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Targeting Tumor Microenvironments for Cancer Prevention and Therapy

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1. Introduction

Solid tumors comprise not only cancer cells but also host stromal cells, such as vascular cells, inflammatory/immune cells, and cancer-associated fibroblasts. The crosstalk between cancer cells and stromal cells plays an important role in tumor growth, metastasis, and response to antitumor therapy (Hanahan and Weinberg, 2011; Joyce and Pollard, 2009; Petrulio et al., 2006). Cancer cells with oncogenic mutations are central to tumor formation. Endothelial cells in tumors form new blood vessels (angiogenesis) which bring oxygen and nutrients to the growing tumor (Ferrara and Kerbel, 2005), and also regulate leukocyte infiltration and tumor cell metastasis (Chouaib et al., 2010). Inflammatory cells have both tumor-promoting and tumor-preventing effects (Grivennikov et al., 2010; Hanahan and Weinberg, 2011). Fibroblasts are the most abundant cells in the tumor stroma and have been demonstrated to have tumor-promoting activities (Bhowmick et al., 2004). Moreover, cancer cells within tumors are heterogeneous and composed of distinct subpopulations with different states of tumorigenicity. One subpopulation of cells that has recently been extensively studied is the cancer initiating cell or cancer stem cell (CSC) (Cho and Clarke, 2008), which exhibits high capacity of generating new tumors.

The microenvironment in solid tumors is very distinct from that in normal tissues. Due to deregulated cancer cell metabolism, highly heterogeneous vasculature and defective blood perfusion, the tumor microenvironment is characterized by hypoxia and acidosis (Cairns et al., 2006; Gatenby et al., 2006; Gatenby and Gillies, 2004). The uncontrolled proliferation of tumor cells results in a growing mass that rapidly consumes oxygen, glucose and nutrients (Gatenby and Gillies, 2004). When an oxygen diffusion limit is reached, some regions of a tumor become hypoxic. Cancer cells rely heavily upon glycolysis ('Warburg effect') to generate ATP and metabolic intermediates for biosynthesis (Gatenby and Gillies, 2004; Vander Heiden et al., 2009). There is much evidence to link the connection between the adaptation to hypoxia and the development of an aggressive tumor phenotype in both experimental and clinical settings (Chang et al., 2011; Gatenby and Gillies, 2004). In addition to hypoxia, the existence of acidosis is a defining hallmark of the tumor microenvironment.

This condition arises mainly due to an increase in the production of lactic acid by glycolysis along with other proton sources (Gatenby and Gillies, 2004; Helmlinger et al., 2002; Yamagata et al., 1998). Acidosis is a selection force for cancer cell somatic evolution, modulates cancer cell invasion and metastasis, and affects the efficacy of some chemotherapeutic drugs (Cairns et al., 2006; Gatenby et al., 2006; Gatenby and Gillies, 2004).

Here we will describe cellular heterogeneity, hypoxia, and acidosis in the tumor microenvironment, and discuss some recent progresses in targeting tumor angiogenesis, inflammation, hypoxia and acidosis-related pathways for cancer prevention and therapy.

2. Tumor microenvironments and cancer progression

2.1 Complex cellular components in solid tumors

Tumor is an aberrantly proliferating tissue that contains cancerous cells and host stromal cells such as vascular cells, inflammatory cells, and fibroblasts. These cells are crucial for cancer initiation, progression and metastasis and have been exploited as targets for cancer therapy and prevention (Ferrara and Kerbel, 2005; Fukumura and Jain, 2007; Hanahan and Weinberg, 2011).

2.1.1 Vascular cells

Tumor blood vessels, like normal vessels, are composed of endothelial cells, pericytes/smooth muscle cells and basement membrane. However, all of these components are morphologically and/or functionally different from the normal counterparts (Baluk et al., 2005).

Tumor-associated endothelial cells (TECs) are the major player in the formation of tumor vasculature through sprouting from pre-existing blood vessels (a process called 'angiogenesis'). During blood vessel formation, endothelial cells proliferate, migrate and form the inner layer of a lumen, followed by basement membrane formation and pericyte attachment. Angiogenesis is stimulated by excessive pro-angiogenic factors secreted by tumor cells or stromal cells in an oxygen-depleted microenvironment. Moreover, bone marrow-derived endothelial progenitor cells recruited to tumor stroma can contribute to blood vessel construction by incorporating into vessels (Lyden et al., 2001). New blood vessel formation is critical for tumor development and progression, as it delivers nutrients and oxygen to growing tumor and removes metabolic wastes. In addition, vascular endothelial cells form a barrier between circulating blood cells, tumor cells and the extracellular matrix (ECM), thus playing a central role in regulating the trafficking of leukocytes and tumor cells (Chouaib et al., 2010). In this regard, endothelial cells are critical for boosting a host immune defense against cancer cells and for controlling tumor metastasis. However, the 'gate-keeping' function of endothelial cells in tumors is heavily compromised. TECs are not tightly associated with each other, resulting in wider inter-endothelial junctions that cause plasma leakage and hemorrhage (Hashizume et al., 2000). Consequently, tumor vasculature is often leaky and less efficient in blood perfusion, leading to high interstitial fluid pressure, hypoxia and acidic extracellular pH that significantly affect the delivery and efficacy of chemotherapeutic drugs. The leaky blood vessels also facilitate the intravastion of tumor cells and promote tumor metastasis.

TECs are different from endothelial cells in normal tissues at several aspects. It has been reported that human hepatocellular carcinoma-derived endothelial cells, when compared to the ones from adjacent normal liver tissue, show increased apoptosis resistance, enhanced angiogenic activity and acquire more resistance to the combination of angiogenesis inhibitor with chemotherapeutic drugs (Xiong et al., 2009). Studies have also revealed distinct gene expression profiles of TECs and identified cell-surface markers distinguishing tumor versus normal endothelial cells (Seaman et al., 2007).

In blood vessels, pericytes are smooth muscle cell-like cells that cover the vascular tube. They are intimately associated with endothelial cells and embedded within the vascular basement membrane, and play an important role in the maintenance of blood vessel integrity. Pericytes in tumors are different from normal ones: in tumors, pericytes are often less abundant and more loosely attached to the endothelial layer (Abramsson et al., 2002; Morikawa et al., 2002). The abnormality in pericytes weakens the vessel wall and increases vessel leakiness. Pericytes express several markers, though none is pericyte-exclusive, including α-smooth muscle actin (αSMA), platelet-derived growth factor receptor-β (PDGFR-β) and NG2 (Gerhardt and Betsholtz, 2003; McDonald and Choyke, 2003). PDGF-B signaling is important for pericyte recruitment and attachment to endothelial cells during vascular development (Abramsson et al., 2002; Abramsson et al., 2003).

2.1.2 Inflammatory/immune cells

Tumors are often infiltrated by inflammatory cells, such as macrophages, neutrophils, lymphocytes, mast cells, and myeloid progenitors. This phenomenon was initially observed by Rudolf Virchow more than a century ago and thought as an immunological response attempting to eliminate cancer cells. Whereas immune cells play a role in recognizing and eradicating early cancer cells (Kim et al., 2007), mounting evidence has also shown that inflammatory cells within tumors can enhance tumor initiation and progression by helping cancer cells acquire hallmark capabilities (Grivennikov et al., 2010; Hanahan and Weinberg, 2011). Inflammation is considered as an 'enabling characteristic' of tumor biology (Hanahan and Weinberg, 2011).

Pathological studies show that the abundance of certain types of infiltrating inflammatory cells, such as macrophages, neutrophils and mast cells, is correlated with poor prognosis of cancer patients (Murdoch et al., 2008). Tumor associated macrophages (TAMs), along with mast cells, neutrophils and other immune cells, produce cytokines (e.g. TNFα and IL-1), chemokines (e.g. CCL2 and CXCL12), angiogenic factors (e.g. VEGF, PDGF, FGF and IL-8), and matrix-degrading enzymes (e.g. MMPs, cathepsin proteases and heparanase) (Grivennikov et al., 2010; Karnoub and Weinberg, 2006). Some inflammatory cells, particularly neutrophils, also generate reactive oxygen and nitrogen species. These bioactive factors promote cancer cell proliferation, invasion and resistance to apoptosis through, for instance, the interleukin-JAK/STAT pathway (Ara and Declerck, 2010), and induce new blood vessel formation in the tumor. Extracelluar matrix-degrading enzymes promote cancer cell invasion and metastasis, whereas accumulation of reactive oxygen and nitrogen species can cause DNA mutagenesis, suppress DNA repair enzymes, increase genomic instability, and aggravate cancer progression.

