose the development of multi-mutational damage and the neoplastic transformations that are recognized as cancer.

DNA repair refers to a collection of processes by which a cell identifies and corrects damage to the DNA molecules that encode its genome. In a normal mammalian body, normal metabolic activities and environmental factors, such as UV light and radiation, can cause structural damage to DNA molecules and can therefore adversely affect or even eliminate the cell's ability to transcribe the gene that the affected DNA encodes. Other noxious stimuli induce potentially harmful mutations in the cell's genome, which affect the survival of its daughter cells after it undergoes mitosis. DNA repair processes are therefore critical to cell and organ survival and maintenance. The process of DNA repair has to be extremely sensitive and very accurate in its response to subtle, often minute, changes in the DNA. When cellular apoptosis does not occur, the loss of DNA repair functions causes permanent DNA damage. This leads to irreparable DNA damage, including double-strand breaks and DNA cross-linkages and further genomic instability and mutation. In many circumstances, this also leads to loss of tumour suppressor gene function and conversion of proto-oncogenes to oncogenes, which in turn leads to increased cancer susceptibility.

The rate of DNA repair is dependent on many factors, including the cell type, the age of the cell and the extracellular environment. A cell that has accumulated a large amount of DNA damage, or one that no longer effectively repairs damage incurred to its DNA, can enter one of three possible states within the cell-cycle:

1. *An irreversible state of dormancy* 'senescence' (see also p. 36): When a cell enters this state, the cell simply grows old gracefully without any harmful consequences and waits for eventual death – similar to a state of 'retirement'!
2. *Cell suicide* 'apoptosis' or 'programmed cell death' (see p. 29): Here the cell is actively and carefully 'decommissioned' or dismantled in such a way as to prevent any concurrent damage arising as a result of cell death and destruction. This is in contrast to necrosis in which harmful cell enzymes and products are released and which is inevitably associated with inflammation (p. 32).
3. *Unregulated cell division*: This can lead to the formation of a tumour that may become cancerous.

The DNA repair ability of a cell is therefore essential to the integrity of its genome and thus to its normal functioning and that of the organ. Many genes that were initially shown to influence lifespan have been found to play a role in DNA damage repair and protection.[6] Failure to correct molecular lesions in cells that form gametes can introduce mutations into the genomes of the offspring and thus can even influence the rate and process of evolution; not all mutations are harmful – some can imbue 'advantage' without significant harm.

Cell destruction

The other side of the life-cycle of a normal cell is cell death. In the same way that cell growth is regulated by growth-promoting and growth-inhibiting genes, cell survival is dependent on genes that control the life-cycle, lifespan and prevent uncontrolled or unnecessary death of the cell.

Cell death occurs by two main mechanisms and one lesser mechanism: the former are necrosis and apoptosis (p. 29) (Figs 4.6, 4.7). The process of autophagy is the third less-understood mechanism for cell destruction (p. 32). Necrosis is a 'passive' response to cellular injury in which the cells swell and lyse. This releases their contents into the interstitial space. This triggers local inflammatory pathways resulting in detectable cellular and often systemic effects, inflammatory responses and, potentially, to serious health problems. In contrast, apoptosis is an 'active' process of planned, coordinated and regulated 'decommissioning' of the various cell components in such a way that there is no local or systemic consequence. This is clearly an advantage to the organism, given the number of cells that are being destroyed in any body system every day. The three mechanisms can be recognized within tumours by their distinctive changes through standard histology (Fig. 4.8) and by ultramicroscopy (see Fig. 4.7).

Apoptosis

Cells undergo apoptosis (natural planned cell death) in response to a variety of cellular 'insults', such as UV light, chemical or physical damage, viral infection and as a result of ageing. Apoptosis is a cellular response process in which cells play an active role in their own death (which is why apoptosis is often referred to as 'cell suicide' or 'programmed cell death'). In this process, the cells are 'dismantled' in a controlled, regulated fashion without release of any significant mediators that might induce an inflammatory response or cause local or remote cell or organ injury. Apoptosis is therefore physiologically and pathologically distinct from necrosis, in which uncontrolled cell death leads to lysis of cells; this 'chaotic' destruction releases chemicals and mediators that induce inflammatory responses, which in turn invariably has remote effects on other organs and tissues. Apoptosis, or programmed cell death, is a normal component of the development and health of multicellular organisms. There is clear advantage in activation of the apoptotic pathway in cells that have sustained significant, irreparable DNA damage in both preventing and minimizing the risks of uncontrolled expansion of mutated/transformed cells and in preventing necrosis.

The triggering of apoptosis in a cell is a complex process that can involve different stimuli; unbalanced growth signals and damage to the cell membrane or the DNA are just some of the possibilities. Although the exact pathways are yet to be determined fully, almost inevitably, the *p53* gene family is fundamentally involved in the process. *p53* is an in important pro-apoptotic gene that induces apoptosis in cells that are unable to repair their genetic damage. When a cell is damaged as a result of oxidative stress or treatment with certain drugs or by exposure to radiation, apoptosis is triggered through expression of the p53 protein. Cells that have a *p53* mutation may not be triggered to undergo apoptosis by such damage. Many cancer cells have a

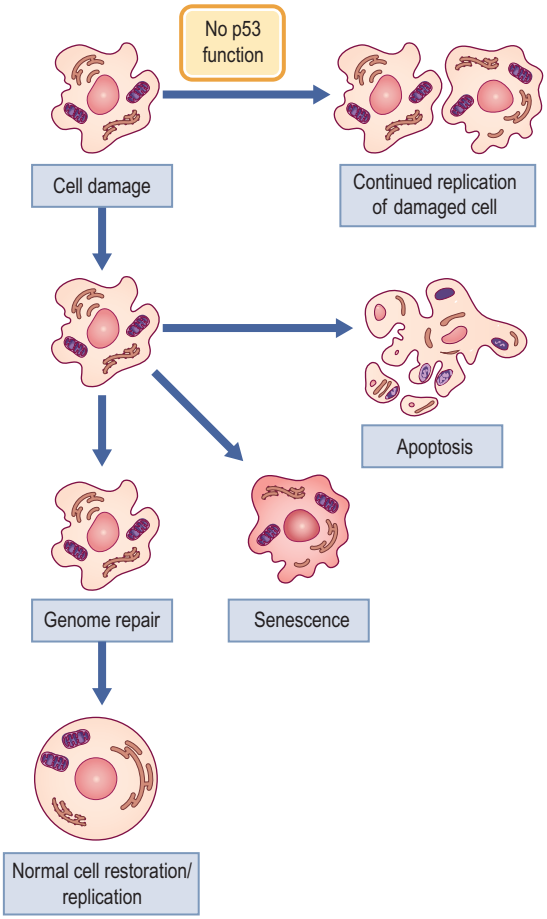


Figure 4.6 Mutation of the p53 gene complex. The *p53* family of genes are recognized as the 'molecular policeman or the guardian of the genome' because of the fundamental role they play in the prevention of progression of abnormal cells through the cell-cycle. Mutation of the *p53* gene complex and other chromosomal losses/ alterations are involved in the progression to malignancy and metastasis. Since the genomic surveillance of cells as they proceed through the cell-cycle is governed by *p53* and other like genes, damage to these will inevitably lead to sustained and replicated abnormality. The genomic instability that follows damage to the controlling genes makes subsequent mutation more likely and more able to bypass the surveillance systems. Simplistically, the role of the surveillance genes can be likened to a motor repair technician who 'triages' the cars to detect faults and passes them to the right kind of repair technicians. The options available to the master technician are: (a) The repair can be completed and the car can be rendered safe and reliable – the cell is 'fixed' and returns to the cell-cycle with no adverse consequence. (b) The technician can recognize that the car is beyond repair and divert the process to the breakers who can dismantle the car with no deleterious effects – the cell is subjected to apoptosis. (c) The damage to the car might be sufficient to warrant simply storage of the car in a state that neither allows use or destruction – the cell is held in a static state without the ability to replicate but is not subject to apoptosis. (d) The car may have 'undetectable damage' that the technician cannot identify and so the cell passes into the cell-cycle to replicate identically damaged cells but future mutations may be more evident. The car can be re-checked at a future date and the damage can be dealt with. (e) Where the technician is damaged, sleeping, drunk or dead – the triage is not carried out and the repair technicians are not called into action and so the cell can pass naturally and persistently through the cell-cycle in a progressively unstable state! Damage to *p53* genes and its 'peers' is an extremely dangerous state.

significantly decreased ability to undergo apoptosis in response to various stimuli including DNA damage because these cells are able to tolerate increased levels of genetic instability. Since some chemotherapeutic agents used in cancer medicine act by inducing apoptosis, the loss of

