INTRODUCTION

The cancer cell's "power plants" as promising therapeutic targets: An overview

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Abstract This introductory article to the review series entitled "The Cancer Cell's Power Plants as Promising Therapeutic Targets" is written while more than 20 million people suffer from cancer. It summarizes strategies to destroy or prevent cancers by targeting their energy production factories, i.e., "power plants." All nucleated animal/human cells have two types of power plants, i.e., systems that make the "high energy" compound ATP from ADP and P_i. One type is "glycolysis," the other the "mitochondria." In contrast to most normal cells where the mitochondria are the major ATP producers (>90%) in fueling growth, human cancers detected via Positron Emission Tomography (PET) rely on both types of power plants. In such cancers, glycolysis may contribute nearly half the ATP even in the presence of oxygen ("Warburg effect"). Based solely on cell energetics, this presents a challenge to identify curative agents that destroy only cancer cells as they must destroy both of their power plants causing "necrotic cell death" and leave normal cells alone. One such agent, 3-bromopyruvate (3-BrPA), a lactic acid analog, has been shown to inhibit both glycolytic and mitochondrial ATP production in rapidly growing cancers (Ko et al., Cancer Letts., 173, 83–91, 2001), leave normal cells alone, and eradicate advanced cancers (19 of 19) in a rodent model (Ko et al., Biochem. Biophys. Res. Commun., 324, 269–275, 2004). A second approach is to induce only cancer cells to undergo "apoptotic cell death." Here, mitochondria release cell death inducing factors (e.g., cytochrome c). In a third approach, cancer cells are induced to die by both apoptotic and necrotic events. In summary, much effort is being focused on identifying agents that induce "necrotic," "apoptotic" or apoptotic plus necrotic cell death only in cancer cells. Regardless how death is inflicted, every cancer cell must die, be it fast or slow.

Introduction

Despite the enormous amount of funding targeted for cancer research during the past half century by funding agencies throughout the world and by private donors, particularly in the U.S.A., a major victory in our ongoing war against this frequently fatal disease does not appear imminent. Thus, some reports predict even today that one in two men and one in three women are likely to die of cancer. Considering there are about 6.5 billion people in the world today (World Fact Book, Central Intelligence Agency, Office of Public Affairs Washington, D.C., July 2006), and about half are men and half are women, it can be estimated that if anti-cancer agents with long-term curative effects are not found soon, particularly for advanced "metastatic" cancers, that the cause of death for about 2.7 billion people, i.e., about 42% of the world's current population, will be cancer.

Although during the past century, a number of diseases have been seriously curtailed including those related to bacterial infection, polio, smallpox, and even heart disease, cancer is rapidly becoming "public enemy number one." Despite this, there is historical solace in the fact that bacterial

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infections that had posed a constant death threat to humans for at least 5,000 years were finally curtailed following the discovery in 1928 of penicillin in a laboratory mold by a single scientist, Sir Alexander Fleming (Fleming, 1929). This led to the isolation/manufacture of penicillin on a large scale and the discovery of other anti-bacterial agents that collectively have greatly benefited the whole world. Therefore, optimistically, if we view cancerous cells as sophisticated types of "infectious-like" cells that have developed and gone awry in our own bodies, i.e., exhibit the capacity to multiply, mutate, invade, spread (metastasize), and kill, we can believe also that natural agents may already exist, or synthetic agents can be made, that will selectively and repeatedly kill the major types of cancer regardless of stage.

Although in the U.S. alone, such success may impact negatively on some major pharmaceutical companies, reduce the size of the National Cancer Institute, and make the newly built, or to be built, Comprehensive Cancer Centers in almost all 50 states in the United States available for other health purposes, it would most importantly reduce greatly human suffering, increase life expectancy and the quality of that increased life, and hopefully also reduce income taxes on the general population who are supporting our never ending losing war on cancer.

Despite everyone's desire for one or more proven cancer cures/preventions in the immediate future, our continuing 36 year war with cancer in the United States, formally declared by President Richard Nixon upon signing the National Cancer Act (U.S. Govt. Print, 1971) shows no signs of ending soon. When applying this prognosis to the rest of the world, the situation is no better and in fact in many cases much worse. Thus, in the United States and in the rest of the world, we are still looking for our first "Magic Bullet" that will consistently destroy in different patients both primary and metastatic cancers regardless of their tissue of origin while exhibiting minimal toxicity to the human host. The major challenge is not to find agents that kill cancer cells. Rather, the real challenge is to find agents that kill cancer cells while leaving normal cells alone. To do this, one must target one or more phenotypes unique to cancer cells.

Cancers' major phenotypes as rational drug targets

Although cancer cells have many phenotypic differences from normal cells, two general categories stand out, i.e., those related directly to enhanced cell division (*molecular biological phenotypes*) and those related to fueling this enhanced cell division (*bioenergetic phenotypes*). Because cancer cells divide more rapidly than normal cells, it is expected that there will be certain enzymes involved in DNA and RNA synthesis that will be up-regulated or subjected to different patterns of regulation than in normal cells. Likewise, it is expected in cancer cells that the two energy (ATP) producing power plants "glycolysis" and the oxygen dependent "mitochondria" will be altered such that tumors comprised of such cells will be able to survive when oxygen is either plentiful or limiting.

In fact, the normal to cancer cell metabolic shifts suggested above do occur (Warburg, 1930; Weber and Lea, 1966; Weber, 2001), and are most evident in the more rapidly growing (more malignant types of cancers). Thus, enzymes involved in nucleic acid synthesis have been up-regulated whereas those involved in nucleic acid degradation have been down-regulated. This helps assure more rapid cell division. Likewise, in the most malignant, rapidly growing cancer cells the mitochondria have been down-regulated frequently resulting in fewer of these organelles (Schreiber et al., 1970; Reviewed in Pedersen, 1978), whereas enzymes involved in glycolysis have been up-regulated (Weber, 1968; Weinhouse, 1972; Bustamante and Pedersen, 1977). This "shift" allows cancer cells to assume an energetic advantage over normal cells in the sense that cancer cells can remain alive either when ample oxygen is present or when oxygen becomes limiting. Thus, cancer cells within tumors that have a limited blood supply (therefore less oxygen) or cancer cells close to a tumor's inner core, i.e., where blood vessels may not reach will remain viable for a longer period of time by relying more on ATP produced by glycolysis. It should be noted also that glycolysis (via intermediates like glucose-6-phosphate) plays a major role in biosynthesis of the cell's building blocks (i.e., proteins, phospholipids, fat, and nucleic acids). Thus, when a cancer cell's glycolysis is enhanced, it not only gains an advantage over normal cells in being able to produce ATP should oxygen become limiting, but also provides increased numbers of building blocks to help make more cancer cells (Arora and Pedersen, 1988).