While the tumor-promoting effects of infiltrating inflammatory cells have been well documented, certain types of immune cells, particularly cytotoxic T cells and natural killer cells, exhibit anti-tumor activities. The high numbers of these cells within a tumor predict a favorable prognosis (de Visser, 2008; Fridman et al., 2011). Immune surveillance is considered as an important mechanism to inhibit carcinogenesis and maintain tumor dormancy (Kim et al., 2007). Evading immune destruction by downregulating tumor antigens, suppressing immune cell function and other means is an emerging hallmark of cancer cells and plays important roles in cancer progression and metastasis (Hanahan and Weinberg, 2011). With regard to cancer therapy, blockade of CTLA-4 (cytotoxic T lymphocyte-associated antigen 4), a negative regulator of T cells, by the monoclonal antibody, ipilimumab, improved overall survival in patients with metastatic melanoma treated in combination with dacarbazine (Robert et al., 2011a). Moreover, expansion of tumor-infiltrating lymphocytes *ex vivo* and adoptive T-cell transfer immunotherapy led to regression of metastatic melanoma and durable responses in patients (Dudley et al., 2002; Rosenberg et al., 2011).

2.1.3 Fibroblasts

Fibroblasts account for the majority of stromal cells within solid tumors and are the principal source of ECM constituents (Chang et al., 2002). Fibroblasts in tumors are termed as cancer-associated fibroblasts (CAFs).

Tumors have been described as wounds that do not heal (Dvorak, 1986). Indeed, it has been observed that tumor-associated fibroblasts are biologically similar to the ones involved in wound healing or fibrosis (Ryan et al., 1973; Schor et al., 1988). Fibroblasts involved in these processes produce more ECM proteins and proliferate faster than the normal counterparts from healthy tissues (Castor et al., 1979; Muller and Rodemann, 1991). Fibroblasts with these properties are referred as "activated fibroblasts" or "myofibroblasts", due to their characteristic expression of α-smooth muscle actin (α-SMA) (Gabbiani, 2003; Ronnov-Jessen et al., 1996). Fibroblasts can be activated by various stimuli, such as transforming growth factor-β (TGFβ), epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and fibroblast growth factor 2 (FGF2) (Zeisberg et al., 2000).

CAFs play an important role in promoting tumor initiation and progression by stimulating angiogenesis and tumor cell growth and invasion (Shimoda et al., 2010). The existence of a large number of CAFs in tumors is often associated with poor prognosis (Maeshima et al., 2002; Surowiak et al., 2006). CAFs produce growth factors, cytokines, chemokines and ECM proteases to stimulate angiogenesis and cancer cell proliferation and invasion. For example, CAFs secrete elevated levels of stromal cell-derived factor 1 (SDF-1; also called CXCL12) that facilitates angiogenesis by recruiting endothelial progenitor cells into the tumor (Orimo et al., 2005). SDF-1 can also interact with the CXCR4 receptor expressed on the surface of cancer cells, thus stimulating tumor cell growth and promoting tumor progression *in vivo* (Orimo and Weinberg, 2006). TGFβ, another factor produced by CAFs, is a critical mediator of the epithelial-to-mesenchymal transition (EMT); therefore, CAFs might contribute to EMT in nearby cancer cells and promote their invasiveness (Shimoda et al., 2010). Moreover, CAFs facilitate cancer cells to invade ECM and metastasize by releasing ECM-degrading proteases, such as matrix metalloproteinases (MMPs) (Boire et al., 2005; Sternlicht et al., 1999).

CAFs can maintain the myofibroblastic properties even after several passages *in vitro* without further signaling from carcinoma cells. How do CAFs acquire and maintain their activated phenotype? There are some controversial results with regard to the presence of somatic genetic alterations in CAFs. It has been reported that stroma microdissected from various human cancers exhibited some genetic alterations, such as chromosomal loss of heterozygosity (LOH) and somatic mutations (Currie et al., 2007; Kurose et al., 2002; Moinfar et al., 2000; Paterson et al., 2003; Tuhkanen et al., 2004; Wernert et al., 2001). Other reports also demonstrated that in the process of tumor development, fibroblasts that have lost p53 activity were clonally selected, leading to a highly proliferative stroma (Hill et al., 2005; Kiaris et al., 2005). In contrast, several genome-wide genetic analyses, including CGH and SNP arrays, were not able to detect any genetic alterations in the myofibroblasts isolated from various human cancers (Qiu et al., 2008; Walter et al., 2008). Other studies have suggested that epigenetic modifications within the genome of CAFs, such as DNA methylation, might be the reason (Hu et al., 2005; Jiang et al., 2008). Further studies are required to clarify these issues.

2.1.4 Cancer stem/initiating cells

Although cancer can originate from a single transformed cell, not all the cancer cells within a tumor are identical; in other words, cancer cells become heterogeneous during the somatic evolution process, reflected by distinct tumor regions with different histopathological characteristics and various degrees of tumor hallmark capacities. Moreover, mounting evidence indicates that tumor cells are also heterogeneous with regard to the capability to generate new tumors (Cho and Clarke, 2008; Lobo et al., 2007). Multiple studies showed that distinct subpopulations of cancer cells could be sorted from primary tumor samples based on their cell-surface antigen profiles. When different subpopulations of cells were injected into immune-deficient mice, only a subset of cells was able to propagate tumor growth, whereas other cells were unable to induce tumor regeneration (Lobo et al., 2007). This population of cancer cells has also been demonstrated to have the ability of self-renewal and differentiation, two hallmark characteristics of stem cells (Clarke et al., 2006). In addition, these cells also expresses some markers of normal stem cells (Al-Hajj et al., 2003); hence, these cells are termed as 'cancer stem cells' (CSCs; also referred as cancer initiating cells or tumorigenic cancer cells).

CSCs were initially identified in leukemia (Bonnet and Dick, 1997; Lapidot et al., 1994) and later in solid tumors that include cancers of breast, brain, pancreas, head and neck, and colon (Al-Hajj et al., 2003; Dalerba et al., 2007; Li et al., 2007; O'Brien et al., 2007; Prince et al., 2007; Ricci-Vitiani et al., 2007; Singh et al., 2004). Studies of leukemia stem cells suggest that, CSCs may arise from normal stem cells that acquire oncogenic mutations and undergo transformation (Fialkow, 1990; Lapidot et al., 1994; Lobo et al., 2007), or progenitor cells that gain the ability to self-renew through oncogenic transformation (Cozzio et al., 2003; Krivtsov et al., 2006; So et al., 2004). However, recent observations suggest that CSCs may also be derived from non-CSCs via the EMT process (Mani et al., 2008; Morel et al., 2008; Singh and Settleman, 2010), which plays an important role in morphogenesis and in promoting tumor cell motility and invasiveness (Hugo et al., 2007; Thiery, 2003). This model indicates that whereas CSCs can differentiate into non-CSCs; non-CSCs may also be reprogrammed and converted to CSCs, suggesting the existence of a dynamic interconversion between CSCs and non-CSCs that is controlled by the tumor microenvironment (Gupta et al., 2009). Such

plasticity of CSC state is absent in the conventional depiction of normal stem cells, and has changed the perception of CSCs biology.

With regard to the frequency of CSC representation in tumors, there are conflicting results and ongoing controversies. Initially, CSCs were described to exist only as small subpopulations within tumors (Bonnet and Dick, 1997; Lapidot et al., 1994); moreover, since normal stem cells are usually rare, it was assumed that CSCs should also be rare. However, recent studies on human melanoma suggested that as many as a quarter of the cancer cells could be CSCs (Kelly et al., 2007; Quintana et al., 2008). This disparity on the frequency of CSCs reported were partially attributed to the experimental xenograft conditions in which the ability of human tumor cells to seed and grow in a mouse tissue may vary (Quintana et al., 2008). The plasticity of CSCs state may also in part account for the differences of CSCs representation. The balance of the interconversion between CSCs and non-CSCs could be shifted in one direction or another in response to microenvironmental signals (Santisteban et al., 2009; Till et al., 1964). It is suggested that the proportion of CSCs may differ between tumor types, dependent on stromal microenvironment and somatic mutations within tumors as well as tumor progression stage (Gupta et al., 2009).

