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The Regulation of Mitochondrial Metabolism by the Bcl-2 Family of Pro-Survival Proteins: New Therapeutic Opportunities for Targeting Cancer Cells

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Abstract

Since the identification of the first tumor oncogenesin the 1970s, advances in our understanding of the molecular basis for cellular transformation have continued at a breathtaking rate. Yet compared to other classical hallmarks of cancer such as evading apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals and limitless replicative potential, the mechanisms by which transformed cells undergo metabolic reprogramming leading to rampant glycolysis (termed the "Warburg effect") remain poorly defined. Several very recent studies have revealed that, in addition to their well-established roles in regulating intrinsic apoptosis programs, the Bcl-2 family of pro-survival proteins are also important regulators of mitochondrial respiration and energy generation in cancers such as Acute Myeloid Leukemia (AML). This article discusses some recent advances in our understanding of how the Bcl-2 family of proteins regulate cellular metabolism and how exploiting metabolic vulnerabilities in transformed cells by direct targeting of the Bcl-2 proteins may provide new clinical avenues for the treatment of cancer.

Keywords: Apoptosis; Bcl-2; Oxidative phosphorylation; Glycolysis; Leukemia

Targeting the Cell Survival Machinery in Cancer

The discovery that Bcl-2 functions to promote cell survival and cooperates with c-myc to promote B-cell lymphomas established the first molecular link between tumorigenesis and deregulated cell survival [1-3]. Since those early studies, Bcl-2 and related pro-survival family members Bcl-xL, Bcl-w and Mcl-1 have been widely shown to promote both autonomous cell survival and drug resistance in cancers of diverse origins and therefore constitute important therapeutic targets [4]. In fact, because the activation of intracellular survival pathways in malignant cells and their ability to over-ride apoptotic triggers is proposed to be a universal feature of all human cancers [5], future success in developing anti-cancer therapies will almost certainly require drugs or approaches that block the pro-survival activity of Bcl-2, Bcl-xL, Bcl-w and Mcl-1.

The recent development of rationally-designed small molecule inhibitors of the Bcl-2 family of pro-survival proteins now provides a therapeutic approach for the selective targeting of the cell survival machinery in transformed cells. Designed to mimic the BH3 domain of the pro-apoptotic Bad protein, ABT-737 and its orally bioavailable analogue ABT-263, bind and neutralize the pro-survival activity of Bcl-2, Bcl-xL and Bcl-w. ABT-737 and ABT-263 have demonstrated activity in animal models of lymphoma and small cell lung carcinoma and ABT-263 has now progressed to clinical trials where it is being used to treat non-Hodgkin's lymphoma and Chronic Lymphocytic Leukemia (CLL) [6,7]. However, despite its clinical promise, ABT-263 induces significant thrombocytopenia due to the requirement of Bcl-xL for platelet survival [8]. To overcome this on-target side-effect, a re-engineered version of ABT-263, ABT-199, has been developed which targets Bcl-2 but not Bcl-xL or Bcl-w. So far, ABT-199 has shown pre-clinical activity in some haematological malignancies including CLL, B-cell lymphoma and myeloma [9-12]. Drugs targeting Bcl-2 such as ABT-199 may also have application in the treatment of Acute Myeloid Leukemia (AML) where it has been reported that the functional level of Bcl-2 determines the clinical response of some primary AML patient samples to chemotherapy [13].

Targeting Metabolic Pathways in Cancer

Over 50 years ago, Otto Warburg described a phenomenon in which cancer cells were widely observed toundergo a metabolic switch from mitochondrial respiration to glycolysis [14]. Since glycolysis is less efficient than oxidative phosphorylation at generating ATP from glucose, tumor cells that derive a significant amount of energy from glycolysis require high rates of glucose uptake to meet their metabolic demands. Warburg's fundamental observation that cellular transformation is accompanied by a dependence on glycolysis for ATP generation continues to have an important impact in cancer biology where the "Warburg effect" is now widely used diagnostically to locate malignant cells with high rates of glucose uptake by positron emission tomography. Furthermore, the metabolic "addiction" to high rates of glycolysis observed in diverse malignancies may offer unique therapeutic approaches for targeting bioenergetic pathways in cancer.