Although the molecular biological and bioenergetic-related phenotypes in cancers seemed to be the most obvious two phenotypes to target in the middle of the last century, it will be noted below that the former was almost exclusively championed, and the latter almost completely ignored. Even when it was clear near the end of the last century that the almost exclusive focus on potential anticancer agents that target molecular biological phenotypes was not winning the war on cancer, the shift in focus was to signal transduction targets (Reviewed in Dancy and Sausville, 2003) rather than targets related to bioenergetics. Only very recently, after it has been learned that cell death pathways (particularly those involving apoptosis), have a close relationship to the cell's bioenergetic machinery (Reviewed in Jiang and Wang, 2004) has there been an overwhelming interest in seriously considering bioenergetic or bioenergetic-related phenotypes as potential anticancer drug targets.

Therapy for cancer, part I: An initial and persistent half century focus on targeting molecular biological phenotypes

Following World War II, the thrust of cancer research focused primarily on the development of new anticancer agents that targeted molecular biological phenotypes that were accentuated in cancer cells. Among these were derivatives of folic acid, a member of the vitamin B complex that is required for DNA synthesis via an enzyme named thymidylate synthase (Reviewed in McGuire, 2003). Such derivatives were first used by Sidney Farber and colleagues (Farber, 1950) at Harvard to treat acute lymphoblastic leukemia in children. This initial thrust into targeting DNA related processes was likely accelerated immensely after 1962 by the awarding of the Nobel Prize to James Watson, Francis Crick, and Maurice Wilkins for their work on the "double helix." Thus, many other agents were later either synthesized or isolated from natural sources to target nucleic acid synthesis/function in cancers. The NCI website (http://dtp.nci.nih.gov/docs/cancer/searches/standard_mechanism.html) list these agents under 6 different categories: *Alkylating agents* (e.g., cis-platinum, mitomycin c), *Antimitotic Agents* (e.g., taxol (paclitaxel), colchicines, vincristine), *DNA Antimetabolites* (e.g., ara c, hydroxyurea, and thioguanine), *RNA/DNA Antimetabolites* (e.g., 5-fluorouracil, methotrexate), *Topoisomerase I inhibitors* (e.g., campothecin and derivatives), and *Topoisomerase II inhibitors* (e.g., doxorubincin, daunorubincin).

Although a detailed analysis of the literature will certainly reveal many success stories with such agents either alone or in combination, cancer has continued to be a major health problem worldwide, the U.S. being no exception. This is despite other notable advances that include earlier detection methods, improved treatment centers, and more knowledge about the disease. Therefore, there has been a continued search for newer, more powerful, and specific agents, and hopefully one or more agents that might be called "the penicillin for cancer," or at the minimum "the penicillin" for one or more cancer types.

Therapy for cancer, part II: A shift toward agents that target signal transduction pathways

The end of the 20th century and our entry into the 21st century has seen some significant movement away from molecular biological phenotypes as the almost exclusive targets for anticancer drug development and some rather significant focus on signal transduction phenotypes (Dancy and Sausville, 2003). This new direction had its origin more than 3 decades earlier following the awarding of the Nobel Prize in 1971 to Earl Sutherland who while working with T. W. Rall on the mechanism of hormone action discovered cyclic AMP (Rall and Sutherland, 1958; Sutherland and Rall, 1958). Subsequently, many signal transduction pathways were discovered that commenced via either hormone binding or other types of induction. This led eventually to the additional discovery that the human genome encodes over 500 protein kinases that can be grouped into approximately 20 known families (Manning et al., 2002), thus revealing the extent to which signal transduction pathways are involved in modulating key events occurring in human cells.

Now, over 30 clinical trials are under way to defeat several types of cancers with either small molecule inhibitors of selected protein kinases or monoclonal antibodies to one or more of their domains (Fischer et al., 2003). Of these novel anticancer agents, Gleevec (Imatinib mesylate) and Herceptin (Trastuzumab) have received the greatest attention (Fischer et al., 2003). Gleevec is a small molecule ATP analogue that targets protein kinase domains in the proteins named BCR-Abl, ckit, and the platelet-derived growth factor receptor while Herceptin is a monoclonal antibody that targets the receptor tyrosine kinase named HER2. Remarkably, Gleevec has been approved by the FDA (http://www.fda.gov/cder/cancer/druglistframe.htm) on 9 different occasions in this new century for different cancers. Among these are chronic myelogenous leukemia (CML), malignant gastrointestinal stromal tumors (GIST), and newly diagnosed Ph+ chronic myelogenous leukemia. Herceptin, approved earlier by the FDA in 1998, is used in the treatment of metastatic breast cancer. Other signal transduction based drugs in clinical development are targeting pancreatic, lung, colorectal, stomach, ovarian, and prostate cancer (Fisher et al., 2003).

Despite the success today with Gleevec as an anticancer agent, particularly in patients with CML, it now seems clear that this agent in some patients can cause cardiotoxicity, i.e., left ventricle dysfunction (Kerkela et al., 2006; Strebhardt and Ullrich, 2006).

Therapy for cancer, part III: Targeting phenotypes related to bioenergetics

Death by necrosis

Necrosis is a term that has been associated more in the literature to death of normal cells as a consequence of tissue injury than to cancer therapy. Such injury to normal tissues may result because of a lack of oxygen, e.g., to the heart resulting in cardiac arrest or to the brain resulting in stroke. In either case, the mitochondria become compromised, glycolysis cannot fully compensate, cell ATP levels drop precipitously and death results. Death by necrosis is a quick way to die. To understand the targeting of cancer via agents that

induce necrosis requires some understanding of the bioenergetics/metabolism of cancers, particularly those that are referred to as "PET positive."

One of the problems with cancer as a disease in humans is that it is frequently asymptomatic and not detected until an advanced stage. At this stage such tumors are likely poorly differentiated and have undergone many changes. The most well known bioenergetic change (phenotype) of such cancers is their capacity to catabolize glucose at elevated rates to pyruvic acid, a significant portion of which is then converted to lactic acid and transported out of the cell (Warburg, 1930). This metabolic property of many cancers occurs even in the presence of oxygen and is referred to as the "*Warburg effect*" or "*high glycolytic phenotype*," the former designation after its discoverer, the German scientist Otto Warburg. In fact, PET analysis for cancer (Reviewed in Shields, 2006; Cherry, 2006) monitors the "Warburg effect" and is one of the most common detection methods for cancer used clinically throughout the world. Warburg received the Nobel Prize in 1931 for his pioneering studies on cell respiration but never lived to fully appreciate the clinical applications of his work.

