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Voltage dependent anion channels (VDACs): a brief introduction with a focus on the outer mitochondrial compartment's roles together with hexokinase-2 in the "Warburg effect" in cancer

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Abstract In recent years there has been renewed interest and focus on mitochondria of animal and human tissues. This interest commenced in the latter part of the past century and has gained momentum during the first eight years of this new millennium. The well accepted reason is that mitochondria are now recognized to represent not only "power houses", i.e., the ATP production factories of tissues essential for cell life, but in response to a variety of different "cues" may participate significantly also in cell death, both that associated with normal turnover and that associated with disease. Conversely, in cancers (particularly the advanced) their mitochondria interact with hexokinase 2 (HK-2) resulting in suppression of cell death while supporting cell growth via enhanced glycolysis, even in the presence of oxygen (Warburg effect). The identification/elucidation of proteins and mechanisms involved in deciding and/or participating in cell fate (i.e., life, death, or cancer) has focused to a large extent on the mitochondrial outer compartment, which is taken here to collectively include the outer membrane, the space between the inner and outer membranes, and contact regions between these two membranes. Among the established proteins believed to be involved in events related to cell fate are "VDACs" that form the basis of this mini-review series. This brief introductory review focuses mainly on the past discovery by the author and colleagues that VDAC located within the outer mitochondrial compartment and its binding partner HK-2 are pivotal players in the "Warburg effect" in cancer. As one case in point, when glucose is added to liver cytosol (mitochondria-free) the rate of glycolysis is very low. However, upon addition of tumor mitochondria containing VDAC bound HK-2, the low glycolytic rate is increased to a high rate near that catalyzed by the tumor cytoplasm from which the tumor mitochondria were derived.

Keywords VDAC · Hexokinase-2 · Mitochondria · Cancer · Warburg effect · Glycolysis

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Introduction

In recent years mitochondrial VDACs (Colombini 2007; Shoshan-Barmatz et al. 2006; Lemasters and Holmuhamedov 2006), of which there are three isoforms (VDAC-1, VDAC-2, and VDAC-3), have received considerable attention because of their now well recognized or suspected roles in cell life, cell death, cancer, and other diseases. As natural mechanisms for inducing programmed cell death involve the mitochondria (Jiang and Wang 2004; Neuzil et al. 2006), in particular the outer compartment, it is not surprising that attention has focused on VDACs (Sampson et al. 1997) as these proteins (three isoforms) reside in this compartment and are recognized as ***v***oltage ***d***ependent ***a***nion ***c***hannels. Although the role(s) of VDACs are still being debated (Baines et al. 2007; Chiara et al. 2008), the interest in this family of outer membrane proteins continues to rise. Thus, this minireview series touches on recent developments in better understanding the structure–function relationships within VDACs as well as their physiological roles and roles in disease. Others leading contributors to this minireview series are Drs. S. Chan, M. Colombini and C. Stein, W. Craigen, V. DePinto, M. Gavish, J. Hoek and J Pastorino, C. Mannella and K. Kinnally, T. Rostovtseva, S. Scheuring, E.

Shimamura, and V. Shohan-Barmatz. Collectively their contributions together with colleagues should provide interested readers with an up-to-date overview about the structure, and functions of VDACs and their roles in health and disease.

Specifically, this introductory minireview focuses briefly on work in the author's laboratory as it relates to the role(s) of the outer mitochondrial membrane in cancer. At this location it remains a fact that hexokinase is found bound in those cancers (Rose and Warms 1967; Bustamante and Pedersen 1977; Bustamante et al. 1981) that exhibit a pronounced "Warburg effect", i.e., high glycolysis even in the presence of oxygen (Warburg 1930, 1956). Here, HK-2 contributes significantly to both the "Warburg effect" (Bustamante and Pedersen 1977; Bustamante et al. 1981) and the suppression of cancer cell death (Gottlob et al. 2001; Pastorino et al. 2002), i.e., immortalization. Also, all available evidence to date indicates that it is VDAC that participates in the binding of hexokinase to the outer mitochondrial membrane (Nakashima et al. 1986; Al Jamal 2005), although such evidence does not rule out the possible involvement in part of one or more other proteins. Below the author indicates why mitochondrial bound HK-2 is the most critical enzyme involved in the "Warburg effect" in cancer and how at this location it contributes in multiple ways to cancer cell growth.

Why mitochondrial bound HK-2 is the glycolytic enzyme most critical for the "Warburg effect" in cancer

The discovery that an isoform of hexokinase, later identified as HK-2 (Nakashima et al. 1988), is the most critical glycolytic enzyme involved in the Warburg effect in cancer was made in the author's laboratory 31 years ago (Bustamante and Pedersen 1977) and further confirmed in the same laboratory 4 years later (Bustamante et al. 1981). Specifically, in the latter study the author and his colleagues showed working with an ascites tumor cell line that addition of its mitochondrial bound hexokinase to liver cytosol is the only glycolytic enzyme addition necessary for this untransformed normal tissue to exhibit a high glycolytic rate in the presence of oxygen, i.e., a "Warburg effect". Subsequent studies (Nakashima et al. 1988) showed that hexokinase is the only glycolytic enzyme that exhibits significant binding to the mitochondria isolated from the AS-30D hepatoma cell line. These studies (Nakashima et al. 1988) showed also that the specific activity ratio (tumor hexokinase/liver hexokinase) is at least eight times higher than that of any other glycolytic enzyme.

