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Review

Hypoxia-induced carbonic anhydrase IX as a target for cancer therapy: From biology to clinical use

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ABSTRACT

The tumor microenvironment includes a complicated network of physiological gradients contributing to plasticity of tumor cells and heterogeneity of tumor tissue. Hypoxia is a key component generating intratumoral oxygen gradients, which affect the cellular expression program and lead to therapy resistance and increased metastatic propensity of weakly oxygenated cell subpopulations. One of the adaptive responses of tumor cells to hypoxia involves the increased expression and functional activation of carbonic anhydrase IX (CA IX), a cancer-related cell surface enzyme catalyzing the reversible conversion of carbon dioxide to bicarbonate ion and proton. Via its catalytic activity, CA IX participates in regulation of intracellular and extracellular pH perturbations that result from hypoxia-induced changes in cellular metabolism producing excess of acid. Through the ability to regulate pH, CA IX also facilitates cell migration and invasion. In addition, CA IX has non-catalytic function in cell adhesion and spreading. Thus, CA IX endows tumor cells with survival advantages in hypoxia/acidosis and confers an increased ability to migrate, invade and metastasize. Accordingly, CA IX is expressed in a broad range of tumors, where it is associated with prognosis and therapy outcome. Its expression pattern and functional implications in tumor biology make CA IX a promising therapeutic target, which can be hit either by immunotherapy with monoclonal antibodies or with compounds inhibiting its enzyme activity. The first strategy has already reached the clinical trials, whereas the second one is still in preclinical testing. Both strategies indicate that CA IX can become a clinically useful anticancer target, but urge further efforts toward better selection of patients for immunotherapy and deeper understanding of tumor types, clinical situations and synthetic lethality interactions with other treatment approaches.

1. Introduction

Development of targeted cancer therapies has always been linked with key advances in understanding biological phenomena that drive cancer growth and progression. Since the early era of oncogenes, the main focus has been given to the activated oncogenic pathways and their major players, which govern out-of-control oncogenic signaling and thereby provide cancer cells with selective proliferative and survival advantages. Recognizing the phenomenon of oncogenic addiction that connects genomic changes to aggressive tumor phenotype gave birth to a number of targeted drugs that are nowadays in routine clinical use, including well known Herceptin, Rituximab, and others [1]. Despite being often more effective and less toxic than conventional chemotherapy and radiotherapy, these drugs cannot cure all patients carrying tumors with corresponding molecular targets. Why is that?

One of the answers to this readily asked question is offered by the recently highly appreciated role of tumor microenvironment in cancer biology. Tumor microenvironment includes a complicated network of physiological gradients (of oxygen, nutrients, growth factors, glucose, metabolites, acid, interstitial pressure, etc.), diverse stromal cells that can crosstalk with each other and with cancer cells, paracrine molecular signals, etc. This “jungle” of factors and signals significantly contributes to plasticity of tumor cells and to intratumoral heterogeneity, which affects cellular expression programs, leads to co-selection of adaptable cells with different genomic backgrounds and endows these cell subpopulations with a range of phenotypic features that result in therapy resistance and increased metastatic propensity. These processes are also governed by important signaling pathways and involve

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molecular players that offer useful targets for novel anticancer strategies [2,3].

2. Hypoxia and beyond

Hypoxia (situation when the supply of oxygen to tumor cells is below their demand) is a key component of tumor microenvironment with strong effects on tumor phenotype and cancer progression. It develops in growing tumors due to the abnormal vasculature that is unable to maintain proper delivery of oxygen to all tumor cells. Depending on regional and temporal status of blood flow through tortuous vessels, hypoxia can vary from moderate to severe, acute to chronic, and intermittent to persistent, and can induce a spectrum of adaptive cellular responses, including glycolytic metabolism, reduced cell proliferation, decreased cell adhesion, increased migration and invasiveness, and increased angiogenesis. These processes result from robust molecular changes in the transcriptome and proteome of hypoxic cells, which induce genes and proteins that facilitate survival of cells, their escape from the primary tumor mass, and dissemination to secondary metastatic sites [4].

The hypoxia-induced transcriptional program is principally activated by the hypoxia-inducible factor (HIF), a master transcription factor consisting of two subunits, each existing in at least two isoforms. The HIF-1α subunit is sensitive to oxygen and is generally absent in well-oxygenated cells because of the negative control by the PHD–VHL pathway, which directs it to proteasome degradation. In hypoxic cells, PHD–VHL control is inactivated, HIF-α is stabilized, enters the nucleus and dimerizes with the constitutively expressed HIF-1β subunit. This results in the formation of the active transcription complex, which interacts with transcriptional co-factors and trans-activates numerous genes through binding to HIF-responsive elements (HREs) in their regulatory regions. Molecular mechanisms that regulate the HIF pathway (isoforms, posttranslational modifications, oncogenic signaling, etc.) are described in detail elsewhere [5,6]. Proteins expressed from the HIF-activated genes (such as glucose or lactate transporters, ion transporters, glycolytic enzymes, pro-angiogenic growth factors and receptors) execute the adaptive responses to hypoxia and are therefore active players in tumor progression. Indeed, some of these proteins are already clinically exploited as hypoxia-associated biomarkers and anticancer therapy targets [7]. Moreover, the relationship of hypoxia to poor response to conventional therapy has already led to the modification of treatment regimens and introduction of new predictive biomarkers to clinical practice [8,9].