It is important to note that in PET scan positive tumors not all the pyruvate derived from glucose catabolism is converted to lactic acid. Some enters the mitochondria and becomes oxidized, at least in part, to carbon dioxide and water, a process that involves the tricarboxylic acid cycle and the electron transport chain. *In fact, in those advanced "highly glycolytic" cancer cells that have been examined carefully, it is found that significant energy (ATP) is derived from both glycolytic and mitochondrial events (Aisenberg, 1961).* In one of the most extreme cases studied, mitochondrial ATP production was estimated at about 40% and glycolytic ATP production at about 60% (Nakashima et al., 1984). This is in sharp contrast to most normal cells where the mitochondria are responsible for over 90% of the ATP production.

From the above, one can see clearly why the "high glycolytic phenotype" serves the advanced cancer cell well and gives it an energetic advantage over those normal cells residing in its tissue of origin. Thus, the advanced cancer cell thrives when oxygen is either plentiful or reduced, and because of its rapid division can "crowd out" surrounding normal cells while subjecting them to a constant stream of acid ("chemical warfare"). In addition, once a solid tumor is formed within one human organ, some of its cancer cells can separate, sometimes as clusters, travel (metastasize) through the blood stream (Elshimali and Grody, 2006) where "food" (glucose) is plentiful, and eventually settle into a comfortable environment in one or more other body organs. Here, it will undergo multiple divisions until a new tumor is developed that will become vascularized and receive both oxygen and glucose from the blood of the human host. Thus, the new tumor in its new body organ will be using also both of its power plants (glycolysis and mitochondria) to provide the ATP essential for its survival and rapid growth (Fig. 1A).

To quickly arrest the growth of a tumor characterized by the "high glycolytic" phenotype where it is in fact fueled by significant amounts of ATP derived from both glycolysis and the mitochondria (See above discussion), it is essential to have an agent that will "selectively" destroy both "power plants" of the tumor while leaving the power plants in the surrounding normal tissue alone. That is, to subject the tumor to a quick death it is important to induce necrosis in each of its cells while doing minimal harm to normal cells.

One approach ("Trojan Horse") is to screen for and identify an agent that will preferentially enter tumor cells, and once inside destroy both its "power plants," i.e., glycolysis and mitochondria. This would cause a rapid decline in cell ATP levels inducing death mainly by "necrosis" (Fig. 1A). The second approach ("Backdoor Block") is to identify an agent that will selectively block the exit of lactic acid from the tumor cells (i.e., inhibit the lactate transporter) without doing the same to normal cells. By blocking the exit of lactic acid, the tumor cells' internal pH will be lowered and this increased acidity will have a deleterious effect on both power plants resulting in death predominantly by necrosis.

The first approach ("Trojan Horse") noted above, i.e., "to identify an agent that will preferentially enter tumor cells but not normal cells, and once inside the tumor cells destroy both power plants (Fig. 1A)," has been accomplished. Thus, following the discovery by Ko et al. (2001) that the lactic acid analog 3-bromopyruvic acid (3-BrPA) inhibits hepatocellular carcinoma cells in tissue culture, advanced cancers (hepatocellular carcinomas) were completely eradicated (Fig. 1B) with this agent in 19 out of 19 animals without harm to the animals (Ko et al., 2004; Also see Supplement to Ko et al., 2004). Moreover, all animals lived thereafter a normal life without tumor recurrence. [A unique aspect of this study that is rarely presented in published work to verify therapeutic success over a given type of cancer is that photographs of animals bearing healthy growing tumors to be treated are presented followed by photographs of the same animal at different stages of treatment. In addition, photographs of the tumor free animals are shown after the tumors have disappeared. Finally, photographs revealing the results of PET, the analysis that monitors the high glycolytic cancer phenotype, are shown before and after treatment. Significantly, these two types of visual data verify tumor eradication. What is more common to see in many animal studies with a potential anticancer agent is the absence of such photographs/data during the treatment process. Rather, a Kaplan-Meier survival curve (Allison, 1995) is shown that reveals, relative to untreated controls, how many of the animals' lives have

been extended and for how long. Unfortunately, this survival curve also reveals for most, if not all cases, that the agent being tested rarely "cured" the animal of its cancer, unlike 3-BrPA in the study noted above by Ko et al., 2004]. Finally, it should be noted also that an earlier study involving the use of 3-BrPA to treat liver implanted dermatoid tumors in rabbits also showed impressive results, although animal survival was not monitored (Geschwind et al., 2002).

The potent anticancer agent 3-BrPA has been called an "energy blocker" (Foubister, 2002) for highly malignant cancer cells as it inhibits both of their "power plants" (glycolysis and mitochondria) while leaving normal cells alone. Like a "Trojan horse" 3-BrPA likely enters cancer cells through the same "gates," i.e., lactic acid transporters, that lactic acid goes out (Fig. 1A). Normal cells are spared as they have a much lower number of such transporters. (The transporter for lactic acid is in the "mono-carboxylic acid transporter family").

Studies involving the second approach ("Backdoor") noted above to selectively arrest the growth of highly glycolytic cancers had its roots three decades ago in studies by Spencer and Lehninger (1976). These investigators showed that lactic acid transport could be inhibited by alpha-cyano-4-hydroxycinnamate (ACCA), alpha-cyano-3-hydroxycinnamate, or DL-p-hydroxyphenyl-lactate. Subsequent studies pursued by Johnson et al. (1980) showed that lactic acid transport could be inhibited also by isobutylcarbonyl lactayl anhydride (iBCLA) resulting in a decrease in intracellular pH. Although these early studies focused on identifying inhibitors of lactate transport out of tumor cells, more recent work has applied this approach to cancer therapy. In these studies one first inhibits lactic acid exit ("Backdoor") from highly glycolytic cancer cells and then allows time for the acid concentration to rise inside the cell, lower the pH, and induce cell death. Specific examples are the recent studies of Mathupala et al. (2004) who were able to inhibit dramatically (92%) the viability of malignant glioma cells (U-87) using small interfering ribonucleic acids specific for monocarboxylic acid transporters 1 and 2 (i.e., MCT1 and MCT2), and the studies of Fang et al. (2006) who were able to diminish cell viability in neuroblastoma cells using the lactic acid transport inhibitor (ACCA) noted above.

