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Review

*mito*Energetics and cancer cell fate

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ABSTRACT

The critical role of mitochondria in cell fate decisions has been well documented over the years. These observations have highlighted the way mitochondrial physiology controls cell survival and growth in the normal settings, the critical role of mitochondrial outer membrane permeabilization and altered *mito*energetics in cell death execution, and most importantly the association of altered mitochondrial metabolism with pathological states, in particular cancer. Reprogramming of cell metabolism, an invariable finding in cancer cells, is tightly linked to *mito*energetics as is evidenced by up-regulation of nutrient uptake and a pro-oxidant tilt in the intracellular milieu. The latter has also been demonstrated in oncogene-induced carcinogenesis models, notably as a functional outcome of Bcl-2 overexpression. Interestingly, even in that model, mitochondria appear to be the target as well. Thus the association of metabolic re-circuiting and altered *mito*energetics with the process of transformation has resulted in a paradigm shift in the way cancer development and progression is viewed today, which has tremendous implications for the development of novel and strategic therapeutic modalities.

1. Introduction

The role of mitochondria in metabolism is well established. From metabolic diseases to cancer, the organelle remains a hotbed for intense research and discovery on the mechanisms leading up to these diseases. Equally important from its central role as the powerhouse of the cell is the involvement of the mitochondria in cell death regulation. These two processes are intimately linked by the generation of reactive oxygen species (ROS) from the mitochondria, the major contributor of free radicals in the cell. Armed with this knowledge and from the standpoint of carcinogenesis, the majority of today's chemotherapeutic drugs are tailored to induce oxidative stress and bring about ROS-based cell death in the cancer cell through their effects on the mitochondria. On the other hand, it is well known that cancer cells have evolved multiple evasive pathways and circuitries to cheat death. One of these is carried out by the Bcl-2 family of proteins, consisting of an extensive network of pro- and anti-apoptotic proteins, of which anti-apoptotic Bcl-2 will be our main focus here. Given its localization to the mitochondria and its huge stake in mitochondrial cell death pathway, it would be plausible to suggest Bcl-2 involvement in mitochondrial physiology. In this review, we seek to redefine the field's current understanding of these areas and provide insights and implications on the integrative nature of these different aspects of the mitochondria. More importantly, we aim to draw a link between mitochondrial metabolism-based ROS production, the resultant impact on cell death and the role of oncogenic Bcl-2 in controlling these intricate pathways.

2. ROS and cell death — an intricate balance

Conventional dogma has long established ROS as agents of detriment. Indeed, a great number of studies have documented the role of ROS as mediators of damage to cellular structures, nucleic acids, proteins and lipids. Modification of the DNA molecule by ROS represents the first step of mutagenesis and if left unchecked, carcinogenesis ensues. Lipid peroxidation and protein modification of Bax monomer to promote oligomerization of Bax are common features of ROS-mediated damage, culminating in the compromise of the mitochondrial outer membrane integrity and the subsequent release of cytochrome *c* to initiate the mitochondrial death pathway [1]. These form the premise for the use of ROS-based chemotherapeutics in cancer intervention and management.

While it is true that overwhelming ROS are harmful to cells, an emergent growing body of evidence indicates the importance of low levels intracellular ROS in physiological signaling, tumor promotion/initiation and its subsequent maintenance and progression. There is sufficient evidence to strongly support a paradigm shift from the convention of ROS as only a mediator of cell damage/death to that of survival/proliferation. Mild elevation in intracellular superoxide (O_2^-) or hydrogen peroxide (H_2O_2) has been demonstrated to promote

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growth responses in a variety of cell types through activation of growth-related genes such as *c-fos* and *c-jun*, alterations in protein kinase activities, oxidative modifications to phosphatases and activation of transcription factors [2,3]. Some of these include ROS stimulatory effect on the PI3-kinase/AKT survival pathway through the oxidative inactivation of PTEN as well as activation of transcription factors such as AP-1 and NF-Kb [4–6]. More importantly, NADPH-dependent generation of ROS has been reported upon growth factor stimulation or cytokine receptor activation, implicating ROS as secondary messengers of survival and proliferative signaling [3,4].