The existence of CSCs has attracted growing attention as CSCs may provide explanations for some puzzled clinical problems and imply novel cancer therapies (Clevers, 2011). CSCs have been shown to be more resistant to a variety of conventional radio/chemotherapies than non-CSCs (Chiu et al., 2010; Diehn et al., 2009; Li et al., 2008). Together with their ability to regenerate tumor and to colonize distant organs (Hermann et al., 2007), CSCs are proposed to be responsible for cancer recurrence following chemotherapy or radiation treatment, and for metastases that appear after surgical removal of a primary tumor. In addition, CSCs hypothesis implies that development of novel and more effective treatments that target the 'seeds' of the tumors might be a promising improvement of current therapy regimen. However, the plasticity of CSC phenotype implies that eliminating CSCs alone may not effectively cure tumors as they can be regenerated from non-CSCs, calling for dual targeting therapeutic regimens (Gupta et al., 2009). Moreover, there are controversies about the CSCs model and the experimental strategy employed to define the existence of CSCs. There are rising concerns about the xenograft assay, a typical experimental strategy in the CSC research, in which sorted cancer cells are xenotransplanted into immunodeficient mice. However, this method could induce cellular stress of the isolated cancer cells; moreover, the species barrier and the transplantation procedure within this approach could complicate the process of CSC identification (Clevers, 2011). It will be of importance to devise new strategies to detect functional presence of CSCs within a tumor.

2.2 Angiogenesis

The growth and progression of tumors rely on blood vessels to acquire oxygen and nutrients and to remove metabolic wastes (Papetti and Herman, 2002). During the early stage of tumor development, once the size of a tumor mass reaches the diffusion limit for oxygen and nutrients, it may stay in a dormancy state with a steady rate of cell proliferation and death (Fukumura and Jain, 2007). Some human tumors can remain dormant for a number of years. However, the steady state may be disturbed as oxygen-deprived tumor cells release angiogenic factors that trigger the 'angiogenic switch' and initiate new blood vessel formation from nearby existing ones (a process called angiogenesis) (Hanahan and

Folkman, 1996; Hanahan and Weinberg, 2000). Angiogenesis expands the tumor vascular network, enabling malignant cell proliferation and metastasis. Therefore, angiogenesis is a rate-limiting step in tumor development and progression.

Angiogenesis is controlled by a fine-tuned equilibrium between angiogenic and angiostatic factors (Baeriswyl and Christofori, 2009; Bergers and Benjamin, 2003; Carmeliet and Jain, 2000). Under normal physiological conditions, this balance is tightly regulated, so that the 'angiogenic switch' is 'on' only when needed and otherwise remains 'off'. Moreover, the newly formed vessels rapidly mature and become quiescent. By contrast, in tumors, the balance between positive and negative controls is disrupted due to an overproduction of pro-angiogenic factors. Consequently, new blood vessels are constantly produced in tumors. To date, more than two dozen pro-angiogenic factors and similar number of anti-angiogenic factors have been identified. Key pro-angiogenic molecules include vascular endothelial growth factor (VEGF), angiopoietin 1 (Ang1), platelet-derived growth factor (PDGF), placenta growth factor (PlGF), fibroblast growth factor 2 (FGF2), hepatocyte growth factor (HGF), among others (Adini et al., 2002; Papapetropoulos et al., 1999). Important angiogenic inhibitors include thrombospondin, angiostatin, endostatin, canstatin and tumstatin (Folkman, 2006; Kazerounian et al., 2008; Nyberg et al., 2005).

VEGF signaling pathway is the most prominent and best characterized pro-angiogenic pathway (Ferrara et al., 2003). The VEGF family includes VEGF-A, B, C, D, and PlGF (Ferrara, 2002; Hicklin and Ellis, 2005). VEGF-A (also called VEGF) is the major regulator of tumor angiogenesis. There are several isoforms of VEGF-A, with 121, 165, 189 and 206 amino acids, which are generated by alternative splicing (Houck et al., 1991; Tischer et al., 1991). VEGF-A mainly binds to VEGF receptor 2 (VEGFR-2) and triggers various downstream signaling pathways to up-regulate genes that stimulate endothelial cell proliferation, migration and survival and increase vascular permeability (Dvorak, 2002; Shibuya and Claesson-Welsh, 2006). VEGF is expressed at elevated levels in most types of human cancer. This can be caused by diverse genetic and epigenetic factors (Kerbel and Folkman, 2002; Kerbel, 2008). Hypoxia, a hallmark of tumor microenvironment, is an important inducer of VEGF through the hypoxia-inducible factor (HIF) 1α and 2α (Semenza, 2003). In addition, inflammatory cytokines, growth factors and chemokines can also induce VEGF expression. Other genetic causes include activation of oncogenes, such as mutant ras (Rak et al., 1995), or inactivation of tumor-suppressor genes, such as the von Hippel-Lindau (VHL) tumor suppressor (Patard et al., 2009).

In addition to VEGF, there are other important signaling pathways that regulate angiogenesis. Endothelial cell-associated delta-like ligand (Dll) 4-notch signaling pathway acts as negative feedback mechanism of VEGF signaling to prevent excessive tumor angiogenesis (Lobov et al., 2007; Ridgway et al., 2006). HGF/c-Met signaling can induce VEGF and VEGFR expression and also promote angiogenic proliferation and survival (You and McDonald, 2008). FGF2 signaling can stimulate angiogenesis independent of VEGF (Beenken and Mohammadi, 2009). PDGF-B signaling is important for the recruitment of pericytes to nascent blood vessels and stabilization/maturation of the vasculature (Lindahl et al., 1997). The angiopoietins (Ang-1, 2), interacting with the Tie2 receptor, act in cooperation with VEGF to promote angiogenesis and stabilize and mature new vasculature (Augustin et al., 2009). PlGF, signaling through VEGFR1, is another growth factor that induces endothelial cell proliferation, migration and survival (Fischer et al., 2007).

Moreover, endothelial progenitor cells can also be recruited and contribute to the formation of new blood vessels.

Due to the imbalanced expression of pro- and anti-angiogenic factors (Jain, 2005), tumor vasculature is often abnormal in architecture and function (Baluk et al., 2005; Fukumura and Jain, 2007). In contrast to the well-organized normal vascular tree, tumor blood vessels are highly variable in size, shape, and branching pattern. They are tortuous, dilated, irregularly shaped, and lack the normal hierarchy of arterioles, capillaries and venules. The structure of vessel wall is also defective, with large inter-endothelial junctions and loose perivascular cells attachment (McDonald and Choyke, 2003; Morikawa et al., 2002). Hence, tumor blood vessels are often leaky and hemorrhagic. Vascular permeability in tumor is generally higher than that in normal tissues, leading to increased interstitial fluid pressure. Also, blood flow in tumor vessels is irregular, slower, oscillating, and sometimes can even reverse the direction. Therefore, in spite of the production of excess blood vessels, the perfusion efficiency in tumor is still low. The aberrant tumor vasculature fails to meet the demand of growing tumor for nutrients and oxygen, as well as to adequately remove waste products. Chronically, the tumor microenvironment becomes hypoxic and acidic (Fukumura and Jain, 2007). As stated in more detail in the following sections, hypoxia and acidosis are selection forces for cancer cell somatic evolution and also significantly affect radiation sensitivity and chemotherapeutic efficacy.

As angiogenesis plays a critical role in tumor growth and progression, anti-angiogenesis therapy has been developed aiming to halt tumor growth by depriving cancer cells of the blood supply (Ferrara and Kerbel, 2005). Most of the angiogenesis inhibitors target the VEGF signaling pathway, including antibodies directly against VEGF and small molecules inhibiting its receptors. These anti-angiogenic agents have provided clinical benefits in patients with various types of cancers. Detailed discussion on anti-angiogenesis therapy is presented in the Section 3.1.