While there are significant ongoing efforts to develop clinical approaches that allow targeting of cancer cells through the disruption of glycolytic pathways, the potential of targeting oxidative phosphorylation has been largely overlooked. This has been, in part, due to the long held view that tumor cells switch to glycolysis as a result of mitochondrial defects that affect oxidative phosphorylation. Thus, tumor cells were thought to undergo selective pressure to increase glycolytic rates due to impaired or defective oxidative phosphorylation. However, a number of recent studies suggest that mitochondrial integrity and

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oxidative phosphorylation are not defective in malignant cells, but rather, may be pivotal to cellular transformation and tumorigenesis. For example, oncogenic BRAF-mediated cellular transformation leads to a Warburg effect in which increased glycolytic activity is accompanied by a decrease in oxidative phosphorylation in melanoma cells [15]. However, while BRAF kinase inhibitors suppressed glycolysis in malignant cells, there was a compensatory re-induction of oxidative phosphorylation that significantly blunted the anti-tumor activity of BRAF inhibitors [15]. Furthermore, impairment of oxidative phosphorylation by down-regulation or deletion of peroxisome proliferator-activated receptor γ coactivator 1 (PPARGC1A; also known as PGC1α) resulted in a sensitization of melanoma cells to agents that induce apoptosis through the induction of Reactive Oxygen Species (ROS) [16]. In other studies, the growth and frequency of oncogenic Kras-driven lung adenocarcinomas in mice was significantly reduced when oxidative phosphorylation was disabled following deletion of the mitochondrial transcription factor A (Tfam) gene [17]. Furthermore, others have shown that high levels of glycolysis were not sufficient to support the growth of human breast cancer xenografts in mice when oxidative phosphorylation was impaired by knockdown of the p32 mitochondrial protein using RNA interference (RNAi) [18]. Together, these reports not only indicate that oxidative phosphorylation performs obligate metabolic functions in malignant cells of diverse origins, but that targeting oxidative phosphorylation may also have therapeutic potential.

The Bcl-2 family of pro-survival proteins lie at the nexus between apoptosis and mitochondrial energy metabolism

A number of recent reports have revealed that in addition to their widely documented roles in regulating apoptosis, the Bcl-2 family of pro-survival proteins also has roles in regulating oxidative phosphorylation. In the 2B4 mouse T-cell line, treatment with Tumor Necrosis Factor alpha (TNFα) resulted in the uncoupling of mitochondrial respiration from oxidative phosphorylation leading to increased ROS production, loss of mitochondrial membrane potential and the induction of apoptosis [19]. However, Bcl-xL expression prevented the uncoupling of mitochondrial respiration from oxidative phosphorylation by TNFα preventing the loss of mitochondrial membrane potential and ROS production [19]. Others have shown that Bcl-2 can also influence mitochondrial biogenesis with Bcl-2 over-expression in leukemia cell lines resulting in increased oxidative phosphorylation while Bcl-2 inhibition resulting in the uncoupling of mitochondrial respiration from oxidative phosphorylation [20].

More recently, Lagadinou et al. uncovered an important role for Bcl-2in regulating oxidative phosphorylation in Leukemic Stem Cells (LSCs: also known as leukemia initiating cells). Primary human AML cells demonstrating low ROS levels were found to be enriched for LSCs and expressed significantly higher levels of Bcl-2 when compared to cells with higher ROS levels [21]. These studies showed that bulk AML cell populations, like many other cancer cell types, demonstrated classical Warburg biogenesis with high rates of glycolysis that could be further increased by inhibitors of oxidative phosphorylation [21]. However, what was particularly revealing was that LSC-enriched populations did not adhere to the Warburg paradigm in two key aspects. Firstly, LSC-enriched cell populations exhibited low rates of glycolysis [21]. Secondly, glycolytic pathways were not be up-regulated in LSC-enriched cell populations following inhibition of oxidative phosphorylation [21].Interestingly, glioblastoma stem cells have also been shown to be highly reliant on oxidative phosphorylation raising the possibility that cancer stem cells may not always utilize the Warburg effect to meet energy demands [22]. Thus, in metabolic terms, cancer stem cells may be unique in that they are highly reliant on oxidative phosphorylation and cannot readily adapt to changing energy demands by increasing glycolysis.

