Adenine nucleotide translocase 2: an emerging player in cancer

Seung Hyun Baik1 and Jongkuen Lee*2

1Division of Reproductive Science in Medicine, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA
2Department of Oncological Sciences and Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Abstract

Adenine nucleotide translocase (ANT) is an integral protein located in the inner mitochondrial membrane and plays an important role in maintaining ATP/ADP ratio, thereby contributing to the energy-supplying function of mitochondria. It also mediates mitochondria-mediated cell death through interactions with Bax and Bcl-2, pro- and anti-apoptotic proteins, respectively. While the overall function of ANT is known, the diverse functions of ANT isoforms have not been investigated in depth. Of the four ANT isoforms (ANT1, ANT2, ANT3, and ANT4), ANT2 has gained many interests due to its aberrant expression in cancers. Here, we discuss the importance of ANT2 in proliferating cells, and highlight its cytoprotective and anti-apoptotic functions in cancer cells. Although the exact tumor-supportive mechanisms of ANT2 remain unclear, accumulating evidence suggests that ANT2 could be a promising target for cancer therapy.

Discussion

Following the observation that cancers prefer glycolytic metabolism to generate energy for cellular processes even in the presence of oxygen (aerobic glycolysis), Warburg proposed mitochondrial respiration defect as the underlying cause of cancer [1,2]. However, we now understand that there are many genetic mutations in cancers, altering cellular metabolism without necessarily impairing mitochondrial function. For instance, oncogenic mutations and amplification of K-ras, c-Myc, and phosphatidylinositol-3 kinase (PI3K) or loss of tumor suppressor genes such as p53 and phosphatase and tensin homolog (Pten) promote glycolysis [3]. Since mitochondria are implicated in various biological activities, including apoptosis and inflammation, the role of mitochondria in cancer is not as simple as Warburg had envisioned [4,5]. Studies of mitochondrial function in diseases have alluded to the significance of maintaining both ATP/ADP ratio and the mitochondrial transmembrane electrochemical gradient (ΔΨm) generated by oxidative phosphorylation [6,7]. The maintenance of the ATP/ADP ratios is partly regulated by the adenine nucleotide translocase (ANT), an integral protein located abundantly in the inner mitochondrial membrane. The physiological role of this protein is to catalyze the exchange of ATP/ADP across the mitochondrial inner membrane [8]. By using carboxyatractyloside, an ANT specific inhibitor, one study solved the crystal structure of bovine heart ANT [9]. The crystal analysis revealed six transmembrane helices with a hexapeptide carrying the signature of nucleotide carriers (RRRMMM) at its bottom even though the exact molecular mechanism of ATP/ADP exchange still remains elusive. ANT structure and earlier biochemical findings altogether imply that ANT plays an essential role in cellular energy metabolism by coupling mitochondrial ATP synthesis to cytosolic delivery. In addition, ANT is a crucial component of the mitochondrial permeability transition pore complex (PTPC) and plays a major role in mitochondria-mediated cell death [10]. ANT and members of the Bcl-2 family create contact sites between the mitochondrial inner and outer membranes [11]. ANT cooperates with Bax, a pro-apoptotic protein, within the PTPC to modulate mitochondrial membrane permeabilization (MMP) and mediate apoptosis responses [12] whereas interaction with Bcl-2 exhibits inhibition of the lethal pore formation [13]. Also, viral protein R, an apoptogenic protein encoded by HIV-1, induces MMP via a direct interaction with ANT [14]. However, Kokoszka et al. [15] showed that mitochondria from livers of ANT1/2-deficient mice still triggered MMP and concluded that ANT1/2 are non-essential components of the PTPC, casting doubt on the role of ANT in MMP. Thus, it remains to be further investigated whether ANT is a central component or just one of the regulators of the PTPC.

Human ANT has four isoforms (ANT1, ANT2, ANT3, and ANT4) and their expressions are various, depending on tissue types, developmental stages, and proliferation status [11] (Table 1). ANT1 is highly expressed in non-dividing, terminally differentiated muscle and heart cells [16]. ANT2 is specifically abundant in tissues that are able to proliferate and/or regenerate such as in lymphoid cells, liver, and various cancers [17-19]. ANT3 is ubiquitously expressed at lower levels and ANT4 is essential for spermatogenesis in testis [16,20]. This suggests that the expressions of the various ANT genes are context-specific and tightly regulated. Indeed, the promoter structure and organization of four ANT genes are different and their expressions can be controlled epigenetically [11]. For example, DNA methylation - that results in transcriptional silencing – of ANT4 promoter is observed in somatic cells whereas its hypomethylation is found in pluripotent stem cells. However, the precise mechanisms of epigenetic regulation of ANT gene expression remain to be elucidated.