2.3 Hypoxia

The defective architecture and functionality of tumor blood vessels results in the occurrence of hypoxic regions in solid tumors (Fukumura and Jain, 2007; Gatenby and Gillies, 2004). Hypoxia is further exacerbated by the uncontrolled and rapid proliferation of tumor cells. These cancerous cells consume large amounts of oxygen and nutrients during their rapid divisions, further dictating the need for ample blood supply. As the oxygen diffusion limit is reached and the partial pressure of oxygen, pO_2, drops towards zero, cells must adapt and rely upon alternative means to acquire energy in this hypoxic microenvironment (Bertout et al., 2008; Cairns et al., 2006; Fukumura and Jain, 2007; Gatenby and Gillies, 2004, 2007). A common adaptation strategy is the dependence upon glycolytic metabolism, coined the 'Warburg Effect' (Gatenby and Gillies, 2004; Warburg, 1956). In cancer cells, a large portion of glucose is utilized through glycolysis, by which each glucose molecule is converted to two ATP and two lactic acid molecules. In contrast, normal cells obtain the majority of their ATP through oxidative phosphorylation, which results in the release of 36 ATP from one glucose molecule (Gatenby and Gillies, 2004; Vander Heiden et al., 2009). While less efficient in ATP production, glycolysis generates intermediate molecules as substrates for nucleotide, lipid and amino acid biosynthesis, which is crucial for rapidly dividing cancer cells (Vander Heiden et al., 2009). It is proposed that the acquisition of glycolytic metabolism offers a

selective advantage for cancerous cells, allowing them to adopt a more malignant phenotype (Gatenby and Gillies, 2004; Vander Heiden et al., 2009).

In addition to the noted alteration in the mode of energy acquisition, hypoxia is also known to regulate gene expression of cells. To proliferate and thrive in a hypoxic environment, cancer cells must modulate numerous cellular pathways. For example, pathways that initiate the acquisition of a motile and invasive phenotype, such as the c-Met pathway (Eckerich et al., 2007), are activated to facilitate cancer cells to leave the primary, hypoxic tumor (Hanahan and Weinberg, 2011). It has been discovered that hypoxia-inducible factor 1 (HIF-1) is a master regulator of many of the pathways that allow cancer cells to thrive in a hypoxic environment (Bertout et al., 2008; Semenza, 2007a, b). HIF-1 is reported to control the transcription of many genes, including those needed for maintaining cell viability, vascularization, glucose uptake, and metabolic reprogramming. HIFs are known to regulate pro-angiogenic and pro-glycolytic pathways. In animal models, HIF-1 overexpression has been associated with invasion, tumor growth and increased vascularization. Furthermore, HIF-1α overexpression has been correlated with an increase in patient mortality (Rankin and Giaccia, 2008; Semenza, 2007a). HIF proteins have also recently been attributed to the survival and self-renewal of cancer stem cells (CSCs), which are involved in cancer cell propagation and the development of aggressive and metastatic phenotypes (Heddleston et al., 2010; Wang et al., 2011). The Notch and Oct4 pathways, responsible for maintaining the stem cell phenotype, have been reported to be under the regulatory control of HIF protein. Due to the immense involvement of HIF-1 in transcriptional regulation of genes that promote survival and progression of cancer cells in a hypoxic environment, it serves as a target of anti-cancer therapies (Semenza, 2007a; Tennant et al., 2010).

Another effect of hypoxia lies in the resistance of cancer cells to chemotherapy and radiation treatment (Cairns et al., 2006; Gatenby and Gillies, 2004). Oxygen is known to increase the effectiveness of radiation therapy as it is a potent radiosensitizer. In turn, hypoxia can invoke a resistance of cancer cells to radiation and some forms of chemotherapy (Cairns et al., 2006). This hypoxia-induced resistance can be attributed, among many factors, to an inability in chemotherapy and radiation to induce cell cycle arrest, DNA breaks, and apoptosis (Wilson and Hay, 2011). Furthermore, hypoxia can up-regulate the expression of genes known to cause resistance to chemotherapeutics, such as multidrug resistance gene (MDR1), and downregulate the expression of apoptosis regulating genes (Bertout et al., 2008).

2.4 Acidosis

As discussed above, cancer cells develop a modified form of energy metabolism in which glucose incorporated by the tumor is mainly converted into ATP and lactic acid through glycolysis even in the presence of oxygen (Gatenby and Gillies, 2004; Vander Heiden et al., 2009; Warburg, 1956). In addition, it is believed this switch to a glycolytic phenotype, although inefficient in ATP production, is overall beneficial for rapidly dividing cancer cells (Cairns et al., 2011; Vander Heiden et al., 2009). However, the glycolytic metabolism directly results in the development of acidic interstitial pH in the tumor microenvironment, another stress that cancer cells must evolve to evade.

Acidosis is another defining hallmark of the tumor microenvironment. Interstitial accumulation of hydrogen ions is due to the production of lactic acid from glycolysis and

other proton sources from, such as, ATP hydrolysis and carbonic acid (Gatenby and Gillies, 2004; Helmlinger et al., 2002; Yamagata et al., 1998). Whereas the intracellular pH of cancer cells is kept neutral, an extracellular pH of 6.5-6.8 is often observed in the interstitial space of tumors (Griffiths et al., 2001). To maintain a relatively neutral intracellular pH, cancer cells utilize an array of acid-base transporters, such as sodium/hydrogen exchangers, vacuolar-type H^+-ATPases, and monocarboxylate transporters, to extrude the excess protons from cancer cells (Izumi et al., 2003; Webb et al., 2011).

Just as the ability for tumor cells to adapt to a hypoxic microenvironment offers a distinct evolutionary advantage towards an aggressive phenotype, so does the ability for cancer cells to survive in an acidic microenvironment. Upon exposure to the low extracellular pH found in and around solid tumors, many of the normal, non-cancerous cells in the surrounding tissue undergo cell death, often attributed to p53-dependent pathways. Cancer cells that have evolved to be immune to this notable acidosis are often left highly invasive and aggressive (Gatenby and Gillies, 2004). Acidosis is also known to contribute towards tumor cell invasion through the release of proteolytic enzymes that degrade extracellular matrix. Hypoxia and acidosis have been reported to increase the secretion and activity of matrix metalloproteinases (MMPs) and other matrix-degrading enzymes (Bourguignon et al., 2004; Johnson et al., 2000; Ridgway et al., 2005).

Acidosis also plays a role in the cytotoxic effectiveness of radiation and chemotherapy. Microenvironmental acidosis has been shown to invoke a resistance to radiation-induced apoptosis of cancer cells (Hunter et al., 2006). Acidic extracellular pH can also modulate the uptake of chemotherapeutic drugs, especially the weak acid and weak base drugs (Cairns et al., 2006; Gerweck et al., 2006). In the acidic tumor microenvironment, weak base drugs, such as doxorubicin, exist in a highly charged state. In turn, the uptake of these drugs across the plasma membrane is inhibited, thereby reducing the ability of the chemotherapeutic drugs to reach their cytotoxic target. In contrast, weak acid drugs, such as chlorambucil, exist in a non-charged state at acidic pH and, therefore, have increased cell permeability.

2.5 Somatic evolution and cancer cell metastasis

As normal cells are transformed to pre-malignant tumor cells and further towards malignant and metastatic tumors, the process of somatic evolution is actively used (Gatenby and Gillies, 2004). Cancer cells arise through gene mutations. Oncogenes with a dominant gain of function arise, while tumor suppressor genes become inactivated through a loss of function. As a result of somatic evolution, some of the traits that promote the cancerous phenotype include the evasion of apoptosis, limitless replicative potential, sustained angiogenesis, the ability for invasion and metastasis, and deregulated energy metabolism (Hanahan and Weinberg, 2011).

Carcinogenesis and Darwinian dynamics draw an analogy as new phenotypes are generated through heritable genetic changes and subsequent selection for the fittest by the environment (Gatenby and Gillies, 2004, 2008). It has been proposed that hypoxia and acidosis both apply extreme constraints and act as selection forces for progressive tumor cells. Cancer cells that have gained immunity to these conditions, such as through the Warburg effect and other adaptations, display a distinct advantage over neighboring normal cells. Cancer cells that are able to thrive in the harsh environment are highly aggressive with

a resistant phenotype. Communication between tumor cells and the microenvironment is crucial for the cells to take advantage of the changes in the microenvironment and develop a malignant phenotype (Gatenby and Gillies, 2004; Lorusso and Ruegg, 2008).