Further studies have gone on to show that the intricate balance between the different species of free oxygen radicals is important in determining the decision of the cell fate. A tilt in favor of O_2^- levels with no appreciable increase in H_2O_2 renders the cancer cell refractory to death execution and preserves viability, irrespective of the trigger [7–9]. In contrast, increased levels of H_2O_2 and a corresponding drop in O_2^- levels create a reduced and acidified environment conducive for the apoptotic signal to filter through [7]. Correlation between increased activity of O_2^- producing systems and proliferative networks as well as the fact that several anti-cancer drugs exert their effects via H_2O_2 mediated killing further lends weight to the pro-oxidant theory of carcinogenesis, prevalent in many tumor types, depending on the intracellular levels and species involved.

Thus, mitochondria being a major site of O_2^- production through its electron transport chain activities are crucial organelles for the study on the potential impact of onco-proteins such as Bcl-2 on its physiology in order to validate the pro-oxidant state, necessary for the transformed phenotype. However, one confounding factor is the notion that cancer cells generally exhibit reduced oxidative phosphorylation and the next section seeks to address this issue.

3. Altered metabolism and cancer cell fate — one size fits all theory?

Early studies on tumor metabolism proposed a unique, signature characteristic in the way these rogue cells obtained their source of ATP to meet the rigorous demands of proliferation, invasion and adaptations to the harsh tumor microenvironment. Back in 1924, Otto Warburg proposed that cancer cells preferentially utilize the glycolytic pathway over oxidative phosphorylation to provide for the majority of the energy supply. This is in stark contrast to normal cells where the reliance on oxidative phosphorylation is far greater than glycolysis for the generation of ATP. Warburg attributed this phenomenon to the dysfunction of the mitochondria whereby these metabolic differences were regarded as an adaptation to the hypoxic environment within the solid tumor [10].

More than 80 years on, the Warburg effect is widely applied as the *de rigueur* metabolic phenomenon to distinguish cancer cells from non-cancerous ones. The idea that cancer cells predominantly utilize glycolysis for energy production is being employed as a parameter in positron emission tomography to assess the prevalence of tumors in the clinical setting. Indeed, extensive studies have shown that fast-growing and highly de-differentiated cancer cell types demonstrated highly modified metabolic patterns compared to their normal counterparts [11–16]. In agreement with these observations, a large body of evidence has emerged suggesting significant up-regulation in the expression of glycolytic genes in aggressive malignancies [17–25].

With these compelling evidence, the concept that enhanced glycolysis is always induced or accompanied by near-defunct oxidative phosphorylation has been ubiquitously and indiscriminately applied to all types of cancer. The universal acceptance of the Warburg effect thus formed the central metabolic dogma that come to characterize all cancer cells. However, it is important to note that fundamental genetic, biochemical and morphological heterogeneity of tumor cells may render the convenient application of the Warburg effect to define all types of cancer as generalization. Tumor cell types such as glioblastoma multiforme, astrocytoma, MCF7 and certain forms of hepatoma utilize both glycolysis and oxidative phosphorylation to an equal extent for energy production [26–29]. More importantly, tumors such as bone sarcoma, lung carcinoma, breast cancer, skin melanoma, cervical, ovarian and uterus carcinomas all primarily make use of oxidative phosphorylation for the generation of ATP [15,28–32]. Moreover, hypoxia could not entirely justify for the assumption of compromised oxidative phosphorylation because the concentration of oxygen in the hypoxic regions of most human tumors is way above the $K_{M\ O2}$ of cytochrome *c* oxidase (COX) [33]. Therefore, it is likely that tumor oxidative metabolism remains unaffected by the level of hypoxia within the tumor microenvironment [33]. Compounded by evidence taken from fast-growing tumors showing large increase in glycolytic flux even in the presence of high oxygen concentration, it is indisputable that all tumor cells possess an increase in glycolytic capacity but not necessarily in response to a defective oxidative phosphorylation system [10,12–17,33]. In fact, the demanding energetic needs of the tumor brought on by its highly proliferative nature may be the main driving force behind the increase in glycolytic flux to boost the ATP supply, together with an intact oxidative metabolic pathway [33]. Enhanced tumor glycolysis may be operating in tandem with oxidative phosphorylation or in some cases; the latter may even predominate to meet the energy requirements of the highly invasive tumor [33].