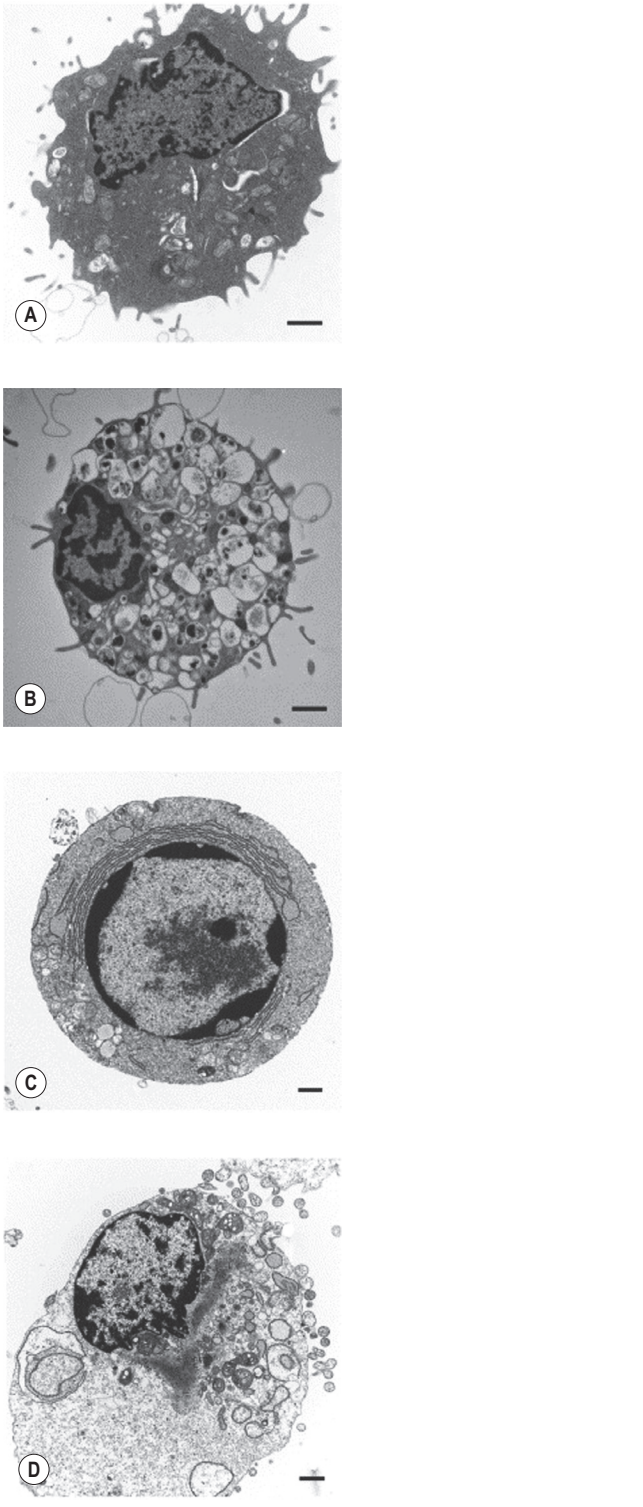


Figure 4.7 Morphological characteristics of cells. Morphological characteristics of a normal cell **(A)**, compared with cells undergoing **(B)** autophagic; **(C)** apoptotic; and **(D)** necrotic cell death. Although the morphological characteristics of an apoptotic cell are well-defined, autophagic vesiculation can be seen in all three forms of cell death. In the context of apoptosis or necrosis, autophagy could be additive or may serve to protect cells from death. Indeed, bioenergetic failure, which will lead to necrosis, can be thwarted by the upregulation of autophagic degradation to maintain proper adenosine triphosphate levels. *(Figure reprinted from Dorsey FC, Steeves MA, Cleveland JL. Apoptosis, autophagy, and necrosis. In: Mendelsohn J, Howley PM, Israel MA, et al., eds. The molecular basis of cancer, 3rd edn. Philadelphia: WB Saunders; 2008 (Fig. 15.1); copyright 2008, with permission from Elsevier.)*

apoptotic genes can have a profound detrimental effect on drug efficacy – some tumours therefore simply do not respond to drugs that act in this way.

Upon receiving specific signals instructing the cells to undergo apoptosis, a number of characteristic changes occur in the cell. A family of proteins known as 'caspases' are typically activated in the early stages of apoptosis. These proteins break down or cleave key cellular components that are required for normal cellular function, including structural proteins in the cytoskeleton and nuclear proteins, such as DNA repair enzymes. The caspases can also activate other degradative enzymes, such as DNAses, which begin to cleave the DNA in the nucleus.

Apoptotic cells have a characteristic histological appearance – in contrast to necrosis, the apoptotic cell has chromosomal condensation and nuclear fragmentation with shrinkage of the cell size and loss of cell contact with its neighbours (see Fig. 4.8). The next stage of the process involves 'blebbing' of the cell membrane and formation of apoptotic bodies that contain small, isolated bits of the cell content (chromatin) surrounded by cellular membranes (Fig. 4.9). These bodies are attractive to local phagocytic cells and are digested without any significant secondary consequence or inflammation. The processes that take place inside the cell to render the blocks of nuclear and other proteins harmless are complex. The process is careful, regulated and self-protective.

Cellular evasion of apoptosis

Normal cell growth is regulated by the opposing growth-supporting and growth-inhibiting genes. Cell survival is also controlled by natural genes that support and inhibit apoptosis. One of the most striking features of cancer cells is their ability to evade the process of apoptosis – this leads to the concept of 'immortality' of cancer cells. The accumulation of cancer cells may arise either as a result of activation of oncogenes or inactivation of tumour suppressor genes. Additionally the cells can accumulate because of the detrimental mutation of genes that control and trigger apoptosis. An extensive list of related genes that regulate apoptosis has been identified in both normal and cancer cells; the first and possibly most significant is the *BCL-2* gene.[7]

The natural lifespan of a cell, organ or body is controlled by the finite number of divisions that cells can undergo (Fig. 4.10). Normal cells eventually become arrested in a

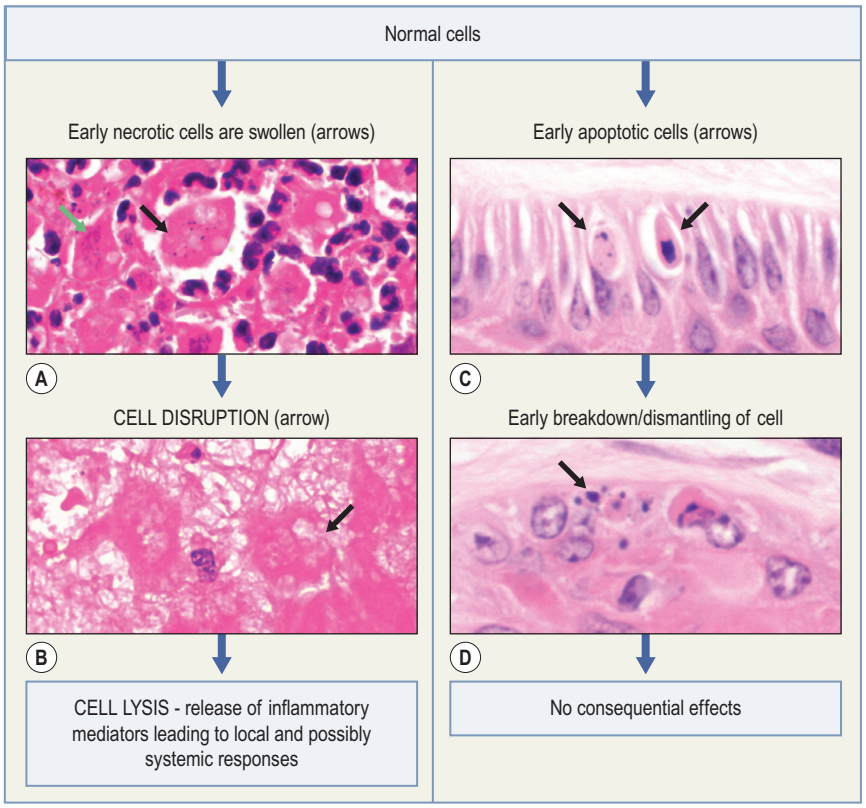


Figure 4.8 Cell death. The manner of a cell's death is a critical feature in respect of the extent of local and possibly remote damage that is caused by the process. Where a cell dies through the process of cell swelling **(A)**, and subsequent disruption (necrosis) **(B)**, there are local consequences that arise as a result of the release of a wide range of intracellular cytokines and mediators. This results in a local inflammatory response and possibly even remote physiological effects. In contrast, apoptosis is a more orderly dismantling of the cell, so that through a process of planned destruction **(C)**, the release of intracellular components **(D)**, is without either local or remote destructive effects and no inflammation follows. **(A)** The necrotic cells are swollen and their hypereosinophilic cytoplasm contains vacuoles, and they are surrounded by neutrophils (with dark-staining, segmented nuclei). The cell indicated by the green arrow shows fading of the nuclear chromatin, defined as karyolysis. The cell indicated by the black arrow shows nuclear karyorrhexis, i.e. a pyknotic nucleus that has undergone fragmentation. **(B)** More advanced necrosis with complete loss of cellular detail and evidence of lysis (arrows). **(C)** Apoptotic epithelial cells (arrowed) with shrunken, hypereosinophilic, rounded cytoplasm. The nucleus of the cell on the left is fragmented, and that of the cell on the right is pyknotic (shrunken and dark-staining). **(D)** These apoptotic cells are fragmenting into small 'apoptotic bodies' that contain dark-staining nuclear fragments (arrow). There are no inflammatory cells.