Death by apoptosis

"Apoptosis" is defined as the process of programmed cell death. Unlike necrosis, apoptosis is not a quick way to die. The pioneers in the discovery of this process were Sydney Brenner, H. Robert Horvitz, and John E. Sulston all of whom shared a Nobel Prize in 2002. The term "apoptosis" was used by Kerr et al. (1972) in order to distinguish this natural type

of cell death from that which results from "necrosis." In biochemical terms the author of the current article has already defined necrotic cell death above as death that results when a living cell is deprived of both its ATP sources (Fig. 1A), i.e., glycolysis and mitochondria. Apoptotic cell death is quite different (Fig. 1A). In this case, the cell suicide program of events commences with a death signal (i.e., "it's time to die") that may originate either within the cell or from external signals acting on membrane receptors. Whatever the case, i.e., an intrinsically initiated pathway for cell death or an extrinsically initiated pathway (Fig. 1A), central players in the death events include mitochondrial proteins, in particular cytochrome c that is released, and a number of proteins that reside outside the mitochondria that include proteases referred to collectively as caspases (Reviewed in Jiang and Wang, 2004).

During cell life cytochrome c is required in our mitochondria to participate in the final stages of the biological oxidation of food that we consume (Reviewed in Hosler et al., 2006). Interestingly, it is loosely bound to the outer surface of the inner membrane where it transfers electrons from complex III (b-c$_1$ complex) of the mitochondrial electron transport chain to complex IV (cytochrome oxidase), the terminal complex that reduces the oxygen that we consume to water. This overall process of electron transport to molecular oxygen via the mitochondrial electron transport chain yields the free energy necessary to drive the synthesis of ATP that is needed for cell growth and development. If cytochrome c is not "in place" within the mitochondrial electron transport chain, the critical process of electron transport to molecular oxygen will come to a screeching halt, and no ATP will be synthesized by the ATP synthase. If glucose is present, which it usually is in a living system, the cells lacking the capacity to make ATP via their mitochondria may still survive, at least temporarily from ATP that is produced by glycolysis. However, to assure that the cell does not survive indefinitely when cytochrome c is released, a number of proteases are present called caspases, and with the help of cytochrome c facilitate the cell death process via what is referred to as the caspase cascade (Jiang and Wang, 2004). Numerous publications (Jiang and Wang, 2004; Neuzil et al., 2006; Cereghetti and Scorrano, 2006; Goodsell, 2004) have dealt with how cytochrome c is released from mitochondria in the initial stages of the cell death program, its interaction with a complex called the apoptosome, and subsequent activation of the caspase cascade leading to cell death.

In contrast to normal cells which turnover, and in so doing must undergo cell death on a programmed schedule, cancer cells have become immortalized. Although there are likely a number of factors involved in this process, one of the most critical is Type II hexokinase (hexokinase II). This crucial metabolic enzyme was first shown in a collaborative

study involving the author's laboratory and that of Marco Colombini at the University of Maryland to bind to the outer mitochondrial membrane "pore" protein VDAC (Nakashima et al., 1986). This hexokinase II –VDAC interaction has more recently been shown to inhibit the release of cytochrome c from mitochondria (Pastorino et al., 2002). Thus, hexokinase

II plays a major role in immortalizing cancer cells, particularly those that exhibit the high glycolytic phenotype and are the most rapidly growing. In a recent article (Mathupala et al., 2006) coauthored with Young Ko, we have referred to hexokinase II as a "facilitator and gatekeeper of malignancy" when bound to mitochondria. In addition, we have predicted