In addition to the high amounts of mitochondrial bound HK-2 that are found associated with numerous malignant tumors, the result of HK-2's gene-related over-expression (Mathupala et al. 2006), there are other HK-2 related events that contribute directly and very significantly to the high production of its product glucose-6-phosphate, a major carbon precursor for all things large and small that must be synthesized in cancer cells. These other very important HK-2 related events are summarized briefly below.

Mitochondrial HK-2 related events that facilitate directly cancer cell growth

1. Mitochondrial Binding of HK-2 Enhances its Apparent Affinity for MgATP.

The HK-2 reaction involves three chemical agents, glucose, Mg^{++}, and ATP. As noted below, the Mg^{++} interacts with ATP to give MgATP, one of the two reaction substrates.

$$\text{Glucose} + \text{MgATP} \rightarrow \text{Glucose} - 6 - \text{P} + \text{MgADP}$$

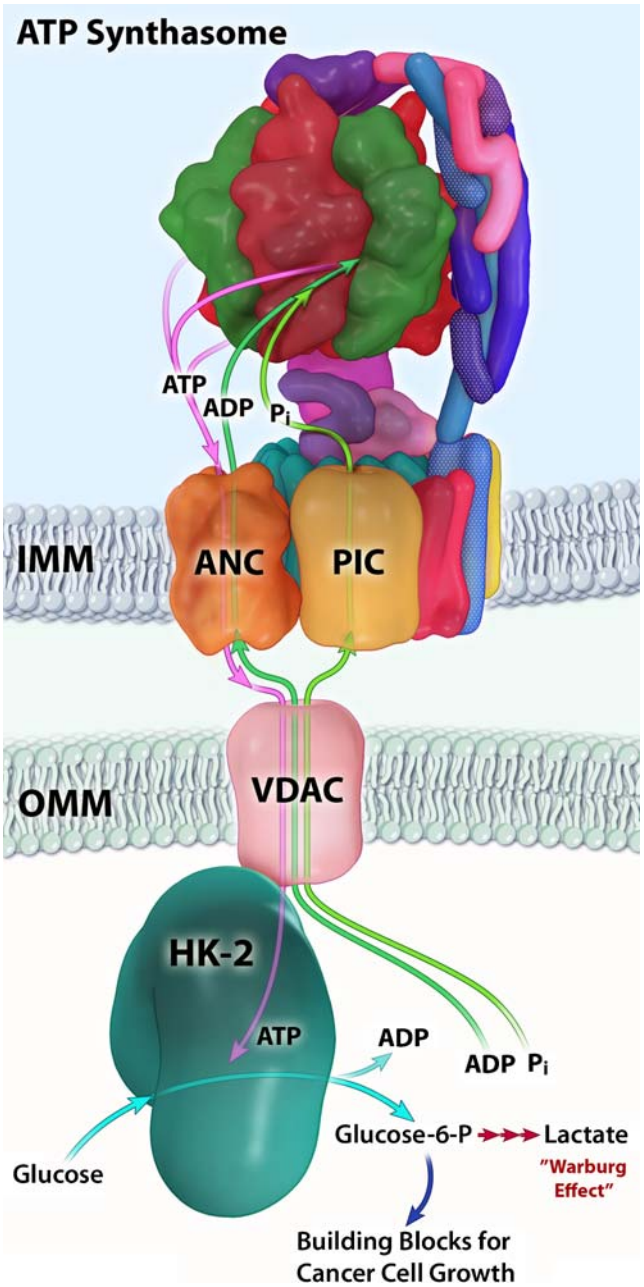
When bound to the outer mitochondrial membrane, HK-2's Michaelis Menten constant (Km) for MgATP is 0.25 mM (Bustamante and Pedersen 1980). However, when HK-2 is removed from the membrane its Km for MgATP is increased to about 1.2 mM (Bustamante and Pedersen 1980). Thus, binding of HK-2 to the outer mitochondrial membrane enhances its apparent affinity for MgATP almost fivefold.

2. Mitochondrial Binding of HK-2 Prevents Inhibition by its Product Glucose-6-Phosphate.

The product of the hexokinase reaction is glucose-6-phosphate (G-6-P), a potent product inhibitor of HK-2. In fact, HK-2 that is not bound to mitochondria (i.e., soluble HK-2) is inhibited over 50% by only 0.2 mM G-6-P (Bustamante and Pedersen 1977; Bustamante et al. 1978). Considering that G-6-P is directly or indirectly a precursor for almost all cell building blocks, the binding of HK-2 to the outer mitochondrial membrane in many types of cancer cells provides such cells with a tremendous growth advantage over most normal cells. That is, at its membrane bound location HK-2, free of G-6-P inhibition, can continue to make this product that is so essential for biosynthesis of cell building blocks.

