The Biology Behind

Targeting the Mitochondria: An Exciting New Approach to Myeloma Therapy


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Introduction

Multiple myeloma, a cancer of bone marrow plasma cells, is considered incurable with median survival being ~4 years. For the last 4 decades, MP 2 has been considered to be the mainstay of treatment for myeloma, producing responses in approximately 50–60% of patients (1). Since the introduction of MP, a number of combinations of cytotoxic drugs have been used to treat myeloma; however, no particular drug combination appears to be significantly superior to MP alone (2). Even the use of high-dose chemotherapy, whereas perhaps extending overall survival by several months, has not dramatically improved overall outcome. Essentially all of the patients who respond to cytotoxic chemotherapy will eventually relapse with drug-resistant disease.

Substantial progress has been made in the last decade to identify cellular mechanisms that confer clinical drug resistance. Multiple cytotoxic drug-resistant mechanisms have been identified in myeloma cells including increased expression of drug efflux pumps, increased apoptotic threshold, and increased ability to metabolize or detoxify cytotoxic agents (3–5). The multifactorial nature of drug resistance has made it difficult to reverse drug resistance, and overcoming one single mechanism is not likely to produce durable remissions (6). Another approach to improving clinical outcome that may be more rewarding is identifying new cellular targets or pathways that regulate myeloma cell growth and survival. One possible target that may offer new advances in the treatment of myeloma, and other cancers, is the mitochondria (7, 8).

Mitochondria have been shown to play a major role in programmed cell death. Moreover, these organelles are critical to cellular survival and growth because they are necessary for generation of energy by synthesizing ATP. Synthesis of ATP requires consumption of high amounts of oxygen, which routinely leads to the generation of ROS such as hydrogen peroxide, superoxide anion, and organic peroxides (9). These ROS can lead to cellular damage if they are not detoxified by antioxidative systems. The GSH redox system represents one of the most important cellular defense systems against oxidative stress, and mitochondria are known to have high levels of GSH. Enhancing production of ROS within mitochondria and inhibiting GSH production may lead to oxidative stress and the induction of apoptosis.

The apoptotic process in cancer cells can generally be classified into three distinct phases. In the first phase, proapoptotic signal transduction pathways are activated by various factors including cytotoxic drugs and cell surface death receptors. After this first phase, an intermediate phase of proapoptotic signals converge at the mitochondria resulting in the final phase of mitochondrial membrane potential loss and the release of cytochrome c, triggering an apoptotic caspase cascade. Unlike conventional cytotoxics, which trigger proapoptotic signal transduction pathways upstream of mitochondria, ATO appears to act directly on mitochondria to induce apoptosis (10, 11). ATO is thought to induce apoptosis by a several mechanisms that involve generation of ROS and loss of the cellular redox system (Fig. 1). In addition to generating ROS, ATO has been hypothesized to block glutathione transferase and glutathione peroxidase enzymes, which detoxify ATO by GSH conjugation and convert hydrogen peroxide into water, respectively. This combination of generating ROS and reducing the cellular redox system appears to directly damage mitochondria of susceptible myeloma cells (12, 13).

Recently, two groups reported that manipulation of the GSH redox system using either AA or BSO can additionally enhance apoptosis of human myeloma cells by ATO (12, 13). By separate mechanisms, both AA and BSO reduce GSH levels, and accentuate mitochondrial damage and apoptosis of myeloma cells. For AA, Grad et al. (12) hypothesized that auto-oxidation of AA to dehydroascorbate can result in the production of H$_2$O$_2$. Dehydroascorbate is then rapidly reduced back to AA by thioltransferase (Glutaredoxin) in a GSH-dependent manner. This rapid reduction of dehydroascorbate to AA results in decreased GSH levels and increased glutathione disulfide (Fig. 1). Indeed, the investigators found that AA potentiates ATO-mediated increases in the production of superoxide and increased disruption of mitochondrial membrane potential. In addition to the observations made in myeloma cell lines, the investigators re-
ported that myeloma cells from patients are more sensitive to the apoptotic effects of ATO when combined with AA. Similarly, both groups found that when ATO was combined with AA or BSO, the combinations were active in drug-resistant myeloma cell lines known to express various mechanisms of drug resistance including overexpression of drug resistance pumps, reduced steroid sensitivity, and overexpression of Bcl-xL. These preclinical studies indicated that ATO is active in myeloma cells resistant to classical myeloma treatments and that modulation of the cellular redox system additionally accentuates the activity of ATO.

As a follow-up of their preclinical observation that AA accentuates the activity of ATO, Bahlis et al. (14) in this issue report results from a limited Phase I clinical trial where ATO was combined with AA in six relapsed/refractory myeloma patients. The investigators found that ATO (0.25 mg/kg/day) plus 1000 mg/day AA was well tolerated during the treatment period, and the coadministration of AA did not appear to alter the degree, or profile, of ATO toxicity. Importantly, the use of AA did appear to reduce intracellular levels of GSH in peripheral blood mononuclear cells of myeloma patients, and the presumption is that the same would be seen in myeloma cells. Also encouraging, the investigators report that two of six patients had partial responses to treatment. The results of this Phase I trial together with results of correlative assays showing a desired effect of AA in reducing intracellular levels calls for a more detailed Phase II trial to determine response rates, and establish biochemical and cellular correlates of efficacy and toxicity.

However, the fact that patients had residual disease despite reduced intracellular GSH levels indicates that other mechanisms besides cellular thios are contributing to ATO resistance, and future studies must examine the development of alternative arsenic resistance mechanisms.

It should also be noted that another new agent has been described in preclinical studies to target mitochondria of myeloma cells. Dvorakova et al. (15, 16) report that Imexon, a cyanoaziridine, induces oxidative stress and apoptosis in human myeloma cells. In contrast to ATO, Imexon appears to directly impair mitochondria function by directly binding cysteine and glutathione, thereby decreasing levels of cellular thios and inducing oxidative damage of mitochondrial DNA and apoptosis. Also of note, whereas ATO is active in U266 myeloma cells and U266 Bcl-xL transfectedants, Imexon is inactive in this cell line. Therefore, whereas both ATO and Imexon appear to perturb mitochondrial function in a direct manner involving the GSH-redox system, these agents appear to be unique in their mechanisms of resistance.

These are exciting times in new drug development for the treatment of myeloma. For the first time in decades, new agents are being described that are unique in their mechanism of action compared with classic cytotoxic agents. Agents that target mitochondria of myeloma cells may represent a new hope for patients with myeloma, a disease that until recently few new advances had been made.

**REFERENCES**


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