A. INDUCING CELL DEATH IN CANCER
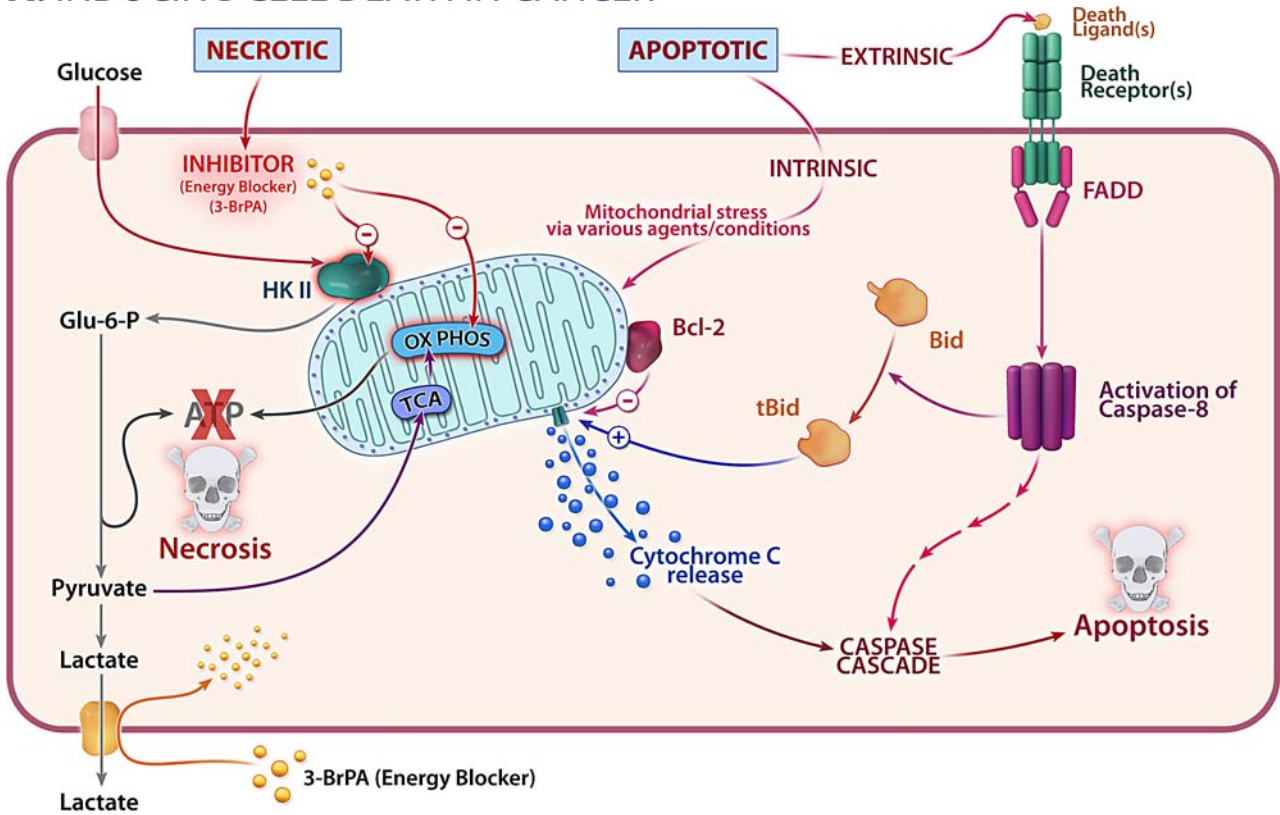
B. THERAPY FOR CANCER TARGETED AT POWER PLANTS
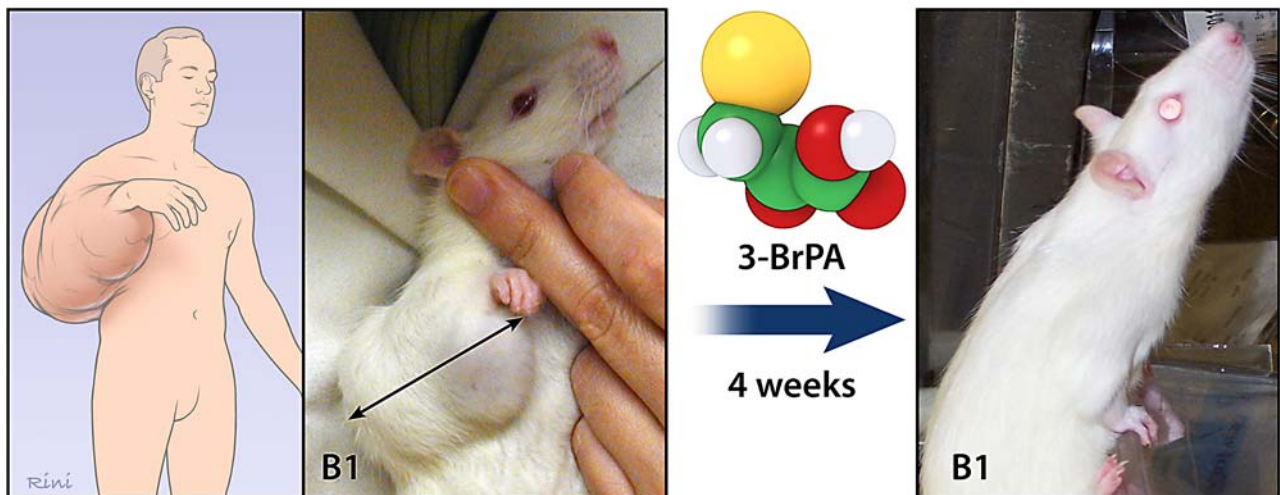
◄ **Fig. 1** (**A**) *Inducing Cell Death in Cancer.* The figure shows in simplified form two different approaches to inducing cell death in cancer via the processes "*necrosis*" and "*apoptosis.*" To induce cancer cell death by the process of "*necrosis*" (left), one must find an agent or condition (or both) that will destroy ATP production by the cancer cells' two distinct types of power plants, glycolysis and mitochondria, while leaving normal cells alone. This approach is best applied to cancers that are advanced (rapidly growing, poorly differentiated, and "PET positive" where "PET" = Positron Emission Tomography). In a collaborative project involving the author's laboratory a small molecule, 3-bromopyruvate (3-BrPA), was selected by Dr. Young Ko from a screen of potential inhibitors of energy metabolism (Ko et al., 2001). It was shown to kill aggressive hepatocellular carcinoma cells (liver cancer cells) in tissue culture and under the same conditions to have little or no effect on normal liver cells (hepatocytes). Cancer cell killing resulted from the rapid decline in cell ATP because 3-BrPA inhibited both power plants (glycolysis and mitochondria) forcing the cells to die mainly by necrosis. Cancer cell killing by 3-BrPA, while sparing normal cells, is believed to occur by a "Trojan horse" type mechanism, where the 3-BrPA enters through the same "gates" (monocarboxylic acid transporter) that lactic acid, the end product of cancer glycolysis, goes out. Once inside the cancer cells, 3-BrPA, an alkylating agent, then inhibits critical enzymes responsible for ATP production by both glycolysis and by the mitochondria. Most normal liver cells (hepatocytes) are likely spared because they have much fewer transporters for lactic acid. To induce death by "*apoptosis*" (right) there are described in the extensive literature on this subject primarily two different pathways "*intrinsic*" and "*extrinsic*" (see right side of figure). Numerous agents, in fact the majority of those shown in Table 1, have been purported to induce cell death by "*apoptosis*." A major feature of apoptotic pathways is the release of cytochrome c from the mitochondria that helps activate proteases known as caspases that then help promote cell death. As indicated in the text, some investigators are now finding that cell death does not always occur by a strictly apoptotic or strictly necrotic pathway, but rather by a combination of the two. (**B**) *Therapy for Cancer Targeted at Power Plants.* The Figure illustrates that advanced cancers can be completely eradicated by destroying both of their power plants (ATP production factories), i.e., glycolysis and mitochondria. In the figure, Rat B1 (center panel) is shown bearing a large (~3 cm) advanced tumor (liver cancer) that was derived by injecting AS-30D hepatoma cells into the upper back. A tumor formed and expanded quickly, extending from the upper back to the paw of the animal. The left panel shows how large the tumor might appear when projected onto a human subject. The tumor is extremely aggressive, and if cells derived from the tumor had been injected into the peritoneal cavity of the animal and left untreated with 3-BrPA, death of the animal would have occurred in less than two weeks. Cells comprising the tumor are known to exhibit an abnormal energy metabolism, i.e., they have a high glycolytic rate where much of the glucose consumed is converted to lactic acid, even in the presence of oxygen (Warburg effect). The mitochondria are also fully functional and together with glycolysis bear a nearly equal role in providing ATP for cell growth. Upon treating the animal with the small chemical agent 3-bromopyruvate (3-BrPA) for about 4 weeks the cancerous tumor is completely eradicated and does not return throughout the life of the animal. The agent 3-BrPA is an "energy blocker" and inhibits both power plants (glycolysis and mitochondria) within each cell comprising the tumor. These studies were carried out in a project led by Dr. Young Ko and resulted in the eradication of advanced cancers in 19 out of 19 animals. Figure 1B has been reproduced in slightly modified form from Figure 3C of the following published article: Ko YH, Smith BS, Wang Y, Pomper MG, Rini DA, Torbenson MS, Hullihen, J, Pedersen PL (2004) "Advanced Cancers: Eradication in all cases using 3-bromopyruvate therapy to deplete ATP" Biochem Biophys Res Commun 324:269–275. (Permission granted from Elsevier, Copyright 2004).

that this is likely the case for most advanced cancers that are found to be PET positive (Mathupala et al., 2006).