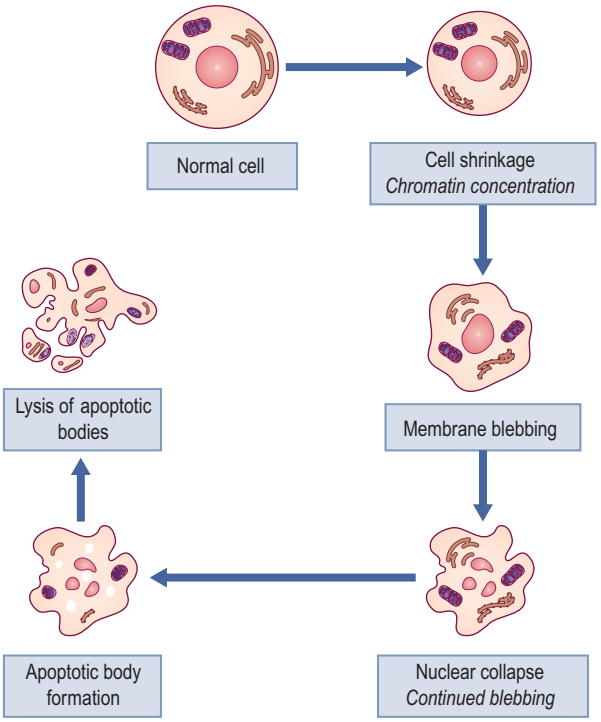


Figure 4.9 Apoptosis. The progress of a cell through apoptosis (K22).

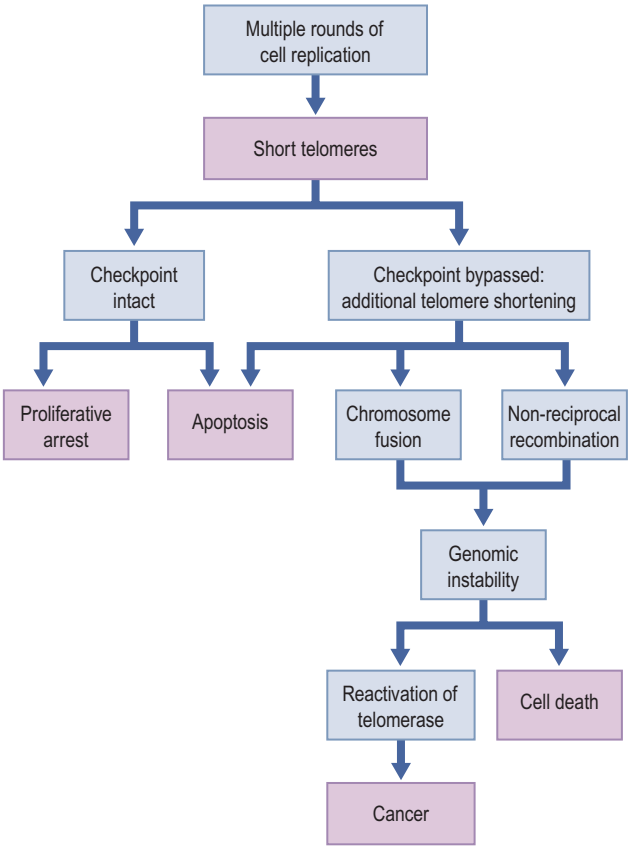


Figure 4.10 Cellular responses to telomere shortening. The responses of normal cells, which have intact cell-cycle checkpoints, and of cells with checkpoint defects. *(Figure adapted from Wong JMY, Collins K. Telomere maintenance and disease. Lancet 362:983; copyright 2003, with permission from Elsevier.)*

terminally, non-dividing state – 'replicative senescence'. It is likely that the finite number of potential divisions for any cell is governed by the shortening of the specialized structures at the end of chromosomes, known as telomeres. When these are shortened to a defined extent, the loss of telomere function triggers activation of p53-dependent cell-cycle arrest.

Telomeres are an essential and universal cellular substructure that is essential for chromosomal stability. They prevent chromosomes from fusing with each other and are involved intimately in nuclear architecture, in chromosomal localization and in the repression of expression of adjacent genes.

The telomere length, in effect, becomes a clock that counts down the available number of cell divisions that the cell type can undergo (Fig. 4.11). The pathways the cell can take from that point include a static 'senescent' state with no further replicative activity (with origin hypoplasia as cells shrink) or entry into an apoptotic state resulting in the reduction in the number of cells present and organ atrophy. Telomerase is the cell enzyme that prevents shortening of the telomeres in germ cells; normal somatic cells do not possess this. If loss of the telomerase is the reason why cells finally die, cancer cells clearly have developed a strategy to overcome this natural process; telomere maintenance is a feature of almost all cancers and, in over 90% of cancers, this is due to the upregulation of telomerase. Cancer cells have therefore found a means whereby the shortening of the telomeres does not occur and this is because telomerase is present in many cancer cell types.[8] This may account for the limitless replicative potential (immortality) of cancer cells.

Autophagy

The term 'autophagy' literally means 'self-eating' and is used to describe the degradation of cellular components by the lysozyme, a cell membrane-bound compartment containing degradative enzymes capable of dismantling macromolecules within the cell. It is a tightly regulated collection of processes that plays a normal part in cell growth, development and homeostasis, helping to maintain a balance between the synthesis, degradation and subsequent recycling of cellular products (Fig. 4.12). Autophagy is a major natural protective mechanism by which a 'starving' cell diverts nutrient resources from unnecessary processes to the more essential, life-preserving processes and this is assumed to be a significant supportive process for rapidly dividing cancer cells that become hypoxic or deprived of the nutritional requirements for rapid growth/replication.

There are inevitable intimate links between autophagy, which is a preservative approach, and apoptosis. It is likely that many of the synthetic processes involved in apoptosis involve the recycling of proteins derived from autophagy. Autophagy is a required pathway for tumour development and/or maintenance. It is a mechanism that can be triggered rapidly and might provide essential nutrients during periods of hypoxia and starvation. Autophagy will of course not always be supportive of tumour survival but rather when the capacity for autophagy is exceeded normal cells will undergo apoptosis whilst cancer cells will undergo necrosis; the latter is clearly a destructive and dangerous state compared to the former.

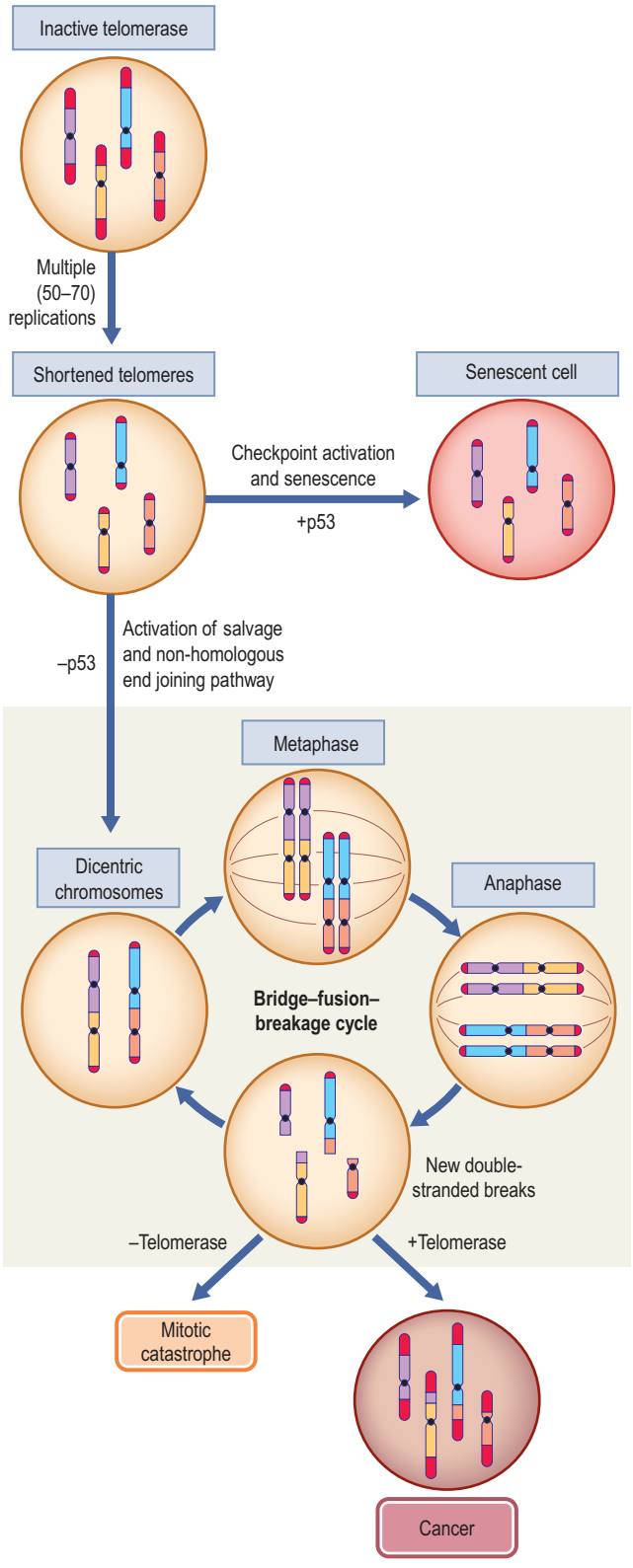


Figure 4.11 Sequence of events in the development of limitless replicative potential. Replication of somatic cells, which do not express telomerase, leads to shortened telomeres. In the presence of competent checkpoints, cells undergo arrest and enter non-replicative senescence. In the absence of checkpoints, DNA-repair pathways are inappropriately activated, leading to the formation of dicentric chromosomes. At mitosis, the dicentric chromosomes are pulled apart, generating random double-stranded breaks, which then activate DNA-repair pathways, leading to the random association of double-stranded ends and the formation, again, of dicentric chromosomes. Cells undergo numerous rounds of this bridge–fusion–breakage cycle, which generates massive chromosomal instability and numerous mutations. If cells fail to re-express telomerase, they eventually undergo mitotic catastrophe and death. Re-expression of telomerase allows the cells to escape the bridge–fusion–breakage cycle, thus promoting their survival and tumorigenesis. *(Figure reprinted from Kumar V, Abbas A, Aster J. Robbins and Cotran pathologic basis of disease, 8th edn. Philadelphia: WB Saunders; 2010: 297 (Fig. 7.35); copyright 2010, with permission from Elsevier.)*

In cancer cell biology, autophagy does appear to be inhibited or altered through genetic mutation, so that cells that would normally be broken down remain, and in that way it has common features with apoptosis. It is important however to recognize that the two processes are not one and the same.

