Advances in Experimental Medicine and Biology 772

Constantinos Koumenis
Ester Hammond
Amato Giaccia *Editors*

Tumor Microenvironment and Cellular Stress

Signaling, Metabolism, Imaging, and Therapeutic Targets

Aegean Conferences

Springer

Advances in Experimental Medicine and Biology

Volume 772

For further volumes:
http://www.springer.com/series/5584

Constantinos Koumenis • Ester Hammond
Amato Giaccia
Editors

Tumor Microenvironment and Cellular Stress

Signaling, Metabolism, Imaging, and Therapeutic Targets

Springer

Editors
Constantinos Koumenis
Perelman School of Medicine
Department of Radiation Oncology
University of Pennsylvania
Philadelphia, PA, USA

Amato Giaccia
Department of Radiation Oncology
Stanford University School of Medicine
Stanford, CA, USA

Ester Hammond
Cancer Research
Gray Institute for Radiation Oncology
Oxford, UK

ISSN 0065-2598
ISBN 978-1-4614-5914-9
DOI 10.1007/978-1-4614-5915-6
Springer New York Heidelberg Dordrecht London

ISSN 2214-8019 (electronic)
ISBN 978-1-4614-5915-6 (eBook)

Library of Congress Control Number: 2013955491

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

The tumor microenvironment has long been recognized for the critical roles it plays in both promoting the malignant progression of solid tumors and modifying the response of solid tumor cells to cytotoxic or targeted therapy. This book comprises 12 chapters that provide critical insights into how changes in the tumor microenvironment affect tumor metabolism, cell stemness, cell viability, genomic instability, immune modulation, and metastasis. In addition, there is also a chapter devoted to magnetic resonance imaging techniques used to visualize changes in tumor invasion, angiogenesis, and inflammation. The work described in these chapters was presented at the first Aegean meeting on the Tumor Microenvironment and Cellular Stress held in Crete, Greece, in October 2012.

Most solid tumors have a microenvironment that differs from their normal tissue counterpart because of malformed vasculature that is insufficient to be able to adequately perfuse tumor tissue. The inadequacy of the vasculature supply to tumors leads to areas that are hypoxic, or low in oxygen. One of the major changes that a tumor cell must surmount is the metabolic changes imposed by a low oxygen environment. Chapters 1, 5, and 10 describe how hypoxic tumor cells adapt and respond to decreased oxygen levels. Hypoxic tumor cells rely heavily on glycolysis, increasing their uptake of glucose by select glucose transporters, increasing the expression of glycolytic genes, inhibiting mitochondrial respiration, and increasing their levels of lactate. The roles of the hypoxia-inducible transcription factors HIF-1 and HIF-2 in regulating these metabolic changes are nicely described in Chapt. 1. The metabolism of glutamine is also discussed, especially as it relates to lipid synthesis. Perhaps the most interesting aspect of this chapter is the proposed therapeutic approaches that may be used to exploit the metabolic changes induced by the hypoxic tumor microenvironment. Chapter 5 describes research on the mitochondria voltage-dependent anion channel (VDAC1). This channel is located in the outer mitochondrial membrane and is responsible for the transfer of a large number of charged and uncharged molecules through changes in membrane voltage. It is interesting to note that hypoxia induces the expression of a C-terminal truncated form of VDAC1

(VDAC1-ΔC). This truncated form is associated with a high output of adenosine triphosphate and resistance to chemotherapy, suggesting that targeting this truncated form of VDAC1 may increase the chemosensitivity of tumor cells. Chapter 10 presents a in-depth summary of the hypoxia-inducible microRNA miR-210, which, among other functions, serves to regulate mitochondrial metabolism and oxidative stress. miR-210 is a robustly induced microRNA under hypoxic conditions, and its role in regulating metabolism may in fact be one of its most critical functions under low oxygen conditions. Taken together, these chapters present a comprehensive picture of metabolic changes induced by hypoxia and the genes that control them.