In terms of developing novel cancer therapies by taking advantage of the cancer cell's capacity to avoid cell death, it seems clear that one must identify agents non-toxic to humans that have the capacity to induce the release of cytochrome c from cancer cells while leaving normal cells alone. Based on the discussion above, a key target for such agents would be hexokinase II, VDAC or both, with the objective of disrupting the hexokinase II – VDAC interaction. [Although the titles of 500–1000 publications appear when one searches Pub Med for agents that induce the release of cytochrome c from mitochondria of cancer cells, *many of these publications fail to provide information about the effect of the same agent on cells or tissue from which the cancer cells were derived.* Although this control is less important when the compound being studied is derived from natural food sources that humans have been consuming for centuries, it is nevertheless important, and much more important for new agents synthesized *de novo* that have no historical background in the diets of animals or humans].

Therapy for cancer, part IV: Death induction mechanisms involving both necrosis and apoptosis

From the above discussions in this introductory mini-review, the reader may come to believe that cancer cells can be induced to die by either a strictly necrotic mechanism (quick death due to rapid dissipation of cell ATP reserves) or by an apoptotic mechanism (slower death involving many players). However, the following example that has its origin many years ago, and others noted thereafter, would lead us to believe that a number of cell death programs may involve both necrotic and apoptotic components.

Long before cell death pathways became dinner table conversation by scientists throughout the world, a single group led by Lan Bo Chen at Harvard Medical School was already selectively targeting the mitochondria of cancer cells with agents that impair their capacity to make ATP. These landmark studies of Chen and colleagues (Lincoln et al., 1980; Weiss et al., 1988; Chen, 1989) that commenced over 20 years ago and are still ongoing today relied on knowledge gained from the chemiosmotic hypothesis of Peter Mitchell (Mitchell, 1961; Greville, 1969), a Nobel Prize awardee in 1978. Significantly, Mitchell's hypothesis and subsequent experiments taught us that ATP synthesis from ADP and P_i at the level of the ATP synthase in mitochondria is driven by an electrochemical gradient of protons across the inner membrane generated by a functional electron transport chain. The net free energy derived from the electrochemical

proton gradient consists of that resulting from the pH gradient (H$^+$ outside, OH$^-$ inside) as well as the membrane potential that results from the positively charged protons being on the outside of the inner membrane and the negatively charged hydroxyl ions remaining on the inside. Using this new knowledge at the time, Chen and colleagues (Reviewed in Lincoln et al., 1980; Weiss et al., 1988; Chen, 1989) demonstrated that positively charged lipophilic compounds are preferentially "sucked up" by cancer cell mitochondria consistent with such mitochondria having a higher membrane potential (plus outside, minus inside) than that of normal cells. As might be expected, Chen's laboratory (Bleday et al., 1986; Weiss et al., 1987; Sun et al., 1994; Koya et al., 1996) went on to show that such agents exhibit anticancer activity, not only in tissue culture but in live animals that resulted in significant reduction in tumor growth. Those human cancers tested in animals (nude mice) included those derived from the ovaries, colon, skin, kidney and prostate.

In one of the above referenced studies Weiss et al. (1987) used the doubly charged lipophilic compound dequalinium that for many years had been used as an antimicrobial agent in over-the-counter mouthwashes, lozenges, and ointments. Significantly, these workers showed that this agent is more effective than seven out of eight established anticancer agents (DNA targeting) in prolonging the survival of mice implanted with a mouse bladder carcinoma. In another of the above referenced studies Koya et al. (1996) found similar results with other positively charged, lipophilic compounds, e.g., MKT-077 that in animal studies inhibits the growth in nude mice of a human renal carcinoma and a prostate carcinoma. In fact, some clinical trials have been conducted with MKT-077 (Britten et al., 2000).

Recently, two decades after the above studies with dequalinium (Weiss et al., 1987), a detailed study of its toxic effects on two different human leukemia cell lines has been performed by Sancho et al. (2007). They concluded that one of the two cell lines (K562) is resistant to apoptosis and dies by necrosis under the conditions studied while the other cell line seems to die by apoptosis followed by secondary necrosis. In other recent work with a variety of anticancer agents investigators are concluding also that a number of these are not strictly apoptotic in their mode of action. Rather, they act via a combination of apoptotic and necrotic events referred to by Lemasters and colleagues (Malhi et al., 2006) in a recent review as "necrapoptosis" or "aponecrosis." For example, in addition to dequalinium, the anticancer agents photofrin (HPD) used in photodynamic therapy (Marcinkowska et al., 2001), and salicylate (Oh et al., 2003) have been shown to produce also both apoptotic and necrotic changes in malignant cells. It is possible that when investigators study in more detail agents that they initially believed induced cancer cell death by apoptosis, they may discover that both apoptotic and necrotic events were involved.

Therapy for cancer, part V: Which anti-cancer agents of the future will prove superior in eradicating the most cancers, those that induce necrotic, apoptotic, or apoptotic + necrotic cell death?

Only time will provide the answer to this question as we are still searching for one or more "penicillins" for cancer. The author's bias is that the best agents will be those that selectively induce cancer cell death by necrosis, meaning any agent that will quickly deplete the cancer cells of their ATP reserves while leaving normal cells alone. One approach to inducing necrosis quickly in cancer cells that exhibit the "Warburg effect" is the use of the lactic acid analog 3-BrPA that has been described already above. The advantage to identifying additional agents like 3-BrPA is that they are quick in dissipating cell ATP levels as they block ATP production by both power plants (glycolysis and mitochondria). In addition, if like 3-BrPA, they may have minimal effects on normal tissues at the dose(s) needed to eradicate tumors. A modification of the approach of Ko et al. (2004) has been used recently by Huang and colleagues (Xu et al., 2005) to kill lymphoma and leukemia cells. In this case, the agent 3-bromo-2-oxopropionate-1-propylester (3-BrOP) is used together with rapamycin, with the former agent being converted to 3-BrPA upon cell entry. Rapamycin is an inhibitor of the mTOR pathway that plays an important role in regulating nutrient metabolism and promoting the growth and survival of the leukemia cells.