There are many mechanisms by which cancer cell somatic evolution is driven by the microenvironmental selection forces. Primarily, various pathways crucial for cancer cell survival under the hypoxic and acidotic conditions are activated, such as those that promote a downregulation of apoptosis, a switch to glycolytic metabolism, and an upregulation of HIFs (Gatenby and Gillies, 2004; Heddleston et al., 2010; Vander Heiden et al., 2009; Wilson and Hay, 2011). Cancer cells resistant to acidosis and hypoxia often acquire p53 mutations. As p53 is important for apoptosis, cells that have a mutation in this gene exhibit an advantage as they are often immune to the cytotoxic microenvironment (Bertout et al., 2008). In addition, many tumor cells develop a very active sodium/hydrogen exchange system and other proton transport mechanisms, which facilitate the extrusion of excess protons (Izumi et al., 2003; Webb et al., 2011). The resulting cytoplasmic alkalinization is thought to be crucial for cell reproduction in the acidic environment. Therefore, the hallmarks of the tumor microenvironment, such as hypoxia and acidosis, actively function as selection forces to shape cancer cell phenotypes during the somatic evolution process (Gatenby and Gillies, 2004; Webb et al., 2011).

The ability to acquire a metastatic phenotype via somatic evolution is one of the most devastating properties of cancer cells, and is directly correlated with an increase in patient morbidity and mortality (Fidler, 2002; Steeg, 2006). Current cancer therapy approaches, such as surgery, radiation and chemotherapy, can be effective in controlling primary, localized tumor. However, these modes of treatment are severely limited in retarding the spread of cancer as they do little to impair metastasis. It is therefore evident that the development of novel means of combating tumor cell metastasis is crucial towards the eradication and control of this disease.

The general steps of tumor metastasis involve the initial acquisition of motility and invasiveness, intravasation, transit in the blood or lymph, extravasation and finally arrest and growth at a new site (Fidler, 2002; Sahai, 2007; Steeg, 2006). The tumor microenvironment plays a large role in the ability of cancer cells to acquire a metastatic phenotype. As previously touched upon, two of the defining characteristics of the tumor microenvironment, hypoxia and acidosis, both actively select for more invasive and metastatic phenotypes (Chang et al., 2011; Gatenby et al., 2006; Gatenby and Gillies, 2004). It is also reported that inflammatory cells, fibroblasts and other stromal cells in the tumor microenvironment can contribute to the progression of a tumor towards a more malignant, metastatic phenotype (Joyce and Pollard, 2009; Lorusso and Ruegg, 2008).

3. Tumor microenvironments as targets for cancer prevention and therapy

Cellular components and molecular pathways associated with tumor microenvironments have been exploited as targets for cancer prevention and therapy. In fact, combination therapy targeting both cancer cells and other related cells and pathways, such as vascular cells and immune cells, can lead to more effective cancer treatment (Cairns et al., 2006; Ferrara and Kerbel, 2005; Luo et al., 2009). Therapeutic approaches modulating angiogenesis, inflammation, and hypoxia and acidosis pathways will be discussed below.

3.1 Anti-angiogenesis cancer therapy

As described in the 'angiogenesis' session, tumors rely on angiogenesis to grow and disseminate. Therefore, it has been proposed that tumor growth can be inhibited by starving tumor cells through angiogenesis blockade (Folkman, 1971). Since VEGF is a major regulator of tumor angiogenesis, a number of agents targeting VEGF and its receptors have been developed and several have been approved by the Food and Drug Administration (FDA) for clinical applications. Among them, bevacizumab (Avastin, Genentech/Roche) is a humanized monoclonal antibody directly against VEGF (Ferrara et al., 2004; Presta et al., 1997), and sunitinib (Sutent, Pfizer) and sorafenib (Nexavar, Bayer) are small molecule inhibitors that target multiple receptor tyrosine kinases (RTK), including VEGF receptors and PDGF receptors (Faivre et al., 2007; Kupsch et al., 2005; O'Farrell et al., 2003).

Bevacizumab was approved by the FDA in 2004 on the basis of the survival benefit observed in a randomized phase III clinical trial, in which bevacizumab was administrated in combination with chemotherapy in patients with previously untreated metastatic colorectal cancer (Hurwitz et al., 2004). The clinical benefit of bevacizumab was also evaluated in other cancer types. The combination of bevacizumab with paclitaxel and carboplatin in patients with previously untreated nonsquamous non-small-cell lung cancer (NSCLC) improved primary endpoint of overall survival (OS) (Sandler et al., 2006). Moreover, the combined regimen of bevacizumab with 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) were used to treat patients with previously treated metastatic colorectal cancers and prolonged progression-free survival (PFS) and OS (Giantonio et al., 2007). More recently, bevacizumab monotherapy was approved as a second-line therapy for glioblastoma multiforme (GBM) (Cohen et al., 2009). Some severe adverse effects of bevacizumab therapy, including gastrointestinal perforation and arterial thromboembolic complications, were observed in a small percentage of patients. Other side effects such as hypertension were also noticed (Eskens and Verweij, 2006; Verheul and Pinedo, 2007). Notably, in addition to the oncologic application, VEGF inhibitors are also used to treat the neovascular (wet) age-related macular degeneration (AMD), as VEGF has been demonstrated to be a mediator of ischemia-induced intraocular neovascularization (Chen et al., 1999; Ferrara et al., 2006; Gragoudas et al., 2004; Ng et al., 2006; Rosenfeld et al., 2006).

Sunitinib and sorafenib are RTK inhibitors that inhibit the tyrosine phosphorylation of VEGFRs, PDGFRs, c-kit, and Flt-3 (Fabian et al., 2005; Smith et al., 2004). Sunitinib has been reported to prolong the time to progression in imatinib-refractory gastrointestinal stromal tumors (Goodman et al., 2007). Sunitinib was also approved by FDA for the treatment of metastatic renal cell carcinoma (Motzer et al., 2009; Motzer et al., 2006). Sorafenib has been shown to increase PFS in patients with metastatic renal cell carcinoma (Escudier et al., 2007). In addition, sorafenib was approved for treating hepatocellular carcinomas (Lang, 2008; Llovet et al., 2008). Pazopanib, another RTKI, was approved for the treatment of metastatic renal cell carcinoma (Sternberg et al., 2010). Moreover, there are other anti-angiogenic agents under investigation that target other signaling molecules involved in angiogenesis, such as antibodies against angiopoietin-2 and PlGF which have been shown to delay tumor growth in preclinical models (Fischer et al., 2007; Oliner et al., 2004).

There were also some clinical trials with angiogenesis inhibitors that did not show significant clinical benefits. For instance, the combination of bevacizumab with gemcitabine didn't show improved PFS or OS in patients with chemotherapy-naïve advanced pancreatic

cancer (Kindler et al., 2010). An earlier phase III trial of bevacizumab combined with gemcitabine and erlotinib for the same type of cancer increased PFS but didn't improve primary endpoint of OS either (Van Cutsem et al., 2009). PFS, although an indicator of the efficacy of drugs, is a poor surrogate for OS, as PFS benefits are not always translated into OS benefits (Wilkerson and Fojo, 2009). The controversies about whether to keep the FDA's approval of bevacizumab for metastatic breast cancer provide such an example. In 2008, bevacizumab was approved for treating breast cancer in combination with chemotherapy based on the results from the clinical trial E2100 (Miller et al., 2007). However, the study was only able to show an improved primary endpoint of PFS but not OS. Several subsequent clinical trials showed similar results (Miles et al., 2010; Robert et al., 2011b), which leads to recent recommended withdrawal of bevacizumab by FDA for metastatic breast cancer (Lenzer, 2011).