Necrosis

Necrosis can be defined as an uncontrolled, chaotic and disordered process of cell destruction. It is a passive response that carries with it significant local and possibly systemic effects that are recognized as inflammatory responses. There are of course many circumstances when cells might become necrotic: toxins, bacterial and viral damage, hyper- and hypothermia, mechanical damage and, importantly, ischaemia and hypoxia are some of the possibilities. Necrosis ensues when there is overwhelming failure at the cell level. Morphologically, necrosis involves plasma membrane disruption with marked swelling of the cellular organelles and, in particular, the mitochondria. Leakage of intracellular contents into the interstitial space occurs and they induce an inflammatory response. Immediately prior to the complete disruption of the cell there may be extensive leakage of cellular degradation products but ultimately the cell is simply destroyed and all the contents are released into the surrounding sites – some may appear in the bloodstream and cause remote effects on other organs and tissues (Fig. 4.13).

Pathways leading to necrosis involve complex cellular and enzymatic processes that in fact may have an ordered series of events that are preceded by disruption of the plasma membranes, mitochondrial dysregulation of calcium concentrations, excessive accumulation of reactive oxygen species (ROS) and disruption of intracellular proteases. The whole process of cell necrosis can be influenced by genetic factors and can also be influenced epigenetically.

Where apoptosis and autophagy are inhibited, necrosis can 'take over' the process of cell destruction and therefore it can be viewed as the last resort for an organism to protect itself from serious cellular damage. This does not always have to be the obvious physical or chemical damage that necrosis is usually attributed to. Rather, it can be the result of any serious cell disruption that cannot be dealt with by one or other (or both) of the other two major mechanisms.

Necrosis is an important part of tumour biology, since many cells will undergo necrosis even when they have begun the process of the more controlled apoptosis or autophagy. If apoptotic cells are not cleared in a timely

fashion or if the cell is sufficiently disrupted to cause more rapid and profound intracellular damage than can be handled by the proper orderly processes, necrosis will ensue – this is commonly termed 'secondary necrosis', but it is important to recognize that it might simply be a fail-safe mechanism for the removal of potentially harmful cells. Tumour necrosis is a common feature of rapidly expanding cancers that can arise through bio-energetic failure or hypoxia (possibly through avascular necrosis); put simplistically, the tumour outgrows its own blood supply and its energy demands exceed supply.

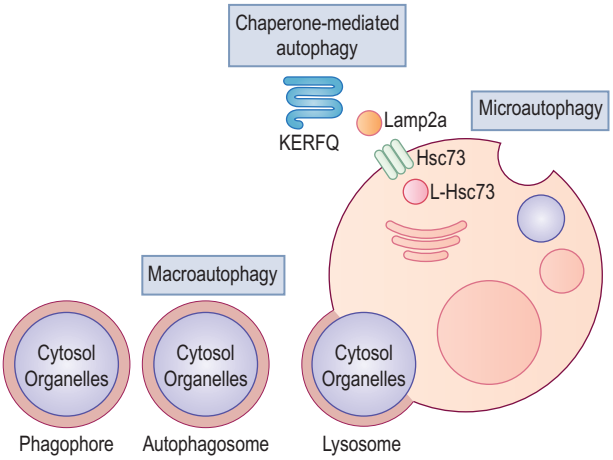


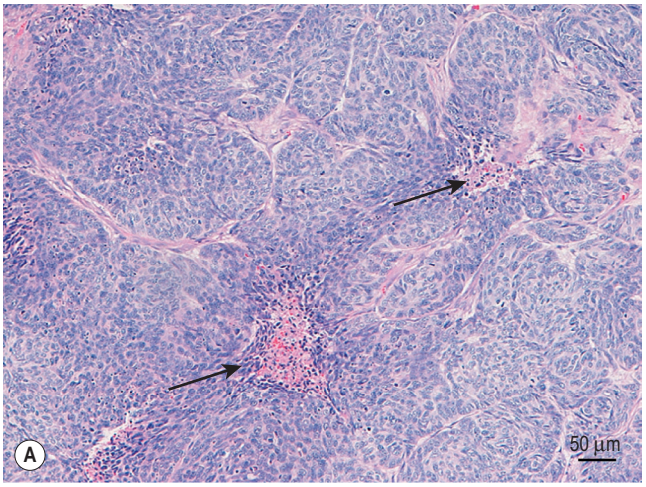
Figure 4.12 Autophagy is lysosome-mediated destruction. Autophagy is the delivery of cytosolic material to the lysosome for degradation/recycling. Three major pathways for lysosomal delivery are known and, as a result, are separated into three classes of autophagy. Microautophagy is the direct invagination of the lysosomal membrane, which engulfs cytosolic material resulting in a vesicle that pinches into the lumen of the lysosome and is subsequently degraded. Chaperone-mediated autophagy is the direct targeting of proteins via a *cis*-peptide sequence (KERFQ) by the chaperone Hsc73, which then unfolds and translocates the protein into the lumen of the lysosome for degradation by Lamp2a and Hsc73. Macroautophagy results from the formation of a double-membrane vesicle (autophagosome) that can engulf both bulk cytoplasm and organelles, such as mitochondria. Once formed, the outer membrane of the autophagosome then fuses with the lysosome delivering the inner vesicle and its contents for degradation. *(Figure reprinted from Dorsey FC, Steeves MA, Cleveland JL. Apoptosis, autophagy, and necrosis. In: Mendelsohn J, Howley PM, Israel MA, et al., eds. The molecular basis of cancer, 3rd edn. Philadelphia: WB Saunders; 2008: 213 (Fig. 15.6); copyright 2008, with permission from Elsevier.)*

Immune evasion

In normal circumstances, immune surveillance is responsible for surveying the body for malignant cells and then, having identified them, destroying them. The evidence that immune surveillance is an important aspect of the defence against tumours is supported by the fact that tumours, in humans at least, are far more common in individuals that have degrees of immunocompromise. However, in spite of this, tumours are still a frequent occurrence in individuals with a normal immune status; this confirms that there must be ways in which the tumour evades the immune system. The reality is that there are no shortages of the mechanisms that tumour cells use to outwit the defences of the host and can find ways of surviving even when there is a fully intact host immune system.

In order to survive either within the tissues or in the lymphovascular channels during metastatic dissemination, tumour cells have to evade the hosts' immune processes. Escape from immune surveillance or immune evasion can be achieved in a number of ways (Fig. 4.14). It is likely that several mechanisms co-exist in any particular tumour type and circumstance and so these defined processes may be up- or downregulated in any particular case:

- *Selective 'evolution' of cloned cells*: Clones that have a strong immunogenicity will naturally be eliminated, whilst those that have a strategy to avoid the immune system will survive. This may simply reflect an evolutionary selection of the cell that has the best survival strategy. Once that clone has been selected, it will continue to develop along the same Darwinistic lines through sequential mutations and generations. Once a tumour starts growing, it seldom stops and the first series of mutations are therefore widely viewed as the critical ones.



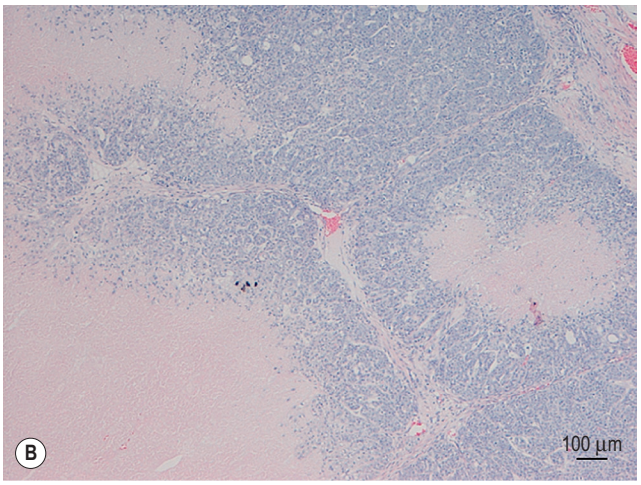


Figure 4.13 Basal cell and mammary carcinoma. (A) Basal cell carcinoma with multifocal small areas of necrosis (arrows) characterized by hyper-eosinophilia and loss of cellular detail, and fragmentation of nuclei (karyorrhexis, karyolysis) (×20). **(B)** Mammary carcinoma with multifocal extensive necrosis, characterized by complete loss of cellular detail and increased eosinophilia (×4).