The importance of niches as regions of tumors that can modulate the growth and aggressive nature of tumor cells is a new concept in the study of the tumor microenvironment. Hypoxia and cancer stem cells are the subjects addressed in Chap. 2. In particular, the concept that hypoxia can modify the "stemness" of a tumor cell is a consequence of hypoxia inducing a niche where tumor stem cells can be arrested in an undifferentiated state through interactions with undifferentiated stromal cells. Furthermore, this chapter proposes that cancer stem cells located in the hypoxic niche may exist in a different state than other cancer stem cells based on analysis of stem cell markers. Hypoxia can affect the generation of cancer cell stemness through the HIF transcription factor as well as chromatin-modifying genes. This is an intriguing hypothesis that is supported by a growing amount of literature and has important therapeutic implications for tumor progression and responses to therapy. Chapter 3 discusses the role of hypoxia in promoting tumor cell metastasis through the increased expression of genes regulating the invasion of tumor cells trough the basement membrane, intravasation of tumor cells into the circulation, survival of tumor cells in circulation, extravasation of tumor cells out of circulation and into tissue, and colonization of a new tissue. The authors also include a circumspect and relevant analysis of hypoxia and the formation of the premetastatic niche, which is formed by tumor cells secreting factors that increase the ability of tumor cells to grow in a site distant from the primary tumor. The role of hypoxia and the genes and proteins it regulates in the formation of the premetastatic niche represents new targets for therapeutic intervention. Thus, these two chapters present new functions for hypoxia in regulating tumor stemness and the premetastatic niche.

Hypoxia has long been recognized as an impediment for cytotoxic therapies such as ionizing radiation and chemotherapy. This information is reviewed well and expanded on in Chap. 7. The role of the microvasculature, tumor stroma, the extracellular matrix, and resident and infiltrating immune cells in influencing the responsiveness of tumors to radiotherapy are described in a logical and clinically relevant manner. This chapter also makes the point that to date there has yet to be a successful targeted therapy against hypoxic tumor cells. The challenge of developing a targeted therapy against hypoxic tumor cells represents the focus of Chap. 6. The chapter is a must-read because it relates the history of developing agents to tackle the hypoxic problem, starting with hypoxic sensitizers, moving on to hypoxic cytotoxins, and ending with targeting the hypoxia response pathway. A different approach to selectively targeting hypoxic tumor cells is brought forth in Chap. 8 through the concept of "synthetic lethality." This concept of cell killing is based on

work with lower eukaryotes, which showed that a mutation in one of two different genes had no effect on cell survival, but if both genes were mutated at the same time, lethality would result. This chapter focuses on the process of autophagy and the genes and pathways that regulate this process. There is ample discussion of autophagy in tumor progression and resistance to therapy. The intriguing concept of activating autophagy to induce cell death and under what circumstances that would be effective is presented in a concise manner. The poster child for targeted therapy has been anti-angiogenic therapy. First described by Folkman and his colleagues many decades ago, anti-angiogenic therapy received approval from the US Food and Drug Administration for treating solid tumors both as a monotherapy and in combination with other agents. Chapter 4 presents the events that led to the development of anti-angiogenic therapy, its current success, and, most important, the reasons underlying the development of resistance to anti-angiogenic therapy. The presentation of a number of possible approaches to overcome the resistance to anti-angiogenic therapy, such as targeting pro-angiogenic myeloid cells, is the most exciting aspect of this chapter. Without question, understanding the mechanism of resistance to anti-angiogenic therapy will drive the next generation of therapeutics that should exhibit more durable benefits. These chapters present an up-to-date picture of the importance of hypoxia in cytotoxic therapy and targeted therapy.

While the conventional thinking is that hypoxic tumor cells are resistant to cytotoxic radiotherapy and chemotherapy based on a reduction of free radical formation and cessation of cell cycling, a different point of view is presented in Chap. 9. This chapter relates the concept that hypoxic cells are deficient in homologous recombination and prone to exhibit gene amplification and chromosome instability. These findings suggest that hypoxia inhibits genome maintenance and integrity and can promote tumor aggressiveness by increasing genomic instability. By understanding the mechanisms underlying the repair deficiency induced by exposure to hypoxia, the potential to selectively target hypoxic tumor cells using synthetic lethality could be a new avenue for fruitful investigation. This chapter provides several examples of how this form of synthetic lethality could be developed for therapy.