4. Bcl-2, *mito*energetics and cancer cell redox status — a unique role

4.1. Classical anti-apoptotic role of Bcl-2

Bcl-2 was one of the earliest regulators of apoptosis discovered in cancer cells, an onco-protein produced via chromosomal translocation in human follicular lymphoma. Bcl-2 is largely localized to the endoplasmic reticulum, nucleus, and the outer membrane of the mitochondria. Functional studies on Bcl-2 revealed its pro-survival properties, mainly through its protective action on the mitochondria. Over the years, various studies have exhaustively demonstrated the physical nature of Bcl-2 anti-apoptotic activity, which acts by sequestering pro-apoptotic proteins such as Bax and Bak at the mitochondria [34,35]. The latter two are responsible for the formation of pores on the mitochondrial outer membrane through oligomerization and thereby perturbing membrane integrity, resulting in the release of cytochrome *c*, a key event leading to the eventual activation of the mitochondrial death pathway [34,35]. Impairment to engage apoptosis to eliminate cells with malfunctioning cell cycle controls not only preserves these aberrant cells but also allow metastasis-causing mutations to accumulate and exacerbate their effects. The accrual of mutations further accentuates the function of Bcl-2 as a death brake, conferring a heightened level of chemoresistance.

The classical role of Bcl-2 focuses on the interactions between the two antagonistic classes of proteins from the Bcl-2 family such as anti-apoptotic Bcl-2/Bcl-xL against pro-apoptotic Bax/Bak, with the bout-winning proteins defining molecular signaling pathways which govern several areas from cell death execution to mitochondrial morphology and physiology [34,35].

4.2. Intrinsic pro-oxidant function of Bcl-2 — involvement of mitochondria

Several landmark advances have been made to change the field's perspective and to re-evaluate the role of oxidative phosphorylation in the cancer cell. Notably, functional p53 has been shown to promote mitochondrial respiration by inducing the expression of Synthesis of Cytochrome *c* Oxidase 2 (SCO2), which is responsible for regulating the COX complex [36]. From the perspective of tumor suppressor p53 where it is often mutated and non-functional in cancer cells, the role

of onco-protein Bcl-2 in promoting tumor cell survival has been designated for further investigation from another perspective, that of ROS and *mito*energetics. Could Bcl-2 replace the role of non-functional p53 in regulating mitochondrial respiration and contribute to oncogenesis? Can Bcl-2 preserve or optimize oxidative phosphorylation to tailor to the survival instincts of the tumor cell from a ROS perspective? Traditionally, Bcl-2 has been portrayed as an anti-oxidant due to its ability to suppress H_2O_2 mediated lipid peroxidation [37]. However, recent work has challenged this belief by demonstrating that under normal conditions, Bcl-2 did not operate as an anti-oxidant on its own but rather, its expression levels was directly associated with a pro-oxidant intracellular milieu that triggered the reinforcement of the endogenous anti-oxidant defense machinery [38]. In turn, this mild pro-oxidant state was connected to the death inhibitory activity of Bcl-2 as shown in leukemia cells [39]. Moreover, inhibition of NADPH oxidase activity by diphenyleneiodonium (DPI) decreased intracellular O_2^- and rendered Bcl-2 overexpressing cells more sensitive to apoptosis, suggesting specificity for O_2^- in Bcl-2 mediated pro-oxidant state [39]. Still, other models employing mouse and bacteria corroborated our findings demonstrating Bcl-2 as a pro-oxidant protein. These reports firmly established the significance of a mild pro-oxidant milieu in tumor progression/initiation through enhanced survival, with Bcl-2 at the heart of this phenomenon.