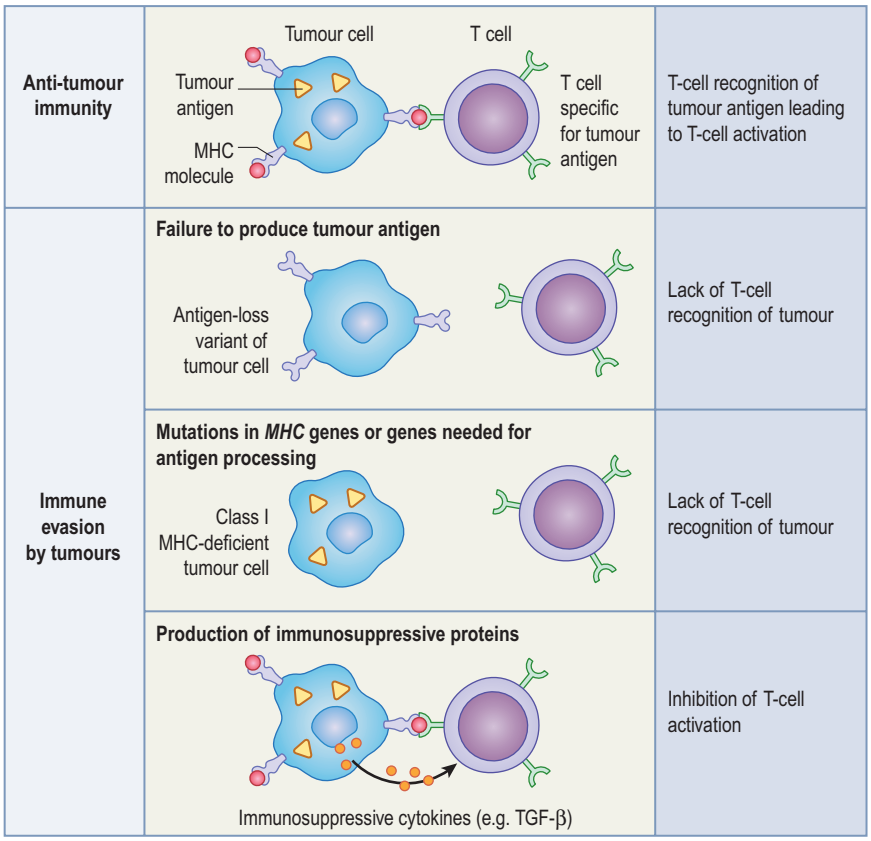


Figure 4.14 Mechanisms by which tumours evade the immune system. Immune evasion is a cornerstone of the pathogenesis of cancer. Loss of recognition of the cancer cells is largely but not exclusively an immunological phenomenon. Normally, tumour cells would be recognizable by T cells and the cell would be destroyed by one means or another. However, where the T cells fail to recognize the antigenic variant of the tumour cell, or where mutations in *MHC* result in an MHC class I deficient tumour cell or where tumours produce immunosuppressive proteins (so-called shield proteins), the immune system will not recognize the cells and so they will be left to replicate unmolested. *(Figure reprinted from Abbas AK, Lichtman AH. Cellular and molecular immunology, 5th edn. Philadelphia: WB Saunders; 2003: 319; copyright 2003, with permission from Elsevier.)*

- *Reduced expression of tumour antigens*: The tumour cells may have masked or suppressed cell surface antigens and this may be a result of excessive production of (normal) autogenous glycocalyx molecules that naturally 'sheath' the tumour cells; they produce a self-masking complex of chemicals that militate against immune recognition and, effectively, this camouflages the cell. Where significant changes occur in the glycocalyx of cancerous cells, the immune system may become capable of recognizing and destroying them, but if the glycocalyx is 'normal', the cells could evade the immune system effectively.
- *Reduction in major histocompatibility complex (MHC) expression*: Tumour cells may fail to express normal levels of MHC class I molecules and so would be capable of evading attack by cytotoxic T cells. Cytotoxic T cells simply overlook the tumour cells because there is no signal to recognize them.
- *Production/overexpression of anti-T-cell factors such as transforming growth factor-beta (TGF-β)*: Some of the natural growth factors produced by the immune system may have a paradoxical effect in supporting tumour growth. Activated lymphocytes and macrophages in particular can produce factors that support tumour cell replication whilst suppressing the immune responses.
- *Direct immunosuppressive effects*: It is accepted that many of the potential oncogenic agents, such as radiation, viruses and chemicals, induce concurrent immunosuppression. Tumours can, however, be directly immunosuppressive themselves. For example, TGF-β is a recognized potent immunosuppressant and so the abnormally high expression of this will inhibit tumour immunity. In effect the tumour is self-preserving.
- *'Hiding' from the immune system in 'safe' sites such as the central nervous system in such a way that the host's immune system has no material access*: This is a mechanism that is dependent on intact 'barriers' such as the blood–brain barrier.

Immune evasion is clearly a target for therapeutic measures. Immunotherapy such as interferon or interleukin-2 may exert their positive effects by modifying tumour antigenicity. Monoclonal antibody systems are similarly used to target specific cancer cells and thereby render them immunologically 'visible'. Harnessing and preferably enhancing the natural protective abilities of the immune system and suppressing the tumour's ability to encourage and support tumour growth are significant targets for the management of some of the most problematic tumours, such as the equine sarcoid and the equine melanoma.

An interesting alternative hypothesis for immune evasion has been proposed by Matzinger.[9] This hypothesis recognizes that even though a newly formed tumour cell produces antigens that are not expressed by its normal tissue fellows, there may still be no immune recognition of the potential harm of that cell. There may in fact be no significant intrinsic differences between the dangerous cell and the normal ones. The cells do not trigger the so-called danger signals in the dendritic cells. Matzinger went further by proposing the concept that antigen-presenting cells respond to 'danger signals' – most notably from cells undergoing injury, or stress or 'bad cell death' (as opposed to apoptosis or

controlled cell death). The alarm signals released by these cells let the immune system know that there is a problem requiring an immune response. The so-called 'Danger Model' covers a broad range of cell interactions including normal physiologic events such as maternal/fetal immunity but also many pathological ones, including autoimmunity, cancer treatments and vaccines and transplantation. Matzinger points out that although it offers an explanation of how an immune response is triggered and how it ends, it does not (yet) offer an explanation of why the immune system responds in different ways to different situations. She hypothesized that tissues send signals to the immune system that determine the immune response appropriate for that tissue. This model has not won universal acceptance. Some immunologists believe that the immune response is mainly fuelled by innate evolutionarily-conserved 'pattern recognition receptors', which recognize patterns expressed by microbes such as bacteria and do not see cell death in the absence of pathogens as a primary driver of immune response. These ideas, however, do not explain how the immune system rejects tumours.

The role of calreticulin in tumour development

Under normal physiological conditions, cellular homeostasis is partly regulated by a balance of pro- and anti-phagocytic signals. Recent research has identified that, subsequent to tumour-transforming mutations, a cell may in some circumstances carry the 'seeds of its own destruction', in that it expresses a surface protein (calreticulin) that signals macrophages around it to engulf and destroy it – a sort of 'EAT ME' signal.[10] Calreticulin is a calcium-binding chaperone that has several functions in the immune response. In the endoplasmic reticulum (ER), calreticulin facilitates the folding of major histocompatibility complex (MHC) class I molecules and their assembly factor tapasin, thereby influencing antigen presentation to cytotoxic T cells.[10]

Although calreticulin is normally ER-resident, it is found at the cell surface of living cancer cells and dying cancer and non-cancer cells; calreticulin promotes phagocytic uptake of the cell expressing the protein on its surface. Calreticulin may also be expressed in normal cells that are damaged in any other non-cancerous manner – a mechanism that would encourage cell destruction and self-preservation. Furthermore, increased calreticulin expression was an adverse prognostic factor in diverse tumours, including neuroblastoma, bladder cancer and non-Hodgkin's lymphoma. These findings identify calreticulin as the dominant pro-phagocytic signal on several human cancers, provide an explanation for the selective targeting of tumour cells by anti-CD47 antibody, and highlight the balance between pro- and anti-phagocytic signals in the immune evasion of cancer. Cancer cells expressing calreticulin may therefore simply be 'behaving' in a normal self-protective manner. In this event one might expect the tumour cells to be engulfed and destroyed as part of the overall natural anticancer/anti-damage strategy. In tumour vaccine models, drugs that induce cell surface calreticulin confer enhanced tumour protection in an extracellular calreticulin-dependent manner.[11] However, it does appear that some of the worst types of cancer are the ones that produce the most calreticulin.

Why then are cancer cells allowed to go beyond this stage and further evade the host's immune system? Most cancer cells are in fact destroyed and this may be one of the significant mechanisms for this self-protective process. If this was a totally efficient system, we would have no cancer! However, it appears that a significant proportion of the cancer cells (but by no means all) also display a 'DON'T EAT ME' signal, called 'CD47'. Where this signal is present it appears to prevent macrophage-mediated cell destruction. CD47, which prevents cancer cell phagocytosis by the innate immune system, is highly expressed on several human cancers including acute myeloid leukaemia, non-Hodgkin's lymphoma and bladder cancer. The cells are now able to efficiently evade the host's immune system and, as further mutations occur, to develop into a more dangerous tumour with the same 'DON'T EAT ME' signal (Fig. 4.15).