Tumor hypoxia has often tried to escape immune surveillance. An important pathway in immune surveillance against cancer has been the complement system. Cancer cells have, unfortunately, developed inhibitory mechanisms against complement activation that allow them to escape immune attack from the body and impede the activity of monoclonal antibody-directed therapy. This Chapter 11 describes our most up-to-date understanding about the activation and function of the complement system in human tumors and the paradoxical role of complement in promoting tumor growth in the face of an inflammatory signal. The impact of inflammation and hypoxia due to poor perfusion and increased interstitial fluid pressure in tumors can be visualized using magnetic resonance imaging. Chapter 12 describes the elegant use of tracking dyes or contrast agents to follow the fate of cells in tumors and their interactions with other components of the tumor microenvironment such as stroma, blood vessels, and immune cells. Imaging of the tumor microenvironment is useful for tumor staging as well as monitoring the efficacy of therapy directed at a specific tumor compartment.

We are grateful to all the authors who contributed these outstanding chapters to this book. We are also grateful to all the staff at Springer, especially Portia Wong, Development Editor, as well as Fiona Sarne and Gregory Baer, who have worked dilligently to get this book to publication. Their assistance has been greatly appreciated.

We hope that this book entices all those who do not study the tumor microenvironment to consider working in this field.

Philadelphia, PA, USA — Constantinos Koumenis
Oxford, UK — Ester Hammond
Stanford, CA, USA — Amato Giaccia

Contents

Chapter 1
Hypoxia and Metabolism in Cancer

Karim Bensaad and Adrian L. Harris

Abstract Interest in targeting metabolism has been renewed in recent years as research increases understanding of the altered metabolic profile of tumor cells compared with that of normal cells. Metabolic reprogramming allows cancer cells to survive and proliferate in the hostile tumor microenvironment. These metabolic changes support energy generation, anabolic processes, and the maintenance of redox potential, mechanisms that are all essential for the proliferation and survival of tumor cells. The metabolic switch in a number of key metabolic pathways is mainly regulated by genetic events, rendering cancer cells addicted to certain nutrients, such as glutamine. In addition, hypoxia is induced when highly proliferative tumor cells distance themselves from an oxygen supply. Hypoxia-inducible factor 1α is largely responsible for alterations in metabolism that support the survival of hypoxic tumor cells. Metabolic alterations and dependencies of cancer cells may be exploited to improve anticancer therapy. This chapter reviews the main aspects of altered metabolism in cancer cells, emphasizing recent advances in glucose, glutamine, and lipid metabolism.

Keywords Cancer • Hypoxia • Metabolism • Glycolysis • Glutaminolysis • Mitochondrial respiration • Lipids • Therapy • Synthetic lethality

K. Bensaad (✉)
CRUK Hypoxia and Angiogenesis Group, The Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford OX3 9DS, UK
e-mail: karim.bensaad@imm.ox.ac.uk

A.L. Harris
CRUK Growth Factor Group, The Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Headington, Oxford OX3 9DS, UK

C. Koumenis et al. (eds.), *Tumor Microenvironment and Cellular Stress*, Advances in Experimental Medicine and Biology 772, DOI 10.1007/978-1-4614-5915-6_1,

1.1 Introduction

1.1.1 Metabolism in Normal and Cancer Cells

Cancer is a genetic disease involving numerous pathways that are mainly induced by gain of function mutations that activate oncogenes or loss of function mutations, which inhibit tumor suppressor genes. These changes primarily lead to the dysregulation of cell proliferation. More than a decade ago, in an influential article titled the "Hallmark of cancer," Hanahan and Weinberg (2000) organized these pathways into six major biological capabilities acquired during the multiple steps of cancer development. These researchers recently published an up-to-date version of their initial article, describing reprogramming of energy metabolism as one of two new emerging hallmarks of cancer (Hanahan and Weinberg 2011). Cancer cells gain an undeniable survival advantage by reprogramming metabolism to respond to environmental stress, a process known as metabolic transformation. Metabolism can be described as the pathways required for the maintenance of life within a living cell. In metabolism, some substrates are broken down to yield energy while other substances necessary for cell survival are synthesized. The amount of adenosine triphosphate (ATP) required for cell proliferation is, surprisingly, not radically different from that required for resting cells (Kilburn et al. 1969). Cancer cells undergo metabolic reprogramming as their energy requirement increases to fuel increased growth and proliferation. It is important to note that tumor cells also have high need of carbon building blocks, which are provided by glucose, glutamine, and fatty acids, and cofactors (nicotinamide adenine dinucleotide phosphate [NADPH] and nicotinamide adenine dinucleotide [NADH]) for growth and proliferation in the changing tumor microenvironment.