In consideration of Bcl-2 localization at the mitochondria, which is a major site of ROS production from oxidative phosphorylation, we demonstrated that Bcl-2 is responsible for promoting tumor mitochondrial respiration and in turn, generating a slight pro-oxidant state through ROS production from electron transport activities [40]. This slight pro-oxidant state has been shown to favor a cascade of survival signaling pathways in cancer cells [38,39,41–43]. Bcl-2 which is almost ubiquitous at the mitochondria has rarely been linked to the bioenergetics aspect of the mitochondria. For the first time, by measuring the enzymatic activity of cytochrome *c* oxidase (COX) which is the rate-limiting complex of the electron transport chain, leukemia cells (CEM) overexpressing Bcl-2 displayed an increase in COX activity and oxygen consumption, which associated with an increase in mitochondrial O_2^- production [40]. Similar observations were obtained in a cervical carcinoma (HeLa) model [40]. Bcl-2 overexpressing tumor cells not only displayed elevated oxygen consumption but also better coupled mitochondrial respiration, suggesting that the increase in O_2^- generation is indeed a function of amplified mitochondrial respiration and not due to uncoupling [40]. Despite treatment of mock-transfected and Bcl-2 overexpressing cells with the uncoupler FCCP, cells with Bcl-2 overexpression continued to display higher oxygen consumption [40]. Increased mitochondrial respiration suggests an increased tendency to leak electrons for the generation of O_2^- as a by-product.

Transient overexpression and silencing of Bcl-2 correlated with the presence and absence of the pro-oxidant effect respectively [40]. Similarly, reduction of electron transport activities through the partial inhibition of COX was able to abrogate O_2^- levels in Bcl-2 expressing cells to that of non-transfected cells, further validating the impact of Bcl-2 on the pro-oxidant state through mitochondrial respiration [40]. Although HA14-1, which is a well-known Bcl-2 inhibitor, has been shown to induce oxidative stress in treated cells, functional inhibition of Bcl-2 led to a reduction in oxygen consumption [40].

4.3. Anti-oxidant function — two faces of Bcl-2?

In spite of conventional acceptance of Bcl-2 as an anti-oxidant, a growing number of studies indicate that the enhanced anti-oxidant cellular defense is a response to the pro-oxidant capability of Bcl-2 and could be crucial in protecting the cell from acute oxidative stress. Various experimental models established the anti-oxidant property of Bcl-2 by triggering cells with death-inducing stimuli or directly overwhelming the cells with oxidative stress before accruing the resultant anti-oxidant response to Bcl-2 expression. At best, these studies accredit a redox regulatory role for Bcl-2 in countering oxidative stress, but do not suffice to confirm Bcl-2 as having innate anti-oxidant characteristics. True to this aspect, pure Bcl-2 has been shown to be devoid of intrinsic anti-oxidant activity [44]. Hence, while Bcl-2 may be pro-oxidant under normal, non-stressed conditions, triggers inducing acute oxidative stress may activate the feedback function of Bcl-2 to fortify anti-oxidant defenses and thus, prevent levels of free radicals from breaching deleterious level. In view of this, our recent data propose a control by Bcl-2 at the level of mitochondrial bioenergetics by tuning COX activity to modify upstream electron transport chain activities, which in turn, affect the level of ROS by-production from oxidative phosphorylation.

Using both physiological and artificial triggers-induced stress approach, early mitochondrial oxidative stress insult by antimycin or serum deprivation led to a down-regulation of COX activity and oxygen consumption in tumor cells overexpressing Bcl-2 [40]. In contrast, similarly treated mock-transfected cells displayed increases in COX activity and oxygen consumption [40]. This observation corresponded to the maintenance of mitochondrial O_2^- levels in the Bcl-2 cells while the levels in non-overexpressing cells continue to accumulate after the initial oxidative burst [40]. Although treatment with rotenone also resulted in an increase in COX activity in Bcl-2 cells, the magnitude of increase was much lower than the control cells [40]. This could be attributed to the fact that antimycin blockade of complex III results in production of O_2^- to the mitochondrial intermembrane space whereas rotenone inhibition of complex I results in accumulation of O_2^- in the mitochondrial matrix [45]. This disparity may account for the difference in Bcl-2 efficiency to 'sense' oxidative stress and regulate COX activity accordingly. Nonetheless, it appears that Bcl-2 regulation on COX activity during normal conditions and in response to oxidative stress does not involve any change in the levels of expression of COX subunits.