3. Mitochondrial Binding of HK-2 Gives it Preferred Access to Its Substrate MgATP that is Synthesized Within the Mitochondria and then Transported out

The two substrates for HK-2 are glucose and MgATP, the latter compound being derived from oxidative phosphorylation in mitochondria involving the ATP synthasome (Ko et al. 2003; Chen et al. 2004) associated with the inner



◀ **Fig. 1** Proposed View for the Molecular Basis of the "Warburg Effect" in Cancer Involving Mitochondrial VDAC bound Hexokinase-2 (HK-2). The figure depicts the intimate relationship in highly glycolytic cancers among the mitochondrial ATP synthasome, the voltage dependent anion channel (VDAC), and the initial enzyme hexokinase 2 (HK-2) of the glycolytic pathway that leads to the "Warburg effect", i.e., high glycolysis in the presence of oxygen. Specifically, HK-2, overexpressed in many cancers, binds to VDAC. This increases HK-2's affinity for ATP, prevents its inhibition by the product glucose-6-P, and provides it with preferred access to the substrate ATP needed to convert glucose to glucose-6-P and therefore to "jump start" the glycolytic pathway and produce the "Warburg effect" (high glycolysis in the presence of oxygen). This provides the cancer cells with robust amounts of glucose-6-P, a precursor for cell building blocks essential for tumor growth. In the figure the globular spherical mass at the top is the catalytic F_1 moiety ("headpiece") of the ATP synthase that makes ATP in mitochondria from ADP, P_i, and Mg^{++}. The ATP synthase is in complex formation with the adenine nucleotide carrier (*ANC*) and the phosphate carrier (*PIC*). The complete ATP synthase/ANC/PIC complex is the "ATP synthasome". Other abbreviations are defined as follows: *IMM* inner mitochondrial membrane, *OMM* outer mitochondrial membrane, *HK-2* isoform 2 of hexokinase, and finally *VDAC*, voltage dependent anion channel, the subject of this introductory mini-review and other mini-reviews that will follow in this volume (40–3) of the Journal of Bioenergetics and Biomembranes. [The author is grateful to Dr. Young H. Ko, who spearheaded work resulting in the discovery, isolation and characterization of the ATP synthasome (Ko et al. 2003) and for conceptualizing the model, and to David Blum, a predoctoral student in Biological Chemistry and professional artist, for working with Dr. Ko to produce the figure.]

mitochondrial membrane (Fig. 1). In a prior study (Arora and Pedersen 1988) we showed by two different methods that glucose, one of the two substrates of the HK-2 reaction, is preferentially phosphorylated by ATP derived from the inner compartment of the mitochondria rather than by ATP located or generated on the cytosolic side of the mitochondria. In fact, the rate of glucose-6-phosphate formation generated by ATP derived from the inner mitochondrial compartment was estimated to be over twice that derived from ATP located externally.

Studies on Mitochondrial VDAC bound HK-2: from benchside to bedside

Mitochondrial VDAC bound HK-2 forms the basis of the major cancer detection system employed worldwide, i.e., Positron Emission Tomography (PET) that uses ^{18}F-2-deoxyglucose (^{18}F-2-DOG). When this labeled sugar enters cancer cells it is converted by VDAC bound HK-2 to ^{18}F-2-DOG-6-P in high amounts that are not further metabolized. ^{18}F-2-DOG was first synthesized by Indo et al. (1978) one year after the discovery in the author's laboratory (Bustamante and Pedersen 1977) that an isoform of HK, later identified as HK-2, plays a pivotal role in the "Warburg Effect" in cancer. Shortly thereafter (early 1980s) the ^{18}F-2-DOG was used at the NIH by DiChiro et al. (1982) for cancer detection in patients via PET. Thus, the discovery of the important role of mitochondrial bound HK in the "Warburg effect" in cancer in the author's laboratory (Bustamante and Pedersen 1977) was translated serendipitously from the basic science "benchside" at Johns Hopkins in Baltimore, MD to the clinical "bedside" at the NIH in Bethesda, MD in less than 5 years. Today, 31 years after the discovery in the author's laboratory that mitochondrial bound hexokinase (HK-2) plays the most pivotal role in the high glycolytic phenotype (Warburg effect) characteristic of numerous cancers, PET imaging is

used in hospitals and clinics throughout the world to detect cancers and to monitor their treatment.

Other medically relevant or potentially relevant roles of VDAC

In addition to VDAC's role in facilitating the "Warburg Effect" in cancer while helping prevent cell death (immortalization) via HK-2 binding, this vital protein has been studied also in relation to several other pathologies including "mixed connective tissue disease" (Kikuchi et al. 2008), refractory epilepsy (Jing et al. 2007), and amyloid beta-induced toxicity (Marin et al. 2007). It has been studied also in relationship to neuroprotection (Jiang et al. 2007a, b) and to superoxide anion formation (Han et al. 2003).

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