The significance of this aspect of tumour cell behaviour lies in the possibility of targeting the 'DON'T EAT ME' signals with directed therapeutic measures, such as an

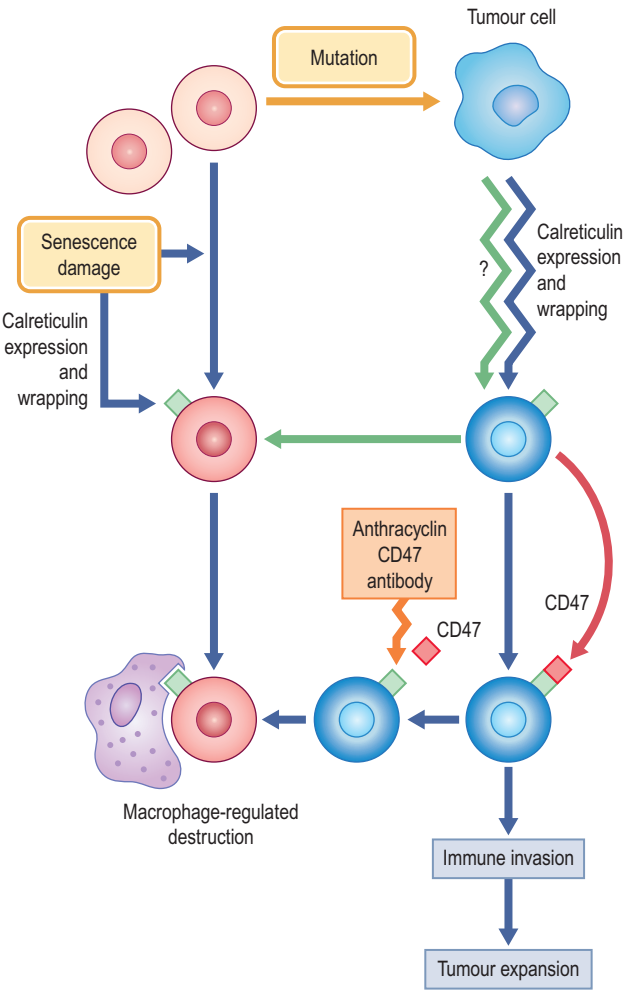


Figure 4.15 Calreticulin. Calreticulin plays an important role in encouraging the macrophage-mediated destruction of senescent and mutated cells. In effect, it imparts an 'EAT ME' signal to the cells destined for destruction. This important protective mechanism is modified by tumour cell expression of CD47. This in effect covers the 'EAT ME' signal with a 'DON'T EAT ME' signal and this, in turn, then prevents macrophage recognition of the mutated cells. The 'DON'T EAT ME' signal can be negated by the use of a specific anti-CD47 antibody or the use of chemicals such as anthracycline. There is little information on this in any equine cancers but it seems plausible that some at least will have this immune-evasion capability, even in the absence of *p53* damage.

anti-CD47 antibody or drugs such as anthracycline; blocking CD47 with a monoclonal antibody results in phagocytosis of cancer cells and leads to in vivo tumour elimination, yet normal cells remain mostly unaffected.[12] This would be a simple way of restoring the immune 'visibility' of the 'EAT ME' signal, so that the tumour cells would be destroyed by macrophages.

Cellular senescence

Cellular senescence stops the growth or replication of cells. Cell senescence is broadly defined as the physiological programme of natural terminal growth arrest, which can be triggered by alterations of telomeres or by different forms of stress. After a fixed number of divisions dictated largely by telomere shortening (p. 30 and Figure 4.11), normal tissue cells become arrested in a terminally non-dividing state called 'replicative senescence'. This process was first recognized in cell culture where cells simply stopped replicating after a defined number of generational divisions (often between 20 and 40 but with a wide variation in the number for different cell types). This phenomenon is also known as 'replicative senescence', the 'Hayflick phenomenon' or the 'Hayflick limit'. The process of natural senescence is largely controlled by the two most potent tumour suppressor genes, *p53* and *pRb*. Mutations in these two genes are by far the commonest changes found, at least in human tumour physiology. It is now confirmed by in vivo evidence that abnormal cell senescence is a pivotal mechanism that constrains the malignant progression of many tumours.

This natural process is assumed to be related to the progressive shortening of the telomeres of the chromosomes (p. 30); this enables the cell to 'count-down' to its state of retirement/senescence. Once the telomeres are shortened to a predefined point, the cell triggers its *p53* gene-related cell-cycle checkpoints. The cell cannot pass into the normal replicative cell-cycle and moves instead to a state of senescence or apoptosis (p. 29). Of course such a defined terminal stage would be counterproductive in germ cell/stem cell lines and so these cells have a sustaining telomerase enzyme. This enzyme is not present in normal somatic cell lines, where replication and repair are not present, e.g. nerve cells.

In most species, cancer incidence increases with age and this is suggested to be fuelled by mutational accumulation and by the age-related changes in tissue integrity that result from the direct functionality of the accumulated senescent cells (Fig. 4.16). This model is well supported in most species, but in the horse it is somewhat less convincing, since the incidence of most of the serious cancer states occur in the middle years of a horse's life. Nevertheless, the contributions that older cells have towards cancer have to be recognized.

Neoplastic transformation involves events that inhibit the programme of senescence and tumour cells were believed, until recently, to have lost the ability to progress though natural senescence – i.e. they were thought to be immortal. However, cancer cells acquire the behaviour of germ cell lines and therefore have limitless replicative potential. It was assumed that cancer cells had to devise a way of preventing telomere shortening and it appears that telomerase enzymes are a critical aspect of cancer cell survival (p. 30). In about 85% of tumours, this evasion of cellular senescence is the result of up-activation of their

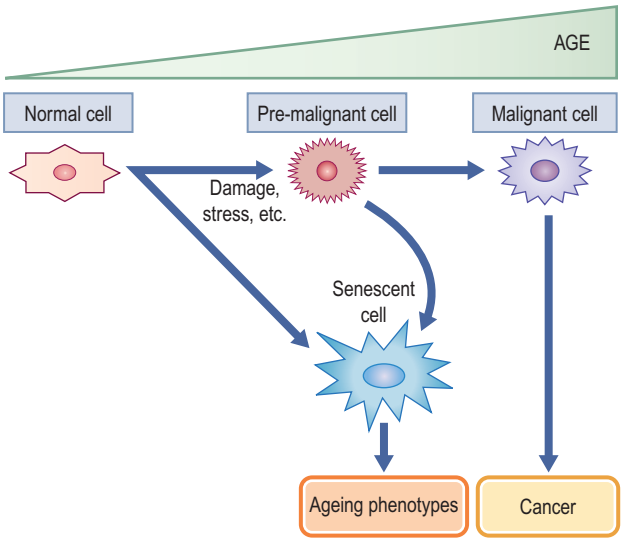


Figure 4.16 Senescence. Both normal ageing cells and tumour cells are influenced by the process of senescence. The natural ageing process induces the process but it can also precipitate mutation into a precancerous state. This possibility is enhanced by the changes within the ageing cell itself and, thus, an ageing phenotypic population of cells develops. Most will be diverted towards apoptosis or autophagy, but a few will mutate and take a path towards cancer development. *(Figure reprinted from Campisi J. Cellular senescence. In: Mendelsohn J, Howley PM, Israel MA, et al., eds. The molecular basis of cancer, 3rd edn. Philadelphia: WB Saunders; 2008: 224 (Fig. 16.3); copyright 2008, with permission from Elsevier.)*

telomerase genes.[8] This simple observation suggests that reactivation of telomerase in healthy individuals could greatly increase their cancer risk.[13]

It has now become apparent that tumour cells can be readily induced to undergo senescence by genetic manipulation or by treatment with chemotherapeutic drugs, radiation or differentiating agents. Treatment-induced senescence, which has both similarities with and differences from, replicative senescence of normal cells, was shown to be one of the key determinants of tumour response to therapy in vitro and in vivo. Although senescent cells do not proliferate, they remain metabolically active and produce secreted proteins with both tumour-suppressing and tumour-promoting activities. Expression of tumour-promoting factors by senescent cells is mediated, at least in part, by a panel of senescence-associated cyclin-dependent kinase inhibitors. Clinical and preclinical studies indicate that expression of different biological classes of senescence-associated growth-regulatory genes in tumour cells has significant prognostic implications. Elucidation of the genes and regulatory mechanisms that determine different aspects of tumour senescence holds out hope for new therapeutic approaches to improving the efficacy and to decreasing the side-effects of cancer therapy.

Cell interactions

Histological examination of tumours will invariably reveal that most masses comprise many different cells – not just the tumour cells and their associated blood vessels. The tumour stromal micro-environment comprises fibroblasts, adipocytes, macrophages, mast cells, vascular elements and a variable mix of inflammatory cells of the innate and

acquired immune system, all embedded and interrelating within the extracellular matrix. It is therefore impossible to ignore the contributions that other cell types and other mediators and bioactive chemicals have on the tumour cells: 'no cancer cell is an island unto itself'. The interaction between the various stromal inhabitants is highly complex and poorly understood at present. Fibroblasts, type-1 collagen, stromal derived growth factor-α, transforming growth factor-β are just some of the possible players in the process. Some will have an enhancing effect on cancer progression in some circumstances but it is also possible that some will have inhibiting or limiting effects also in other circumstances. It is well recognized, for example, that mast cell tumours 'attract eosinophils' into the site and so they may in fact be a predominate cell type; tumour recognition will often require a skilled histopathologist who can recognize the congregations of mast cells with an abnormal morphology and a higher than normal mitotic rate (Fig. 4.17). It is probably safe to say, however, that overall cancer progression is significantly affected by the cells that surround the tumour itself.