Table 1 provides a sampling of agents that target one or both of the cancer cell's power plants to produce cell death. A number of these agents are believed to cause cell death via apoptosis. In other cases, however, cell death is caused primarily by necrosis, e.g., 3-BrPA. Finally, in other cases cell death likely occurs via a combination of apoptotic and necrotic events that will be revealed in future studies.

In the papers that follow in this review series Dao M. Nguyen and Mustafa Hussain (NIH, Bethesda, MD, USA) and Valdimir Gogvadze and Boris Zhivotovsky (Karolinska, Institutet, Stockholm, Sweden) *provide more in depth overviews of the cell death process and the role of mitochondria*. Kang-Beom Kwon, Byung-Hyun Park and Do-Gon Ryu (Wonkwang University, Jeonbuk, Korea) *review the use of nutritional supplements and herbs in chemotherapy* and their capacity to induce mitochondrial apoptosis. Helen Pettersson, Jenny Karlsson, Alexander Pietras, Ingrid Ora, and Sven Pahlman (Lund University, Malmo, Sweden and associated hospitals) *review the capacity of arsenic trioxide to induce neuroblastoma cytotoxicity*. Kevin Cullen, Zejia

Table 1 A "sampling" of agents with anticancer potential that directly or indirectly target "power plants" to induce cell death

The "sampling" of agents listed below have been shown to induce cancer cell death. Where cell death has been demonstrated, it resulted in some cases by what is called "necrosis," i.e., via an inhibitory effect of the agent or condition on the ATP producing capacities of both "power plants," glycolysis and mitochondria. The agent 3-bromopyruvate (3-BrPA) is used as an example in this introductory article. In other cases, cell death resulted by what is called "apoptosis." Here, direct or indirect effects of the listed agent on the mitochondria induce the release of the electron transport chain member named "cytochrome c," a protease (caspase) activator. Finally, in a third case a listed agent may induce cell death via processes that involve both "apoptotic" and "necrotic" components. Dequalinium (lipophilic cation), salicylate, and the photosensitive agent photofrin are examples. An asterisk tagging an agent indicates that it has been tested clinically for its capacity to inhibit one or more human cancer types or is currently being tested. The cancer killing action of those agents that are shown in "*italics*" are discussed or referred to in the minireviews that comprise this volume (39-1) of the *Journal of Bioenergetics and Biomembranes*. Below in this review some brief comments are made about the effect of some of the agents listed in this table on the two power plants of tumor cells.

AA1 (lipophilic cation), ABT-737*, *Arsenic Oxides**, Betulinic Acid, *3-Bromopyruvate, 3-Bromo-2-oxopropionate-1-propylester (3-BrOP)/Rapamycin, Berberine, Capsaicin, Carotene (beta), Cisplatin*, Dequalinium* (lipophilic cation), 2-Deoxyglucose, *Diallyl disulfide,* Dichloroacetic Acid, *δ-Elemene, Eugenol*, Epigallocatechin gallate (EGCG), Gallium nitrate, GX015-070*, *Jasmonates (salicylate, acetyl salicylic acid, i.e., aspirin, methyl jasmonate, jasmonic acid and cis-jasmone), Hematoporphyrin derivative (HpD, Photofrin II*), Lonidamine*, Lycophene, MKT-077* (Lipophilic cation)*, Motexofin Gadolinium*, Oblimersen*, PGMtide, *Reservatol*, Retinoids (ATRA)*, *RNA_i, Selenite, Selenodioxide, Scopoletin (Coumarin derivatives), α-tocopheryl succinate (α-TOS), Triptolide*, Triptycene Analogs, *Thymoquinone, Velcade (bortezomib)**.

Yang, Lisa Schumaker, and Zhongmin Guo (University of Maryland, Marlene and Stewart Greenebaum Cancer Center, Baltimore, MD) *review evidence that the mitochondria are a critical target of the chemotherapeutic agent cisplatin* in head and neck cancer. Nataliea Goldin, Alina Heyfets, Dorit Reischer, and Eliezer Flescher (Tel Aviv University, Israel) *discuss mitochondria-mediated ATP depletion by anticancer agents of the jasmonate family*. Andrea Lisa Holme and Shazib Pervatz (National University Medical Institutes, Singapore (Holme and Pervatz) and Yong Loo Lin School of Medicine and NUS Graduate School of Integrative Sciences and Engineering, National University of Singapore (Pervatz) *review the role of the increasingly popular reservatol in cell fate decisions*. Jeri Neuzil, Ruth Freeman, L-F Dong, X-F Wang, Jeffrey Dyason, S.J. Ralph (Griffith University, Southport, Qld., Australia), Lubomir Prochazha (Veterinaary Research Institute, Brno, Czech Republic) and Immuo Scheffler (Univ. California, San Diego, CA.) *discuss the role of "Mitocans" as anticancer agents with special emphasis on vitamin E analogues*. Saroj Mathupala (Karmanos Cancer Institute, Detroit MI.), Chaim Colen, Prahlad Parajuli (Wayne State University, Detroit, MI) and Andrew Sloan (H. Lee Moffitt Cancer Center, Tampa Fl.) *review their work on targeting the exit of lactate from gliomas*. Pratima Nangia-Makker, Susumu Nakahara, Victor Hogan, and Avraham Raz (Karmos Cancer Institute, Wayne State University, Detroit, MI) *discuss the role of Galectin-3, a member of a family of carbohydrate binding proteins, in the regulation of apoptosis*. Russell Hilf (University of Rochester) *reviews photodynamic therapy with mitochondria as the target*. Finally, Hidemi Rikiishi (Tohoku University Graduate School of Dentistry, Aoba-ku, Sendai, Japan) *reviews apoptotic cellular events for selenium compounds involved in cancer prevention*.

Additional comments related to anticancer agents summarized in Table 1

As it concerns anti-cancer agents listed in Table 1 that are not covered in the mini-reviews comprising this volume (J. Bioenerg. Biomemb. 39-1) or have been mentioned only briefly, some additional information is listed below.

AA1 is a monovalent lipophilic cation that expresses potent anti-tumor activity in animal models implanted with cells from the following cancers: mouse bladder, human melanoma, or human ovarian (Sun et al., 1994). *ABT-737* is a bcl2 antagonist that is being tested clinically for therapy for chronic lymphocytic leukemia (Del Gaizo Moore et al., 2007). *Betulinic acid* (BA) is a pentacyclic triterpene found in many plant species including the bark of the white birch Betula alba (Rzeski et al., 2006). It decreases the expression of bcl-2 and cyclin D1, inhibits proliferation, migration and induces apoptosis in some cancer cells. *Berberine* is a natural cholesterol reducing product that exerts anti-tumor effects independently of the mevalonate pathway (Issat et al., 2006). *Capsaicin,* a component of red peppers and a vanilloid receptor agonist, inhibits the growth of some prostate cancer cells by a mitochondrial mediated process (Athanasiou et al., 2007).