These unique findings suggest a novel feature of Bcl-2 that steers away from the field's conventional knowledge of the protein by focusing on the regulation of mitochondrial ROS production via its metabolic control on COX activity. With this in mind, an increase in Bcl-2 expression might be a double-edged sword, counteracting against cell death machinery by enhancing energy production as well as generating more O_2^- as a by-product to provide a slight pro-oxidant, survival-inclined milieu, on top of its established anti-apoptotic functions. Investigations reporting the anti-oxidant function of Bcl-2 could be due to the physiological role of Bcl-2 as a ROS modulator [41]. These studies provided an alternative opinion of ROS, away from the classical notion of detriment to a more astute and discerning view of its effects in carcinogenesis that is dependent on the levels and species involved, particularly the implication of O_2^- in tumor cell survival.

On the contrary, physiological stress stimuli or chemical ROS inducers triggered a homeostatic response from Bcl-2 to reduce mitochondrial respiration through the rate-limiting, terminal enzyme COX; hence, preventing the buildup of O_2^- to a lethal level after the early insult, while possibly striving to sustain the basal energy requirement and slight pro-oxidant milieu necessary for survival [40] (Fig. 1). Ongoing studies seem to advocate the promise of Bcl-2 ability to optimize both the energetic demands and redox status of the cancer cell by adapting mitochondrial metabolism so as to craft an environment best suited for survival and proliferation. However, the exact molecular mechanism remains unknown.

4.4. Bcl-2, p53 and HIF-1 in relation to ROS and metabolic adaptation

Recently, several reports have emerged implicating various key proteins to cancer metabolism. More notably, HIF-1 has been demonstrated to play a central role in regulating the efficiency of mitochondrial respiration during hypoxia via its effect on altering the COX subunits' composition by transactivating COX-4-2 and LON

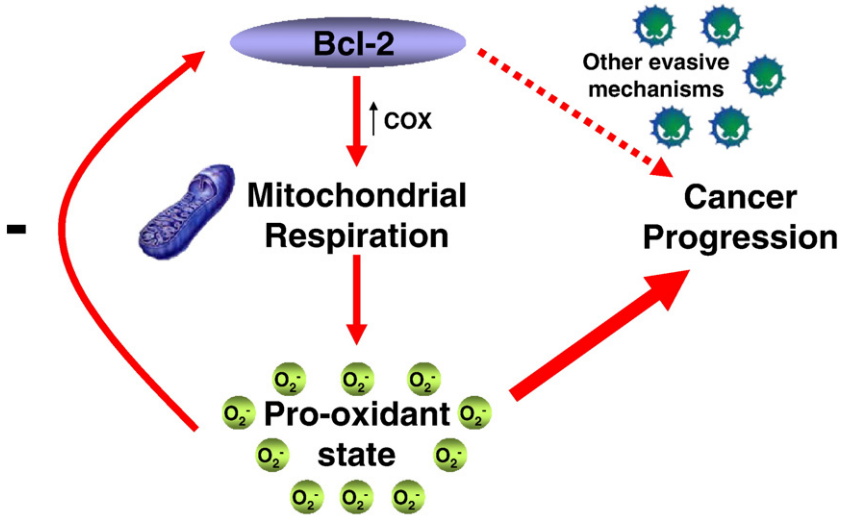

Fig. 1. Bcl-2 regulation of tumor redox states is mediated through its effect on mitochondrial respiration, which suggests an alternative oncogenic mechanism employed by Bcl-2. Oxidative stress serves as a negative feedback by signaling via Bcl-2 to decrease mitochondrial respiration.