Additionally, the altered gene expression of the tumour cells will inevitably influence the behaviour of the surrounding cells. There are the obvious inter-relationships, such as those that control vascularization (p. 48), but all the components in the region will communicate with each other and with the neoplastic cells to contribute to the overall uniquely variable aberrant tumour organ. In many cases, the trigger for neoplastic progression (the critical shifts that move the tumour from a benign to malignant state) may even derive from signals within the stromal micro-environment. Fibroblasts are now recognized as an influence on cancer progression and it has been said that the tumour micro-environment is 'wound healing gone awry'.[14,15] The fibroblasts within the tumour micro-environment are now termed 'carcinoma-associated fibroblasts' and their role in cancer progression may relate to increased expression of proteolytic enzymes and other proteins including S100. This aspect can be used during histological examination to confirm the presence of a cancer state. There are alternative hypotheses for the role of the fibroblast in particular (Fig. 4.18).

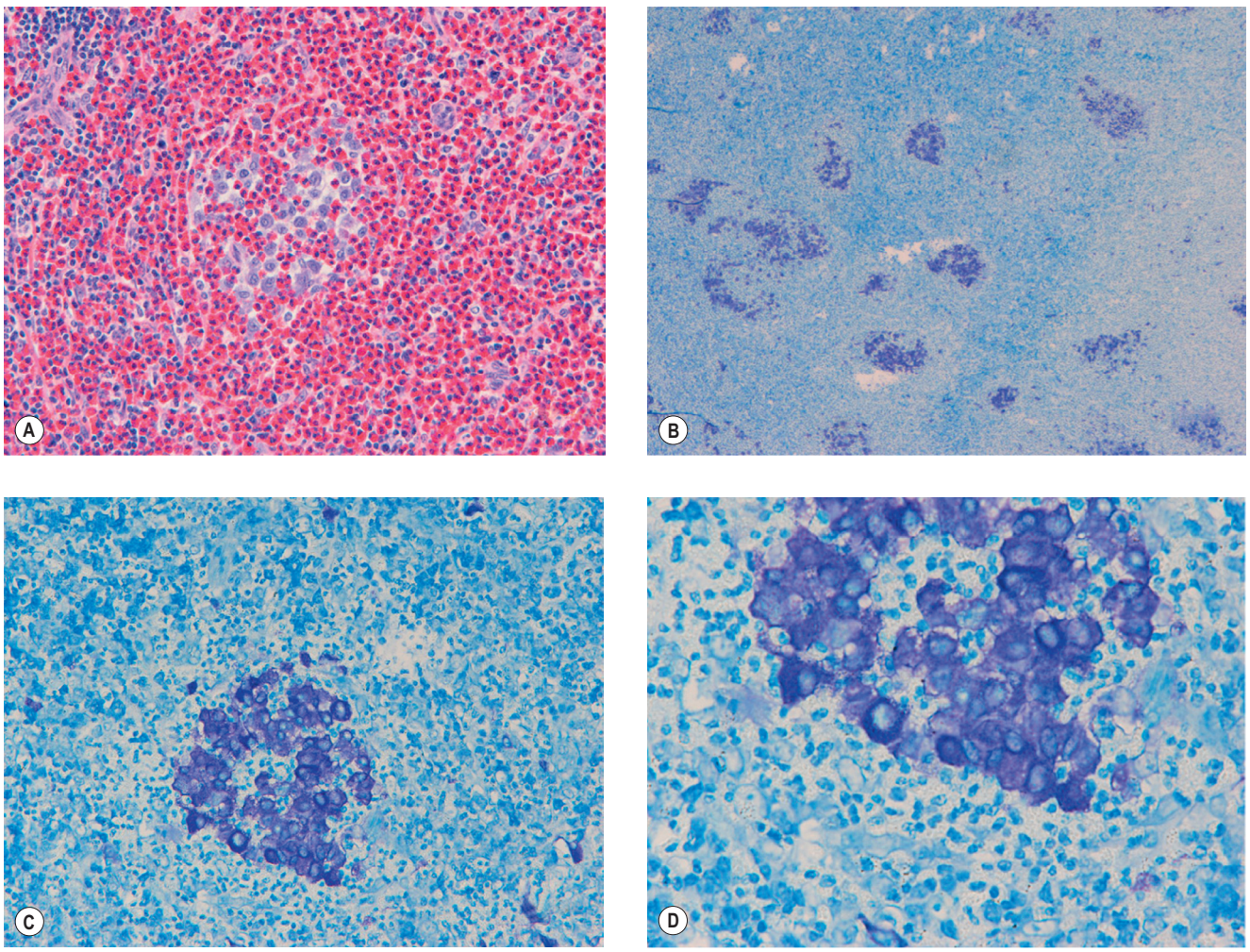


Figure 4.17 The complexity of the relationship between neoplastic cells and normal cells. **(A)** A mast cell tumour of the eyelid showing a large accumulation of eosinophilic polymorphonuclear cells (×100). It is easy to misinterpret this unless a special stain (in this case toluidine blue) is used to identify congregations of mast cells **(B)** (×100) and then to examine those specifically for evidence of neoplastic change (**C,D** ×200 and ×400, respectively). This illustrates the complexity of the relationship between neoplastic cells and normal cells; in this case, the mast cells result in an abnormal accumulation of eosinophils.

It is also clear that matrix metalloproteinase enzymes (MMPs) play an important role in the remodelling of the extracellular matrix (ECM) of the tumour micro-environment. Epithelial to mesenchymal transition occurs when there is break down of cell–cell adhesion and decreased expression of epithelial markers and increased expression of mesenchymal markers, such as vimentin. These changes are associated with increased motility of the abnormal cells and this is another histologically useful characteristic. MMPs support cancer progression by direct stimulation of tumour replication and also by regulating apoptosis, inflammation, angiogenesis, invasion and metastasis. Mainly, the issue is that they are overexpressed and the MMP profile in the environment of a tumour can be abnormal in many ways; what is certain, however, is that where MMPs are over-expressed there is a significantly higher tendency towards angiogenesis, tumour progression and metastasis.

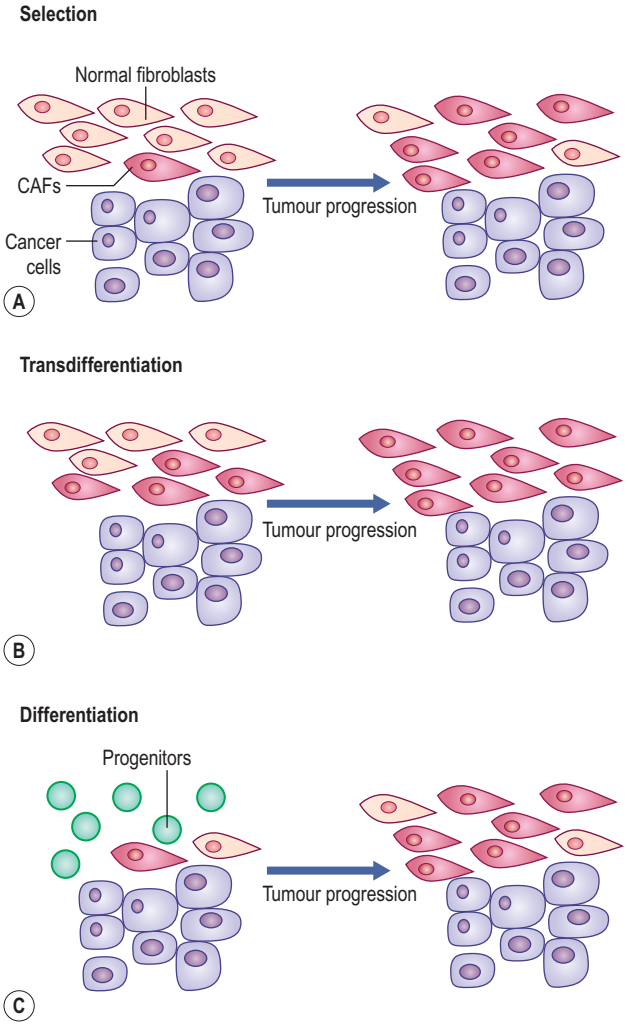


Figure 4.18 Possible models for generation of carcinoma-associated fibroblasts within carcinomas. (A) Clonal selection from a small population of fibroblasts or progenitors that have undergone genetic alterations. **(B)** Transdifferentiation from normal cells, such as normal fibroblasts. **(C)** Differentiation from progenitor cells. *(Figure reprinted from Orimo A, Weinberg RA. Stromal fibroblasts in cancer: a novel tumor-promoting cell type. Cell-cycle 2006; 5:1597–1601, with permission.)*

Carcinogenesis

Ever since the medical and veterinary professions have recognized cancer, there has been a determined search for its causes – '*What causes cancer*?' – in the expectation that if a 'cause' could be identified, it could be prevented, avoided or at least treated specifically.