Another major problem is that, even in those successful trials, the VEGF pathway inhibitors can only generate transitory clinical responses in most patients, with increased survival typically measured in months (Kerbel, 2008). Almost inevitably, temporary tumor shrinkage or stasis was followed by tumor relapse and progression. The modest responses to the anti-angiogenic agents are partly due to the existence of resistance to the therapeutics (Bergers and Hanahan, 2008). Tumor cells and the stroma they reside in can adapt to the presence of angiogenesis inhibitors by acquiring means to evade angiogenesis blockade and sustain blood vessel and tumor growth. One important compensatory response is that, when the VEGF signaling pathway is inhibited, tumor may activate or up-regulate the expression of alternative pro-angiogenic factors, such as FGF2, PlGF, and PDGF pathways (Fernando et al., 2008); or in other cases, there are pre-existing redundant pro-angiogenic factors in the treated tumors (Bergers and Hanahan, 2008).

Furthermore, a growing list of studies indicates that anti-angiogenic therapies may even lead to increased tumor invasiveness and metastasis (Ebos et al., 2009; Paez-Ribes et al., 2009). Questions and concerns have been raised about the efficacy and safety of antiangiogenic agents in blocking different stages of tumor progression. Potential mechanisms of the increased metastasis may involve both tumor-dependent and host-mediated responses (Ebos and Kerbel, 2011), such as increased expression of pro-metastatic proteins (Pennacchietti et al., 2003; Rofstad and Halsor, 2002), induction of tumor cell EMT (Higgins et al., 2007), and pericyte dysfunction (Bergers et al., 2003), among others. Perhaps the most prevailing proposition is that inhibition of angiogenesis could elicit an elevated level of tumor hypoxia, which selects for cancer cell populations that are able to grow in low oxygen environments and promote tumor invasion and metastasis (Rapisarda and Melillo, 2009). Studies have demonstrated that hypoxia-induced mechanisms, such as the up-regulation of c-Met and interleukin 8 (Pennacchietti et al., 2003; Rofstad and Halsor, 2002; Steeg, 2003), can promote cancer cells to disseminate to distant locations (Kienast et al., 2010).

As a breakthrough in cancer treatment, anti-angiogenesis therapies have provided survival benefits in certain cancer types and represent an important complement to the traditional chemotherapy strategies. However, there are ongoing challenges, such as the lack of lasting benefits for the majority of patients and an emerging, though still controversial, possibility that increased tumor invasion and metastasis might in some instances be induced by anti-angiogenesis therapy. It will be of importance to understand the molecular basis of the

treatment limitations and to formulate improved strategies to overcome them. For example, combining anti-angiogenic therapy with anti-hypoxia agents or anti-metastatic agents might help overcome the metastatic phenotype induced by increased tumor hypoxia.

3.2 Anti-inflammation in cancer chemoprevention and therapy

Numerous studies show that inflammation plays an important role in cancer initiation, progression and metastasis. As described in the Section 2, infiltrating inflammatory cells such as macrophages, mast cells and neutrophils produce reactive oxygen and nitrogen species, growth factors, cytokines, chemokines, proteases and other bioactive factors in the tumor microenvironment (de Visser et al., 2006; Grivennikov et al., 2010). These bioactive factors can induce DNA mutagenesis, inhibit DNA repair enzymes, stimulate cancer cell proliferation, degrade extracellular matrix, and promote cancer cell invasion and metastasis. On the other hand, certain types of infiltrating immune cells, such as cytotoxic T cells and natural killer cells, can inhibit tumor progression.

Chronic inflammation is closely associated with the development of some types of cancers. For instance, patients with inflammatory bowel disease or ulcerative colitis have an increased risk of developing colorectal cancer (Xie and Itzkowitz, 2008). Chronic *Helicobacter pylori* infection and ulcers are associated with gastric cancer and mucosa-associated lymphoid tissue lymphoma. Infection with hepatitis B virus increases the risk of developing liver cancer (Pages et al., 2010). Furthermore, epidemiological studies indicate that the use of aspirin and other nonsteroidal anti-inflammatory drugs is associated with a reduced cancer incidence. These widely documented observations provide the rationale to assess anti-inflammatory agents in cancer chemoprevention and therapy (Kashfi, 2009).

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been extensively evaluated for cancer chemoprevention. Aspirin (acetylsalicylic acid), a prototype NSAID, inhibits the cyclooxygenase (COX) enzymes and suppress the production of prostaglandins and thromboxanes. Aspirin exhibits chemopreventive effects on colon cancer in several randomized trials. A recent study performed a 20-year follow-up of 5 randomized trials to investigate the long-term effects of aspirin (75-300 mg daily) on colorectal cancer incidence and mortality (Rothwell et al., 2010). 391 of 14,033 patients (2.8%) had colorectal cancer during a median 18.3-year follow-up. Compared to the control group, the incidence of colon cancer, but not rectal cancer, was lower in the aspirin group. Furthermore, the benefit of aspirin increased with the duration of treatment. Allocation to aspirin treatment for 5 years or longer reduced the risk of proximal colon cancer by ~ 70% and also decreased the risk of rectal cancer. Moreover, a meta-analysis of 3 randomized controlled trials showed that, in more than 2,000 patients with previously resected colorectal adenomas, the aspirin groups (dose range from 81 to 325 mg per day), in comparison to the placebo groups, had a lower rate of tumor recurrence (Gao et al., 2009). In addition to colorectal cancer, a recent study showed that daily aspirin treatment for 5 years or longer reduced the mortality from several common types of solid cancers (Rothwell et al., 2011). However, not all studies supported the protective role of aspirin in cancer prevention. In the Woman's Health Study, a low dose (100 mg) of aspirin every other day for an average 10 years of treatment did not reduce the risk of total, breast, colorectal, and other cancers (Cook et al., 2005). The major adverse effects of aspirin treatment are gastrointestinal bleeding and ulceration.

In addition to aspirin, COX-2 specific inhibitors have been tested as cancer chemopreventive agents. There are two COX genes in the cell: COX-1 is constitutively expressed in many

tissues and COX-2 is induced upon inflammation and other stimuli (Botting, 2010). Earlier clinical studies showed that the COX-2 inhibitor, celecoxib (400 mg twice per day for 6 month), resulted in approximately 30% reduction in polyp number and size in familial adenomatous polyposis (FAP) patients (Steinbach et al., 2000). However, studies found that prolonged use of COX-2 inhibitors increased the risk of cardiovascular events in a dose-related manner (Baron et al., 2008; Solomon et al., 2005; Solomon et al., 2006). These findings led to the discontinuation of the clinical trials and the withdrawal of the drugs from the market. Clearly, drugs with improved safety profiles are required for the long-term use in cancer chemoprevention. In this respect, the "old" NSAID, aspirin, has been widely used to prevent cardiovascular diseases and, therefore, has a favorable cardiovascular profile.

Inflammatory chemokines and their receptors have also been exploited as targets for cancer therapy. Chemokines are a family of chemotactic cytokines that bind to cognate G protein-coupled receptors. Forty-seven chemokine members have been identified and are divided into 4 subfamilies: the CC subfamily, the CXC subfamily, the CX3C subfamily, and the XC subfamily. The chemokine receptors are comprised of the CCR subfamily, the CXCR subfamily, CX3CR1, and XCR1 (Lazennec and Richmond, 2010). In the tumor microenvironment, various chemokines are produced by cancer cells, inflammatory cells, fibroblasts, and endothelial cells. Chemokines can stimulate cancer cell growth and metastasis, recruit inflammatory cells, and promote tumor angiogenesis.