Metabolic adaptation represents a canonical response to hypoxia, which includes a more or less dramatic shift toward the glycolytic metabolism that enables the sustained, although less efficient production of energy in conditions of reduced oxidative phosphorylation. This metabolic shift promotes the survival of hypoxic tumor cells. Moreover, glycolysis also provides substrates for biosynthetic reactions (e.g. nucleotides, amino acids, and lipids) and thereby facilitates cell proliferation in the process of tumor expansion. This is the reason why tumor cells maintain glycolytic metabolism even in the presence of oxygen [10,11]. In addition, hypoxia leads to selection of inherently glycolytic cells developed through oncogenic activation. Nevertheless, some tumor cells strongly rely on uptake of glutamine and glutaminolysis, which supports the mitochondrial TCA cycle and pentose phosphate pathway and thereby facilitates the synthesis of fatty acids, nonessential amino acids and nucleosides [12].

The oncogenic metabolism, which is usually composed of a variable proportion of respiration, glycolysis and glutaminolysis, leads to the excessive production of acidic metabolic products, including lactate, protons and carbon dioxide. The accumulation of these acidic metabolites in the cytoplasm results in intracellular acidosis, that is incompatible with survival and proliferation. Therefore, tumor cells activate mechanisms of pH control, that re-establish slightly alkaline intracellular pH at the expense of extracellular acidification, resulting in the reversed pH gradient in tumors versus normal tissues. This process includes activation of ion exchangers, pumps, and transporters, such as sodium-proton exchangers (NHE1), anion exchangers (AE2), sodium-bicarbonate transporters (NBCe1), monocarboxylate transporters (MCT4), etc., which are often pH-sensitive and in some cases can operate in inverse ion flux modes compared to those existing in normal cells. Regulation of intracellular pH is principally based on the export of lactate and protons and on the import of bicarbonate ions generated by the hydration of CO_2. Pericellular accumulation of the acidic metabolites exacerbated by the ineffective removal through the aberrant tumor vasculature generates acidosis, which can reinforce the impact of hypoxia through multiple effects of both biological and clinical relevance as reviewed in more detail elsewhere [13]. For example, extracellular acidosis can activate the proteolytic enzymes (some of them being transcriptionally induced by hypoxia) that degrade extracellular matrix and facilitate invasion of tumor cells across the basal membrane and through the surrounding normal tissue.

Another important adaptive response to hypoxia affects cell adhesion and induces epithelial–mesenchymal transition that initiates the metastatic cascade. Hypoxia activates expression of Snail and Slug transcriptional repressors of E-cadherin, thereby diminishing intercellular contacts [14]. At the same time, hypoxia increases expression of growth factors and/or their receptors including HGF and c-MET, cell surface proteolytic enzymes such as TACE/ADAM17, regulators of cell-matrix adhesion and cytoskeletal rearrangement such as FAK, intracellular kinases (PKA) and ion and water transporters (MCT4, NHE1, AQP), which then induce cell motility, migration and invasion [15].

All the above-described phenomena include either abnormal expression or the functional contribution of the enzyme called carbonic anhydrase IX (CA IX).

3. Carbonic anhydrase IX – basic facts

CA IX is one of the 15 human isoforms of the α carbonic anhydrase family [16], which are expressed in virtually all cells/tissues of the human body and participate in physiological processes that require water and ion transport and acid–base balance. These metalloenzymes catalyze the reversible hydration of carbon dioxide to bicarbonate ions and protons and their activity ranges from no/low to high. The diverse isoforms are present in various subcellular compartments and contribute to the production of gases, body fluids, bone resorption, and biosynthetic reactions [17]. Excluding CA IX, all CA isoforms are expressed mainly in specialized cells of the differentiated tissues, albeit some isoenzymes are also present in certain tumors, such as CA II in GIST and endothelium of brain tumors and CA XII in kidney, breast cancer, etc. [18–21].

Importantly, CA IX is not expressed in the majority of normal tissues. Actually, it is abundant only in the stomach and gallbladder epithelia [22]. On the other hand, it is very often and strongly expressed in tumors, generally in their more aggressive variants as described in more detail below. In the normal tissues, CA IX and CA XII principally do not overlap and although some tumors co-express CA IX and CA XII, CA XII is rather associated with less-aggressive, well-differentiated tumor phenotype [23–28].

CA IX protein is produced from the *CA9* gene placed on the 9p12–13 chromosomal locus and composed of 11 exons coding for distinct structural domains of the CA IX protein [29,30], see Fig. 1. Exon 1 encodes the N-terminal domain homologous to