Otto Warburg, a German biochemist and Nobel laureate, first observed increased glycolytic flux and lactate production in tumor ascites (Warburg 1956). Under aerobic conditions, normal cells mainly produce energy through mitochondrial oxidative phosphorylation using pyruvate derived from glucose through the glycolytic pathway. Under anaerobic conditions, through a process called the Pasteur effect, energy is essentially provided by glycolysis, and pyruvate is mostly converted to lactate that is preferentially exported from the cells. Most cancer cells undergo a metabolic shift toward glycolysis to produce energy and toward anabolic pathways using metabolic intermediates from the glycolytic pathway to synthesize proteins and lipids independently of oxygen availability. These different processes favorably promote rapid growth and proliferation of tumor cells (Cairns et al. 2011). In cancer cells, the upregulation of glycolytic flux, with lactate production from pyruvate, even in the presence of abundant oxygen, is now known as the Warburg effect (Koppenol et al. 2011; Vander Heiden et al. 2009). The Warburg effect is the molecular basis of the diagnostic tumor imaging technique called fluorodeoxyglucose positron emission tomography (^{18}F-FDG-PET), which allows fluorodeoxyglucose metabolism in tissues with high metabolic activity, such as most types of tumors, to be assessed.

Substrates other than glucose can be used in the mitochondria for energy production, including glutamine as well as long-chain fatty acids (LCFAs) (Dang 2012; Locasale and Cantley 2011). In this chapter, we describe these various metabolic pathways, their regulation under oxygen deprivation, and their importance in the development of cancer.

1.1.2 Hypoxia and Cancer

Oxygen is an essential molecule for cell survival because it is used as the final acceptor in mitochondrial respiration for energy production. Hypoxia refers to lower-than-normal oxygen conditions, with oxygen (O_2) concentrations around 21 % (150 mmHg) in ambient air and 2–9 % (around 40 mmHg) in most healthy mammalian tissues. Hypoxia is defined as less than 2 % O_2, whereas anoxia (or severe hypoxia) is defined as less than 0.02 % O_2 (Bertout et al. 2008). Low oxygen availability is associated with inflammation (Murdoch et al. 2005), necrosis, and/or abnormal neovascularization. In addition, highly proliferative cancer cells can outgrow their blood supply and trigger hypoxia. In the latter situation, hypoxia has a major role in metabolic reprogramming of tumor cells and is also considered to be a hallmark of cancer (Hanahan and Weinberg 2011). Hypoxia is thought to promote invasiveness and metastasis (Harris 2002). The hypoxic environment of tumors leads to the stabilization of hypoxia-inducible factors (HIFs).

HIFs are dimeric protein complexes that consist of an α-subunit (HIF-1α or HIF-2α) and a β-subunit (HIF-1β). HIF-1α is expressed ubiquitously, whereas HIF-2α, also known as endothelial PAS domain protein 1 (EPAS1), was initially detected in endothelial cells but is also selectively highly expressed in a smaller number of tissues (Patel and Simon 2008). HIF-1α and HIF-2α activities are regulated by levels of oxygen. Under normoxic conditions, both of these proteins are degraded by the proteasome machinery. HIFs are targeted for ubiquitination by oxygen-sensitive prolyl-hydroxylases (PHDs) and the von Hippel–Lindau (VHL) tumor suppressor protein. In normoxia, Factor Inhibiting HIF-1 (FIH) also leads to inactivation by hydroxylation of HIFs. In hypoxic conditions, there is stabilization of HIF-1α and HIF-2α because hydroxylases, the VHL tumor suppressor protein, and factor-inhibiting HIF-1 are all inhibited by low oxygen availability. When stabilized, HIFs can bind to specific regulatory elements in the promoter of their target genes and induce their expression (Semenza 2012).