The studies of Lagadinou et al. were also striking in that they showed that Bcl-2 was an important regulator of mitochondrial respiration. Inhibition of Bcl-2 in either LSC-enriched cell populations or bulk AML cell populations using either ABT-263 or RNAi approaches resulted in a rapid impairment of oxidative phosphorylation indicating that Bcl-2 expression is important for maintaining mitochondrial energy production [21]. Importantly, while the bulk AML cell population was able to respond to Bcl-2 inhibition and compensate for the impaired oxidative phosphorylation by increasing glycolysis (Warburg effect), no such compensation was observed for the LSC-enriched population leading to a rapid decrease in intracellular ATP concentrations and the induction of apoptosis [21]. Thus, these studies reveal a metabolic vulnerability in LSCs that, quite remarkably, can be therapeutically targeted by neutralizing Bcl-2. As a consequence, targeting Bcl-2 pro-survival proteins may induce apoptosis by two distinct (although perhaps not entirely mutually exclusive) mechanisms. Firstly, the binding of BH3-mimetic drugs such as ABT-263 to Bcl-2 would liberate pro-apoptotic proteins such as Bax and Bak ultimately leading to mitochondrial outer membrane permeabilization and the induction of cell death. In addition to this canonical mode of cell death, targeting Bcl-2 pro-survival proteins may also enforce an unsustainable mitochondrial energy crisisdue to a rapid block in oxidative phosphorylation leading to apoptosis.

We and others have shown that Phosphatidyl inositol 3-Kinase (PI3K) inhibition (that blocks glucose uptake and glycolytic pathways) is relatively inefficient at reducing intracellular ATP concentrations and is only a modest inducer of apoptosis in primary human AML cells [23-25]. One explanation for such results is that AML cells retain a reserved capacity for generating ATP through non-glycolytic pathways such as oxidative phosphorylation. Thus, significant apoptosis can be induced in primary AML cells when PI3K inhibition is combined with neutralization of Bcl-2 pro-survival proteins at the mitochondria highlighting at least one therapeutic approach for overcoming such bioenergetic obstacles deployed by malignant cells [23].

Future Perspectives

If the dependency on oxidative phosphorylation is a universal feature of cancer stem cells across many tumor types, then the mechanisms by which the Bcl-2 family of pro-survival proteins influence oxidative phosphorylation are of great clinical importance. It remains to be determined precisely how the Bcl-2 proteins influence oxidative phosphorylation and ATP production by the mitochondria and whether such effects are truly distinct and independent from their canonical roles in regulating apoptosis. Bcl-2 has been shown to directly interact with COXVa, a key component of the electron transport chain [26], possibly allowing direct roles in the regulation of oxidative phosphorylation. Others have shown that Bcl-xL can interact with the β subunit of the F (1) F (O) ATP synthase and increase its enzymatic activity thereby enhancing mitochondrial ATP generation [27]. In the case of Mcl-1, the full length protein is proposed to regulate intrinsic apoptotic pathways while a truncated proteolytically cleaved form has been shown to be important for mitochondrial respiration and ATP production [28]. Thus, it is possible that the Bcl-2 proteins may perturb two classical and universal hallmarks of cancer: the resistance to the activation of apoptotic pathways and the deregulation of cellular

metabolic pathways [5]. The future challenge will be to find therapeutic approaches that allow effective targeting of both oncogenic pathways that provide significant and sustained clinical responses.

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