protease to degrade COX-4-1 [46]. The outcome is an optimized efficiency in mitochondrial respiration with a control on ROS production from the initial burst generated by hypoxia. A major stakeholder in the cancer field, p53 is able to promote mitochondrial respiration while keeping ROS in check by inducing the transcription of anti-oxidant genes during low-stress state of normal growth and development [47,48]. In times of extended stress and irreparable damage, stress-induced p53 is able to up-regulate pro-oxidant genes such as PIG-3 and proline oxidase as well as down-regulate anti-oxidant enzymes such as the Nrf-2 dependent ones along with driving up the activation of mitochondrial respiration, in order to induce p53-dependent cell death by ROS, thus removing aberrant cells from transforming into rogue cancerous cells [36,49–52].

Thus, while the normal cell type relies on p53 for normal mitochondrial and redox regulation during different growth conditions, the transformed phenotype which often contains the loss-of-function mutant p53 and high levels of Bcl-2, may possess an entirely different set of metabolic regulation which favors a survival-inclined oxidant level and energy production by employing Bcl-2 as a rheostat acting on mitochondrial respiration during normal and stress conditions [40,48] (Fig. 2). The fact that p53 is an upstream repressor of Bcl-2 expression lends further credit to this theory with regard to their opposing roles through employing different strategies on a single mechanism to achieve their means in normal and cancerous cells respectively.

The link between Bcl-2 and tumor mitochondrial metabolism also begs the question if it is closely connected to HIF-1 action on COX. Studies have shown that silencing of HIF-1α under normoxia or hypoxia correlated with decreased expression of Bcl-2 and subsequent attenuation in cell proliferation and induction of apoptosis

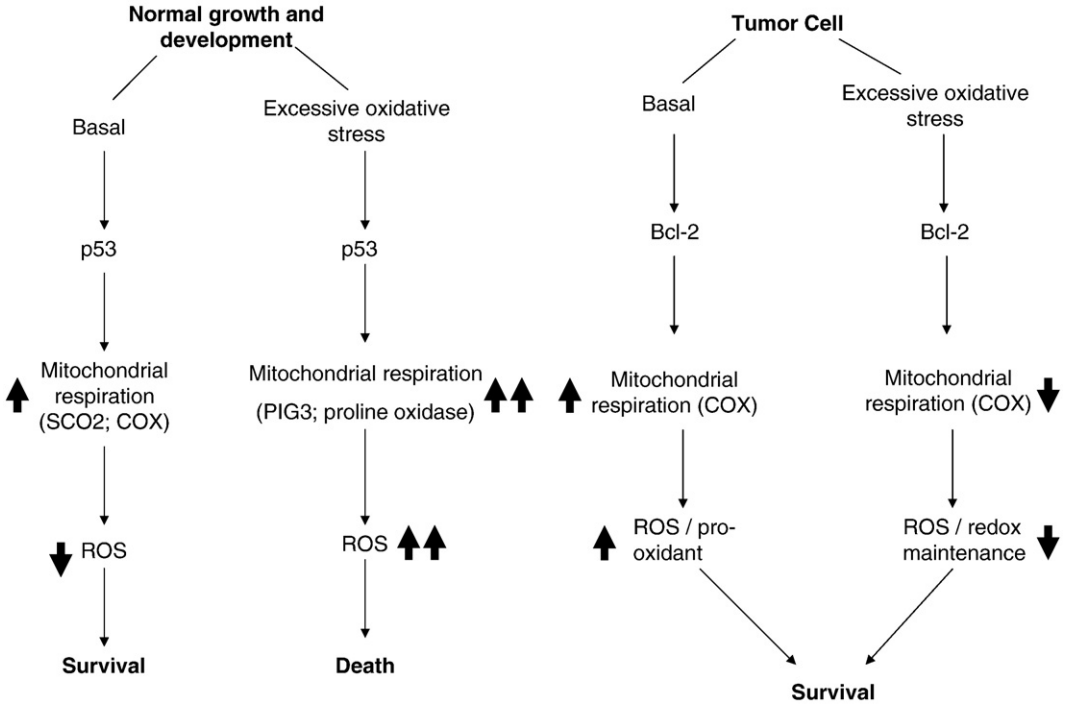

Fig. 2. Comparison between p53 and Bcl-2 regulated *mito*energetics.