Stromal cell-derived factor-1 (SDF-1, also named CXCL12) and its receptor CXCR4 play important roles in the mobilization and homing of hematopoietic stem cells and the metastasis of cancer cells. AMD3100 (plerixafor), developed by Genzyme, is a small molecule that antagonizes the binding of CXCL12 to its receptor CXCR4, and was recently approved by FDA for stem cell mobilization in non-Hodgkin's lymphoma and multiple myeloma patients (Pusic and DiPersio, 2010). Clinical trials demonstrated that AMD3100 (plerixafor), together with G-CSF, significantly increased the mobilization of hematopoietic stem cells for autologous stem cell transplantation in patients with multiple myeloma and non-Hodgkin's lymphoma (DiPersio et al., 2009a; DiPersio et al., 2009b). AMD3100 (plerixafor) can also be used as a chemosensitizing agent. Studies showed that AMD3100 (plerixafor) impeded the interaction between leukemia cells and the bone marrow microenvironment, mobilized cancer cells into peripheral blood, and increased the sensitivity of multiple myeloma cells and acute myeloid leukemia cells to chemotherapeutic drugs (Azab et al., 2009; Nervi et al., 2009). Furthermore, CXCL12/CXCR4 inhibitors have therapeutic effects on other non-hematological cancers. In pre-clinical models, blockade of CXCR4 has been shown to inhibit the migration and metastasis of melanoma cells, oral squamous cell carcinoma cells, and gastric cancer cells (Kim et al., 2010; Uchida et al., 2011; Zhao et al., 2011). CXCR4 inhibitors could also chemosensitize and suppress the growth of pancreatic and ovarian cancer cells (Righi et al., 2011; Singh et al., 2010). Besides CXCL12/CXCR4, inhibitors for other chemokines/receptors, such as CCL2 and CCR4, are also undergoing clinical development for the treatment of leukemia and solid tumors (Lazennec and Richmond, 2010).

3.3 Molecular targeting of hypoxia pathways

As described in the Section 2, hypoxia inhibits the tumor killing effects of radiation and also regulates cancer cell apoptosis, invasiveness and metabolism (Cairns et al., 2006; Gatenby

and Gillies, 2004). Hypoxia-inducible factors (HIFs) are master regulators of cell hypoxia responses and control the expression of numerous genes involved in angiogenesis, glycolytic metabolism, glucose transport, erythropoiesis, and other processes (Semenza, 2007b). Increased expression of HIFs is correlated with a worse prognosis in many types of cancers. Thus, HIFs have been proposed as a potential target for cancer therapy. Inhibitors of the HIF pathway have been developed and tested in preclinical models and/or clinical trials (Semenza, 2007a).

PX-478, a HIF-1α inhibitor, showed remarkable antitumor activity in human cancer xenograft models (Welsh et al., 2004), and also enhanced radiosensitivity of human pancreatic cancer xenografts (Schwartz et al., 2009). It has been shown that the degradation of HIF-1α can be induced through inhibiting the chaperone protein HSP90 (Isaacs et al., 2002). The HSP90 inhibitor, 17-AAG (Tanaspimycin), exhibited anti-tumor activities in multiple cancer cell models. A recent phase II clinical trial showed that 17-AAG (Tanaspimycin) plus trastuzumab had significant anti-cancer activity in $HER2^+$ breast cancer patients previously progressing on trastuzumab (Modi et al., 2011). It should, however, be noted that HIF-1α degradation is only one of the effects of HSP90 inhibitors as HSP90 is required for preventing the degradation of many other proteins.

As a response and adaptation to hypoxia in the tumor microenvironment, cancer cells rely substantially on glycolysis for ATP production. It was discovered by Otto Warburg decades ago that cancer cells preferentially utilize glycolytic metabolism even under aerobic conditions (known as 'Warburg effect') (Warburg, 1956). Since glycolysis only generates 2 ATP molecules per glucose, tumor cells evolve a compensatory mechanism by up-regulating the level of glucose transporters and significantly increasing glucose uptake.

Genes involved in glycolytic metabolism have been proposed as potential cancer therapeutic targets (Tennant et al., 2010). Studies demonstrate that HIFs directly activate the transcription of glucose transporters (e.g. GLUT1) and several key glycolytic enzymes (Semenza, 2007b). Some oncogenes and tumor suppressors, such as Myc, PI3K, p53 and PTEN, can also regulate the expression of genes important for glycolysis and cell metabolism (Dang et al., 2009; Tennant et al., 2010). A recent chemical screening identified a compound, STF-31, which could inhibit the glucose transporter 1 (GLUT1) and the Warburg effect, induce cell death of renal cell carcinoma and inhibit the growth of tumor xenografts (Chan et al., 2011). Agents have also been developed to target the enzymes in the glycolysis pathway. One such agent is the hexokinase inhibitor, 2-deoxyglucose, which has been shown to have anti-tumor activities in multiple cancer cell models (Loar et al., 2010; Zhang and Aft, 2009). Other potential anti-cancer targets in the glycolysis cascade includes pyruvate kinase, the tumor-specific pyruvate kinase M2 (PKM2) isoform, pyruvate dehydrogenase kinase 1 (PDK1), among others (Tennant et al., 2010; Vander Heiden et al., 2009). Inhibitors of these enzymes, such as TLN-232 and dichloroacetate (DCA), are being evaluated in clinical trials. A recent study showed that DCA had anti-tumor activities in glioblastoma patients (Michelakis et al., 2010).

Furthermore, major regulators of metabolic pathways, such as mTOR (mammalian target of rapamycin) and AMPK (AMP-activated protein kinase), have been employed as important targets for cancer therapy. In particular, the mTOR inhibitors, everolimus and temsirolimus, have been approved by FDA for the treatment of advanced renal cell carcinoma with clinical

benefits in prolonging progression free survival and overall survival (Kwitkowski et al., 2010; Motzer et al., 2008). Moreover, epidemiological studies indicated that the cancer incidence is lower in diabetic patients treated with the AMPK agonist metformin (Bo et al., 2011). In a short-term prospective clinical trial, metformin treatment reduced colorectal aberrant crypt foci in non-diabetic patients (Hosono et al., 2010). These results indicate that metformin may be a useful agent for cancer prevention and therapy (Gonzalez-Angulo and Meric-Bernstam, 2010; Li, 2011).

In addition to targeting hypoxia-related molecular pathways, hypoxia itself can be utilized for cancer therapy. Bioreductive prodrugs, which are activated in hypoxic environments, have been evaluated as potential anti-cancer agents to selectively kill hypoxic tumor cells. For instance, apaziquone (EO9), a bioreductive prodrug, was used through instillation to treat bladder cancer and significantly reduced the rate of tumor recurrence in the clinical trials (Hendricksen et al., 2009; Jain et al., 2009).

Hypoxia as a hallmark of the tumor microenvironment has also been exploited to develop imaging approaches for cancer diagnosis. A family of nitroimidazole derivatives has been used as chemical tracers to detect hypoxia in tissues. A PET probe, ^{18}F-fluoromisonidazole (FMISO), has been applied to detect hypoxic regions in solid tumors and to assess the change of tumor hypoxia status in response to therapy such as anti-angiogenesis treatment (Szeto et al., 2009; Valable et al., 2011). In addition, the up-regulation of hypoxia-responsive glucose transporters in tumor cells has been utilized for [^{18}F] fluorodeoxyglucose positron emission tomography (FDG-PET). As the uptake of glucose is substantially increased in cancer cells, the radioactive [^{18}F] fluorodeoxyglucose tracer is preferentially accumulated in the tumor and can be detected by positron emission tomography (Buerkle and Weber, 2008). FDG-PET has been widely used in the clinic to detect tumors and metastases and assess the response of tumors to therapeutics.

3.4 Molecular targeting of acidosis-related pathways

In addition to hypoxia, extracellular acidosis is another major hallmark of the tumor microenvironment. To avoid the harmful accumulation of protons and decrease of intracellular pH (pHi), cancer cells must use the cellular transporter system to expel excess acids from the cells. Carbonic anhydrases (CA), monocarboxylate transporters (MCT), vacuolar-type H^+-ATPase proton pump (V-ATPase), and sodium/hydrogen exchangers (NHE) play important roles in cellular pH regulation (Izumi et al., 2003; Swietach et al., 2010; Webb et al., 2011). Furthermore, recent studies have shown that a family of proton-sensing G protein-coupled receptors regulates the behavior of tumor cells, immune cells, and blood vessels (Ludwig et al., 2003; Mogi et al., 2009; Yang et al., 2007). Targeting these pH regulators may be utilized to kill cancer cells or to augment the effects of other anti-cancer agents.

Carbonic anhydrase (CA) enzymes catalyze the reversible reaction between carbon dioxide and bicarbonate: $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$, and facilitate the transport of CO_2 and H^+ ions for pH regulation (Swietach et al., 2010). There are 15 CA isoforms, I to XV, in mammalian cells, located in the cytosol, mitochondrion, extracellular plasma membrane, or secreted. In particular, the isoform IX (CAIX) has been extensively studied in cancer biology (Swietach et al., 2010). The expression of CAIX is strongly induced by hypoxia and regulated by HIF-1.