Carcinogenesis or oncogenesis (literally 'the creation of cancer') is a process by which normal cells are transformed into cancer cells. It is generally true that genetic mutations form the basis of cancer (see above) but whilst it is accepted that spontaneous mutations do occur in all living creatures, the vast majority of these do not have any harmful effects. Spontaneous mutation and inherited mutations do certainly contribute to the overall pathology, but the large majority of significant mutations are acquired, probably in response to environmental mutagen challenge. Carcinogenesis is characterized by a progression of changes that occur at cellular and genetic levels that ultimately re-programme a cell to undergo uncontrolled cell division and therefore produces a malignant mass. The concept of multistep carcinogenesis and the two-hit hypothesis are the basis of modern cancer understanding.

The Knudson hypothesis

Also known as the 'two-hit hypothesis', the Knudson hypothesis suggests that cancer is the result of accumulated mutations to a cell's DNA and is possibly the most basic approach to the significance of mutations (Fig. 4.19).[16] In

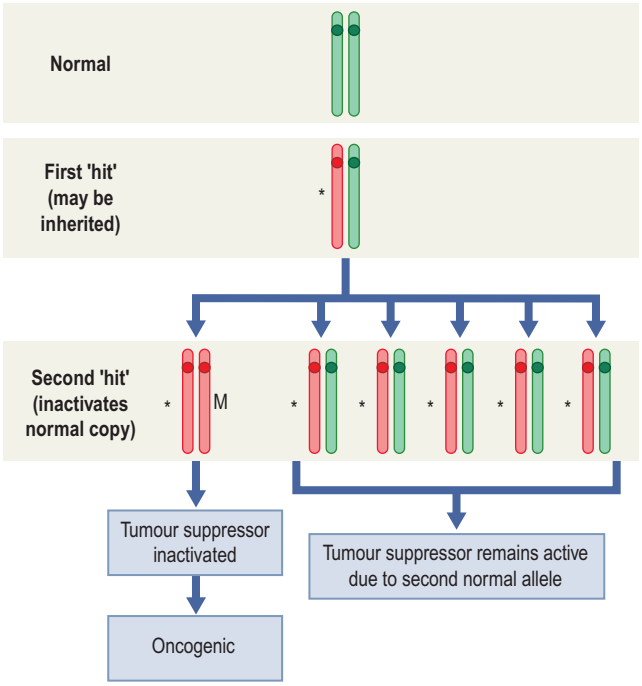


Figure 4.19 The so-called 'two-hit hypothesis' of cancer pathogenesis. This probably applies to most cancers in the early stages, but genomic instability predisposes further mutation and progression. It is most unlikely that a single mutational change will result in a dramatic and serious tumour, although that has been described in some rare tumour types. *(Figure reprinted from Haslett C, Chilvers ER, Hunter JAA, Boon NA, eds. Davidson's principles and practice of medicine, 18th edn. Edinburgh: Churchill Livingstone; 1999: 21 (Fig. 1.17); copyright 1999, with permission from Elsevier.)*

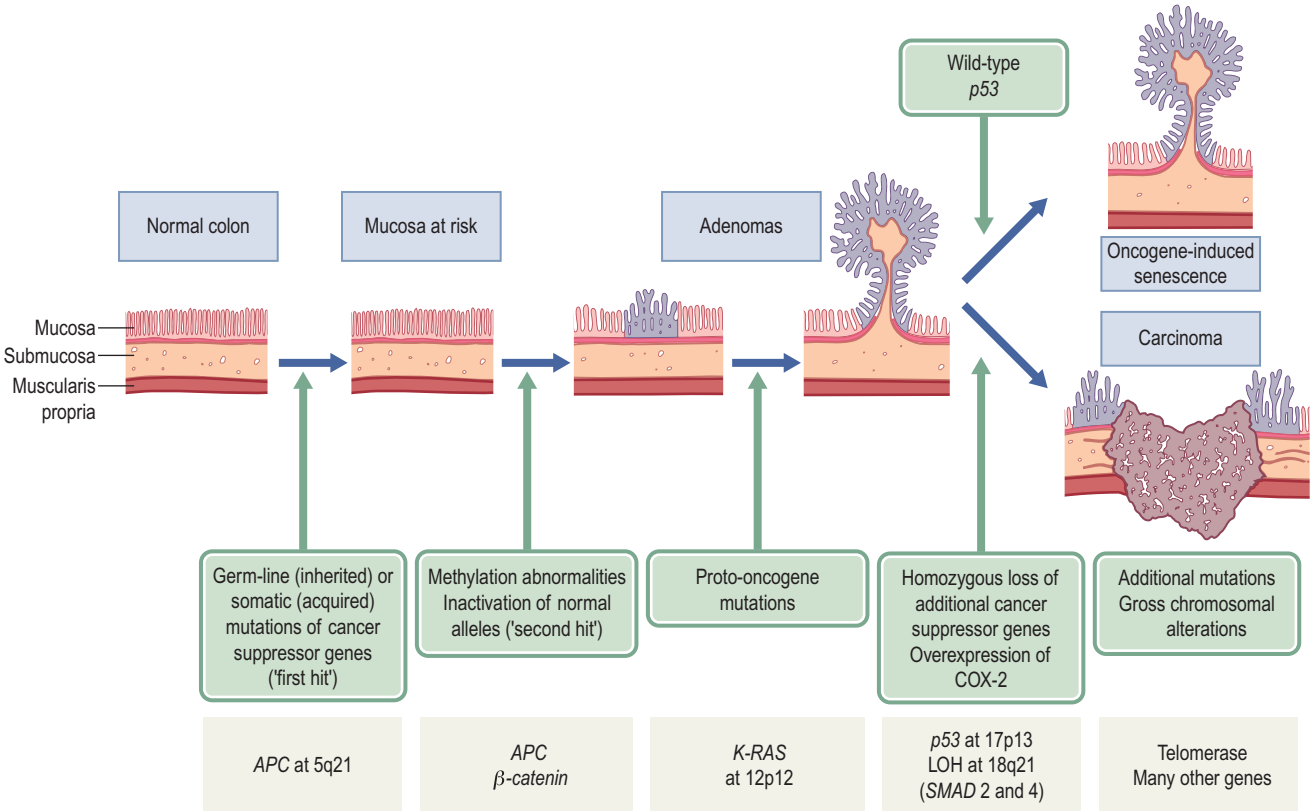


Figure 4.20 Molecular model for the evolution of human colorectal cancers through the adenoma–carcinoma sequence. Although *APC* mutation is an early event and loss of *p53* occurs late in the process of tumorigenesis, the timing for the other changes may be variable. Note also that individual tumours may not have all of the changes listed. Top right, cells that gain oncogene signalling without loss of *p53* eventually enter oncogene-induced senescence. *(Figure reprinted from Kumar V, Abbas A, Aster J. Robbins and Cotran pathologic basis of disease, 8th edn. Philadelphia: WB Saunders; 2010: 308 (Fig. 7.40); copyright 2010, with permission from Elsevier.)*

order that a mutation is expressed, it probably has to be reflected in both alleles of the gene. A single gene mutation, which might be inherited or acquired, is not enough to trigger the progressive development of a tumour. Where a single gene is inherited, the patient can be assumed to be 'more liable' to cancer development, since the next stage will require that only one mutation occurs. There are difficulties with this approach, since the triggering mutation would have to affect the same gene on the other chromosome – random mutations would possibly make that less likely. This may, however, explain why the development of tumours in 'susceptible' families may take many years to develop. DNA repair genes are clearly a significant target for carcinogenic agents.

It is now thought likely that whilst the two-hit theory applies for the basic conversion of a cell to a neoplastic genotype, further mutations are almost inevitable, since the most prominent genes that are mutated are the 'housekeeper genes' – *p53* and its family (p. 26). There are likely to be epigenetic factors involved as well, which alter the expression of genetic changes.

The multistep carcinogenesis proposal

The multistep carcinogenesis proposal involves alterations in sequential steps involving mutational changes that overcome the cell-cycle's self-regulatory mechanism. This then leads from early mild changes that may or may not be clinically detectable to the development of metastatic tumours (p. 52) (Figs 4.20, 4.21).

Recent advances in molecular biology have increased our understanding of cancer and the malignant process. It is now widely recognized that cancer results from mutations of genes that regulate cell division and cell growth. The scope for genetic mutation is very high given the number of cells that are undergoing cell-cycle processes at any time and, in addition, some of the genetic abnormalities can be inherited. Fortunately, only a few genes are integrally involved in the development of cancer.

A large number of agents cause genetic damage and this can be of a type and severity that can result in neoplastic transformation of individual cell types; usually the faster dividing cells are thought to have an increased susceptibility to mutation (p. 47). It is generally accepted that some cells are more liable to induced mutations than others.

Mutations are taking place in all cell types all the time – but few have any harmful consequences. Mutations impart the genetic instability that in turn makes the cell more sensitive to further insults, and mutations become increasingly likely as cells undergo multiple genetic alterations.

Tumour growth and progression is a tissue and cellular phenomenon. The individual cells that make up a multicellular organism are governed by endocrine and paracrine signals and messages deriving from local and remote sites, and affect cell surface receptors. These receptors