[53]. Reinforcing this, a number of studies demonstrated a link between resistance to hypoxia-induced apoptosis and increased Bcl-2 expression [54]. In addition, overexpression of HIF-1α in combination with Bcl-2 resulted in poor treatment outcome in esophageal cancer [55]. However, it remains to be seen whether these two proteins are indeed linked or merely correlative, with each having its own unique repertoire of mechanisms to bring about a common purpose of tumor metabolic regulation. Nevertheless, the intricate controls on tumor mitochondrial respiration and oxidative phosphorylation by onco-proteins and tumor suppressors cannot be denied. Tumors that are able to harness energy from both enhanced glycolysis and oxidative phosphorylation, fashion a redox environment favoring the transformed phenotype and display extensive metabolic plasticity and adaptability under different redox state to provide a survival edge over their counterparts that simply rely on amplified glycolytic flux and compromised oxidative phosphorylation.

4.5. Redox regulation by Bcl-2 and ROS-based chemotherapeutics

Moderate levels of ROS are obligatory by-products of cellular metabolism and indispensable for normal cellular growth and functions. On the other hand, excessive ROS production leads to severe compromise of cellular structures and integrity, which eventually brings about the demise of the cell. Based on this principle, many anti-tumor agents were developed including doxorubicin, cisplatin and vinblastine among many others, in the hope of exerting their anti-tumor effect through ROS-dependent induction of apoptotic cell death [56]. Many of these agents aim to induce massive ROS production either directly or via the inhibition of cellular anti-oxidant defenses. More recently, these compounds have been further developed to enhance their tumor-targeting specificity, in order to avoid severe side effects and improve drug efficacy [56].

In view of this, high Bcl-2 expression may affect the efficacy of these ROS-inducing anti-tumor agents not only through inhibition of apoptotic cell death but also via direct regulation of the resultant oxidative stress through mitochondrial metabolic pathways, keeping the redox status constant while obliterating the death-inducing levels of ROS from these anti-tumor agents.

The discovery of this non-canonical function of Bcl-2 presents a paradigm shift in the understanding of tumor metabolism as well as the conventional roles that onco-proteins were expected to play in carcinogenesis. More importantly, it further underscores the importance of Bcl-2 inhibitors-based drug therapies as well as the significance of Bcl-2 expression levels in various tumors, which will define their metabolic and ROS profiles. These may prove to be predictive of the efficacy of ROS and metabolic-based therapies, whereby the inherent level of Bcl-2 expression may determine the treatment prognosis of the tumor. In this respect, Bcl-2 and mitochondrial metabolism come across as attractive therapeutic targets in the treatment of cancer.

5. Conclusion

Despite numerous reports implicating Bcl-2 as an anti-oxidant, a growing body of evidence clearly indicates Bcl-2 as a pro-oxidant with no intrinsic anti-oxidant properties under normal conditions. However, during oxidative stress conditions, apart from the heightened anti-oxidant defense response previously reported, Bcl-2 is also able to modulate mitochondrial respiration by regulating the activity of the rate-limiting, terminal enzyme COX to bring about an overall reduction in the by-production of O_2^-, keeping levels of ROS from reaching the point of detriment. With the increasing debate over the role of Bcl-2 as an anti- or pro-oxidant, the homeostatic behavior of Bcl-2 that we now understand, may justify and reconcile both schools of thought. This novel understanding of Bcl-2 extends its classical anti-apoptotic role into the realm of mitochondrial metabolism and redox biology, where its involvement further accentuates neoplastic progression and maintenance.

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