CAIX has been used as an endogenous marker to delineate hypoxic regions in solid tumors and is a prognostic marker of aggressive cancers. Inhibition of CAIX has been shown to suppress tumor growth in xenograft models (Chiche et al., 2009). CAIX small molecule inhibitors and antibodies have been developed and evaluated as potential anti-cancer therapeutics. A CAIX monoclonal antibody exhibited anti-tumor activities in the mouse xenograft model of colorectal cancer (Zatovicova et al., 2010). Moreover, CAIX inhibitors were shown to increase the therapeutic effects of tumor radiation (Dubois et al., 2011).

Monocarboxylate transporters (MCT) facilitate the efflux of lactate and protons from cells. The up-regulation of MCTs has been observed in a variety of tumors such as breast, colorectal, ovarian, prostate, and central nervous system carcinomas, and associated with cancer progression and poor prognosis in some instances (Fang et al., 2006; Froberg et al., 2001; Pertega-Gomes et al., 2011; Pinheiro et al., 2010; Pinheiro et al., 2008). MCT1 and MCT4 were related to the invasiveness of human lung cancer cells and drug resistance of ovarian cancer cells (Chen et al., 2010; Izumi et al., 2011). Inhibition of MCT1 could retard the growth of cancer cells in culture and animal models (Fang et al., 2006; Sonveaux et al., 2008). MCTs may, therefore, represent potential targets for cancer treatment.

Vacuolar-type H^+-ATPases (V-ATPases) are multi-subunit, complex enzymes that transport protons from the cytoplasm to vacuolar lumens or to extracellular space (Izumi et al., 2003). These proton pumps are important for maintaining intracellular and extracellular pH homeostasis. V-ATPases overexpression has been detected in many types of tumors such as oral squamous cell cancer and melanoma (Nishisho et al., 2011; Perez-Sayans et al., 2010). Studies showed that V-ATPases increased cancer metastasis and drug resistance (Nishisho et al., 2011; You et al., 2009). Inhibition of V-ATPases by small molecule inhibitors or small interfering RNA suppressed tumor growth and metastasis, induced tumor cell apoptosis, and overcame chemoresistance in several cancer models (De Milito et al., 2007; Lu et al., 2005; Nishisho et al., 2011; You et al., 2009).

Sodium/hydrogen exchangers (NHE) regulate the pH homeostasis of cells by extruding intracellular H^+ in exchange of extracellular Na^+ at a 1:1 ratio. Nine NHE isoforms have been identified in mammalian cells (De Vito, 2006). NHEs were found to regulate cytoskeletal structures and tumor cell migration and invasion (Paradiso et al., 2004). Treatment with NHE1 inhibitors sensitized the paclitaxel-induced apoptosis of human breast cancer cells (Reshkin et al., 2003). Moreover, increased activity of NHE was observed in doxorubicin-resistant human colon cancer cells and the treatment with the NHE inhibitor 5-(N-ethyl-N-isopropyl)-amiloride (EIPA) sensitized the resistant cells to doxorubicin (Miraglia et al., 2005). Inhibition of NHEs has also been shown to reduce the proliferation and VEGF production of leukemia cells (He et al., 2007; Turturro et al., 2007).

Proton-sensing G protein-coupled receptors (GPCRs), including GPR4, TDAG8 (GPR65), OGR1 (GPR68), and G2A (GPR132), can be activated by acidic extracellular pH to transduce multiple downstream signaling pathways such as the G_s/cAMP, G_q/phospholipase C/Ca^{2+}, and G_{13}/Rho pathways (Ludwig et al., 2003; Murakami et al., 2004; Radu et al., 2005; Tobo et al., 2007; Wang et al., 2004; Yang et al., 2007). Different from the proton transporters, the proton-sensing GPCRs do not directly transport protons but, instead, perceive acidic extracellular pH to trigger signal transduction. Potential roles of the proton-sensing GPCRs in cancer biology have been emerging, with differential roles for each family member in a cell context-dependent manner. Activation of GPR4 by acidic pH has been shown to inhibit

tumor cell migration, invasion and metastasis and suppress microvascular outgrowth (Castellone et al., 2011; Yang et al., 2007). Overexpression of OGR1 also inhibited the migration and metastasis of prostate cancer cells and the effects were attributed to the constitutive activity of the receptor but not pH sensing function (Singh et al., 2007). Overexpression of TDAG8, however, enhanced the development of lung cancer cells (Ihara et al., 2010). TDAG8, as well as GPR4, exhibited transforming activities when ectopically overexpressed in immortalized cell lines (Sin et al., 2004). Furthermore, the proton-sensing GPCRs have been shown to regulate immune cell function and inflammatory responses (Ichimonji et al., 2010; Mogi et al., 2009; Onozawa et al., 2011). These observations suggest that the proton-sensing GPCRs may represent novel targets for cancer treatment, inflammation inhibition, and chemoprevention.

Taken together, the acid-base transporters and proton-sensing receptors described above are important for cancer cells to sense and adapt to the acidic tumor microenvironment. Further research is warranted to validate these pH regulators as potential targets for cancer therapy and chemoprevention. Moreover, acidity itself in the tumor microenvironment can also be exploited for cancer detection and treatment. Recent studies showed that a technology using the pH low insertion peptide (pHLIP), a peptide that forms α-helix at acidic pH and inserts across cell membrane, could be applied to image prostate cancer xenografts in mice by positron emission tomography (Vavere et al., 2009). Acidity in the tumor microenvironment may also be utilized to design pro-drugs that are activated or become more potent at acidic pH to differentially kill cancer cells.

4. Concluding remarks

Cancer cells do not exist in isolation; instead, they closely interact with blood vessels, inflammatory cells, and fibroblasts in a unique tumor microenvironment characterized by hypoxia and acidosis. The interaction between cancer cells and the tumor microenvironment plays a pivotal role in cancer progression and somatic evolution, which follows very similar principles of Darwinian selection. It is increasingly recognized that in addition to killing cancer cells, targeting the components of the tumor microenvironment can help develop more effective approaches for cancer prevention and therapy. For instance, anti-angiogenesis therapy, combined with conventional chemotherapy, has shown significant clinical benefits in multiple cancer types (Ferrara and Kerbel, 2005; Kerbel, 2008). Furthermore, a number of agents targeting inflammation, cancer cell metabolism, and hypoxia and acidosis pathways have been developed and added to the arsenal for cancer treatment, detection, diagnosis, prognosis and chemoprevention.

While significant progress has been made to understand the tumor-microenvironment interaction, considerable knowledge gaps still remain. This aspect is exemplified by the lessons learned from anti-angiogenesis therapy. Whereas angiogenesis inhibitors have offered therapeutic benefits in cancer patients, some unexpected adverse effects deserve a close attention. In certain experimental settings, anti-angiogenesis therapy has been shown to promote tumor invasion and metastasis (De Bock et al., 2011; Ebos and Kerbel, 2011; Ebos et al., 2009). The underlying cause is largely attributed to the anti-angiogenesis therapy-induced hypoxia, which is known to stimulate cancer cell metastasis. These observations illustrate that cancer cells constantly evolve and adapt to the changing tumor microenvironment during therapeutic interventions and/or tumor development. In

addition to hypoxia, other microenvironmental factors, such as acidosis and low nutrients, are also important selection forces that have a significant impact on cancer cell somatic evolution. The experience of anti-angiogenesis therapy once again underscores the fact that tumors comprise not just cancer cells and these cancer cells are continuously evolving. It is necessary to target multiple cell components and molecular pathways (both cancer cell-intrinsic and microenvironment-related) in order to devise more effective strategies for the treatment and prevention of cancer.

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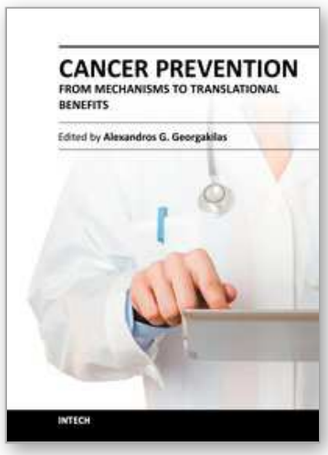

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