Carotene (Beta) obtained from fruits and vegetables induces apoptosis in some human tumor cells (Palozza et al., 2003). The glucose analog *2-deoxyglucose* blocks one of the cancer cells' two power plants, i.e., glycolysis. It does this at the first enzymatic step, i.e., the hexokinase step that normally uses ATP to phosphorylate glucose to glucose-6-P. When 2-deoxyglucose is present it is phosphorylated by hexokinase to give 2-deoxyglucose-6-P that cannot be further metabolized. Recently, 2-deoxyglucose has been tested on a variety of cancer cell lines (Zhang et al., 2006). Depending

on the cell line, one or more of the following effects was observed: proliferation arrest without apoptosis, strong cell cycle arrest accompanied by moderate apoptosis induction, and finally massive apoptosis. *Diallyl disulfide,* a component of oil based garlic extracts, induces apoptosis and histone hyperacetylation in prostate cancer cells (Arunkumar et al., 2006). *Dichloroacetic acid* (DCAA) is a by-product of drinking water disinfection. It has been shown recently to suppress a mitochondrial-K^+ channel axis with its normalization promoting apoptosis and inhibiting cancer growth in an animal model (Bonnet et al., 2007). *Elemene (delta)* derived from the Chinese herb Curcuma Wenyujin has the capacity to induce apoptosis in Hela cells (Wang et al., 2006a). *Epigallocatechin gallate*, a component of green tea, has been found to promote apoptosis in bladder cancer cells (Qin et al., 2007) while *eugenol* derived from cloves has been shown to have apoptotic and anti-proliferative effects on malignant melanoma (Pisano et al., 2007). Gallium nitrate is a *metallodrug* with clinical efficacy in treating non-Hodgkins lymphoma via what appears to be a mitochondrial directed apoptotic process (Chitambar et al., 2006). *GX15-070* is a Bcl-2 inhibitor that has the capacity to induce apoptosis in chronic lymphocytic leukemia cells (Campas et al., 2006).

Lonidamine is an indazole-3-carboxylic acid used in clinical trials for the treatment of advanced breast, ovarian, and lung cancer (Reviewed in Di Cosimo et al., 2003). Similar to 3-BrPA discussed earlier in this review lonidamine has the capacity to inhibit energy (ATP) production. It has been reported also to induce apoptosis in human HepG2 (hepatocarcinoma) cells by a mechanism involving release of cytochrome c (Li et al., 2002), a process that appears to be facilitated by prior knockdown by RNAi of the adenine nucleotide translocator 2 (ANT2) (Le Bras et al., 2006). Thus, lonidamine would appear to have the capacity to promote cancer cell death by a combination of mechanisms involving necrosis and apoptosis.

Lycophene, found in tomatoes, has been shown to exhibit growth inhibitory effects on prostate cancer cells, i.e., those that are androgen-independent, and to induce apoptosis (Tang et al., 2005). *Motexafin gadolinium,* a porphyrin related compound, has been shown to induce the mitochondrially-mediated apoptotic pathway in the HF-1 lymphoma cell line following loss of cytochrome c from the mitochondria (Chen et al., 2005) and appears also to show promise in treating brain metastasis (Richards and Mehta, 2007). *Oblimersen,* a Bcl-2 antisense oligonucleotide, has been used clinically as therapy for metastatic melanoma (Eggermont, 2006) and Waldenstrom's macroglobulinemia (Frankel, 2003). *PGM-tide* is a peptide derived from phosphoglycerate mutase an enzyme required for the glycolytic pathway to function. It has been shown to inhibit glycolytic flux and to induce growth arrest in several tumor cell lines (Engel et al., 2004).

Retinoids, especially all-trans-retinoic acid *(ATRA)*, has been used clinically in the treatment of patients with leukemia, e.g., non-Hodgkin lymphoma, and also in the treatment of patients with neuroblastoma. In the non-Hodgkin lymphoma cell line (HT58), ATRA has been shown at an early stage to depolarize the mitochondrial membrane and is believed to induce apoptosis (Barna et al., 2005), whereas in neuroblastoma cells it is known to induce apoptosis in two different cell lines CHP 134 and NB-39-nu (Niizuma et al., 2006). Retinoids are also believed to be involved in cancer chemoprevention (Shen, 2004). *Triptolide,* a diterpenoid triepoxide, has its origin in Chinese herbal medicine (Chen, 2001) and has been shown to induce growth arrest and apoptosis in colon cancer cells (Ko et al., 2006), leukemia cells (Carter et al., 2006), and cholangiocarcinoma cells (Panichakul et al., 2006). *Triptycene analogs,* i.e., triptycene bisquinones, are currently being studied in some detail for their anticancer properties (Wang et al., 2006b), and have been shown to trigger apoptosis in the HL-60 myeloblastic leukemia cells (Perchellet et al., 2004). *Thymoquinone* is derived from a herb called the "black seed" that grows in countries near the Mediterranean Sea and India, and has been shown to induce apoptosis in HL-60 myeloblastic leukemia cells (El-Mahdy et al., 2005) and also in colorectal cancer cells (Gali-Muhtasib et al., 2004). Finally, *Velcade (Bortezomib),* a proteasome inhibitor has been shown to trigger caspase-dependent apoptosis in multiple myeloma cells (Roccaro et al., 2006) and is used clinically in the treatment of multiple myeloma (Badros et al., 2006).

Final Note: The author apologizes for not being able to do justice to the vast literature on the subject of this introductory mini-review, in particular that which is related to Chinese herbal medicine. In addition to the informative mini-review by Kang-Beom Kwon, Byung-Hyun Park and Do-Gon Ryu (Wonkwang University, Jeonbuk, Korea) in this mini-review series, the author would like to refer readers also to the recent review of Efferth (2006). Also, the author notes that there have been other reviews that have touched on the topic of this introductory article. For a more complete picture or a different point of view, the reader may wish to consult one or more of these earlier reviews (Galluzzi et al., 2006; Pelicano et al., 2006; Andre et al., 2006; Bouchier-Hayes et al., 2005; Don and Hogg, 2004).

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