Correspondence to: Jongkuen Lee, Department of Oncological Sciences and Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, Hess Center for Science and Medicine, 1470 Madison Avenue, New York, NY 10029, USA, Tel: 212-824-9230; E-mail: jongkuen.lee@mssm.edu

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Table 1. Human ANT isoforms are differentially expressed depending on tissue types, developmental stages and proliferation status.

| Protein Isoform | Gene   | Location | Apoptosis Induction | Expression                          |
|-----------------|--------|----------|---------------------|-------------------------------------|
| ANT1            | SLC25A4| Chr4     | Pro-apoptotic       | Non-dividing, terminally differentiated cells/tissues; skeletal muscles, hearts and brains |
| ANT2            | SLC25A5| ChrX     | Anti-apoptotic      | Proliferative and regenerative undifferentiated cells/tissues; lymphocytes, kidneys, livers, and hormone dependent cancers |
| ANT3            | SLC25A6| ChrX     | Pro-apoptotic       | Ubiquitous expression in all tissues  |
| ANT4            | SLC25A3| Chr4     | Anti-apoptotic      | Testes                              |

While there are four ANT isoforms, ANT2 has gained most attention from many research groups due to its markedly upregulated expression in cancers. ANT2 overexpression has been observed in a wide variety of cancers including stomach, lung, liver, ovary, and breast cancers, suggesting its significance in cancer development [24,25]. Jang et al. have described that the two ANT isoforms-ANT1 and ANT3-are barely detectable in breast cancer (MCF7, MDA-231, and SK-BR-3) and ovarian cancer (SK-OV-3 and SNU8) cells, emphasizing that ANT2 overexpression could be a unique feature at least in breast and ovarian cancer development [24]. The role of ANT2 in cancer development is not restricted to its anti-apoptotic behavior. One study proposed that ANT2 could have cytoprotective impacts in cancer cells by reversely importing ATP into the mitochondrial matrix to compensate for the decreased mitochondrial ATP generation [26]. Furthermore, it has been shown that ANT2 is implicated in the PI3K/AKT pathway in breast and liver cancers [18,27,28]. While the overexpression of ANT2 correlated with increased Akt phosphorylation, the level of phosphorylated Akt decreased in response to ANT2 suppression. The PI3K/Akt signaling pathway is important in cancer growth, metabolism, survival and motility, and it is likely the most frequently activated pathway in cancers, affecting 30-50% of tumors [29-31]. Thus, its association with the PI3K/Akt signaling pathway could be important for cancer development although the exact role of ANT2 in this pathway still remains to be elucidated. In addition, ANT2 is shown to regulate multiple microRNAs (miRNAs), which are negative regulators of gene expression, and deregulation of specific miRNAs is commonly found in various tumors [18,28]. Previously, we reported that ANT2-regulated miR-19a and miR-96 enhanced the proliferation of human liver cancer cells, and the knockdown of ANT2 negatively regulated miR-19a and miR-96, thereby suppressing tumor growth [18]. Also, one study showed the metabolic role of ANT2 in obesity [32]. The authors demonstrated that saturated fatty acids stimulate adipocyte mitochondrial ANT2, elevating O2 consumption. Increased adipocyte O2 consumption leads to inflammation and insulin resistance in obesity by inducing hypoxia. Since a great deal of research shows a significant association with obesity for several cancers, its involvement in insulin sensitivity and glucose tolerance could be relevant to cancer. Altogether, numerous studies suggest that ANT2 is a key player in tumor development although the tumor-supportive mechanisms of ANT2 are still poorly understood.

As cancer cells depend on the high rate of glycolysis and ANT2 is involved in the glycolytic metabolism, it is suggested as an anti-cancer therapeutic target. Indeed, many studies demonstrate that suppression of ANT2 by shRNA successfully kills cancer cells [17-19]. However, gene therapy using RNA interference has been hindered owing to the inefficiency of delivery in vivo. To solve this problem, a study by Park et al. suggests a method of ANT2 shRNA gene delivery with ultrasound [33]. This combination has led to significant tumor regression upon knockdown of ANT2 in vivo without toxicity, and therefore it provides evidence that ANT2 could be a promising target for cancer therapy.

Conclusion and future directions

ANT functions as the ATP/ADP carrier that facilitates the exchange of mitochondrial ATP for ADP from the intermembrane space, thus influencing cellular metabolism. Recent studies indicate that ANT isoforms have different effects on cell fate decisions; ANT1 and ANT3 are pro-apoptotic and ANT2 and ANT4 are anti-apoptotic. Given the similarities of sequences between four ANT isoforms, it is surprising that there is functional diversity. One possible explanation is that each isoform has distinct interacting partners or various topological distributions within the mitochondrial inner membrane, regulating MMP in different ways. Thus, a better understanding of the complicated interplay between the ANT isoforms and their partners in diverse situations is required. Moreover, the differences in the gene structures and epigenetic modifications of ANT isoforms may provide a new layer of their specificity for inducing different cellular behaviors. In various cancer types, aberrant expressions of ANT2 are frequently observed. Since cancer cells prefer utilizing the glycolytic pathway, ANT2 could be crucial as ANT2 imports glycolytically generated ATP into the mitochondria, contributing to the maintenance of the mitochondrial membrane potential and prevention of apoptosis. However, the exact role of ANT2 in cancer is not fully understood. Based on the increasing evidence that ANT2 has a positive correlation with tumor progression, ANT2 could be a promising target for cancer therapy. Nevertheless, the fact that normal proliferating cells also express ANT2 should be taken into account and it is required to conduct therapeutic window evaluation to prevent possible adverse effects.

Competing interest statement

Author has no conflict of interest.

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