HIF-1α and HIF-2α are differentially regulated by the NAD^+-dependent deacetylase sirtuin (SIRT) 1, a known stress-activated factor. SIRT1 is activated by elevation of the NAD^+-to-$NADP^+$ ratio and directly couples NAD^+ hydrolysis to the deacetylation of numerous transcription factors and cofactors, including HIFs. As a consequence, SIRT1 directly links metabolic status to gene expression by acting as a redox sensor, and it plays an important role in various pro-survival and metabolic activities (Haigis and Yankner 2010; Schug and Li 2011). SIRT1 deacetylates

specific lysine residues in HIF-1α and HIF-2α proteins, resulting in opposite downstream outcomes (Dioum et al. 2009; Laemmle et al. 2012; Lim et al. 2010). During normoxia, SIRT1 binds to HIF-1α and deacetylates it at Lys674. This deacetylation inactivates HIF-1α transactivation function by blocking p300 recruitment. During hypoxia, SIRT1 activity is reduced because of decreased NAD^+ levels associated with increased glycolytic flux. This results in HIF-1α retaining its acetylation status and remaining activated (Lim et al. 2010). Therefore, if glycolysis is inhibited and, as a consequence, NAD^+ levels are increased, even under hypoxia, SIRT1 is activated and results in HIF-1α inhibition (Lim et al. 2010). SIRT1 has a surprising opposite effect on HIF-2α: deacetylation stimulates activity of HIF-2α during hypoxia (Dioum et al. 2009).

Tumor hypoxia is mainly caused by defective vasculature in fast-growing solid tumor tissues, leading to diminished supply of oxygen and nutrients. This local lack of nutrients and oxygen triggers the formation of new blood vessels in the growing tumor. HIF-1α initiates angiogenesis by inducing Vascular Endothelial Growth Factor (VEGF, also know as VEGF-A) and many other angiogenic factors such as stromal-derived factor 1 (SDF1), placental growth factor (PGF), platelet-derived growth factor B (PDGFB), and angiopoietin 1 and 2 (ANGPT 1 and 2) (Chen et al. 2009; Hickey and Simon 2006; Rey and Semenza 2010). Tumor neovasculature is poorly developed and effective and thus leads to nutrients shortage and hypoxic stress. Adaptation to these conditions of intermittent hypoxia is essential for the survival and progression of cancer.

1.2 Glucose Metabolism

1.2.1 Glycolysis in Normal and Cancer Cells

Glucose is transported from the circulation into cells via glucose transporters; it then is phosphorylated to form glucose-6-phosphate (G6P). G6P is then further phosphorylated and, after a series of reactions, is broken up into dihydroacetone-phosphate (DHAP) and glyceraldehyde-3-phosphate (G3P), which is converted to glycerol-3-phosphate for lipid synthesis or sequentially transformed to produce pyruvate. Pyruvate can be converted to acetyl-coenzyme A (CoA) in the tricarboxylic acid (TCA) cycle in the mitochondria or converted to lactate in the cytosol. G6P can take an alternative metabolic pathway, the pentose phosphate pathway (PPP), which generates ribose-5-phosphate for nucleotide synthesis and the byproduct NADPH for reductive biosynthesis. Finally, G6P can also be converted to glycogen for storage (Fig. 1.1).

Fig. 1.1 (continued) glycogen phosphorylase liver form; *ROS* reactive oxygen species; *R5P* ribose-5-phosphate; *SFA* saturated fatty acid; *SCD1* stearoyl-CoA desaturase 1; *SDH* succinate dehydrogenase; *SCO2* synthesis of cytochrome C oxidase 2; *TIGAR* TP53-inducible glycolytic and apoptotic regulator; *TCA* tricarboxylic acid; *TPI* triose-phosphate isomerase; *TG* triglyceride; *UDP-GlcNAc* UDP-N-acetylglucosamine