ADVANCES IN DRUG DEVELOPMENT

Current Developments in Oncology Drug Research

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Targeting the Anti-apoptotic Signaling Pathway

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H&O What are the key processes that control apoptosis?

CT What we have learned over the past 20 years of studying apoptosis is that it is a core signaling pathway available to all cells in the body. This was a surprising discovery because it meant that cells that we want to live in our bodies for many years without being compromised or dying, as well as all cells that we would like to be able to jettison whenever they are not needed (eg, excess immune cells after an infection is over or excess skin cells after they have served out their usefulness), have an ability to execute themselves or be executed by the apoptotic process within 15 minutes.

There are 2 basic mechanisms that initiate the same core program of what is called programmed cell death or apoptosis: the intrinsic pathways and the extrinsic pathway. In the intrinsic pathway, cells initiate the decision to die on their own through intrinsic sensing mechanisms and signal transduction. The molecules that act in this form of cell death involve the BCL-2 family, which regulates the integrity of the intracellular organelles to which they are distributed; this includes the mitochondria and the endoplasmic reticulum. Cell death is initiated by the pro-apoptotic BCL-2 family members, the best characterized of which are BCL-2 associated X protein (BAX) and BCL-2 homologous antagonist killer (BAK).

The BCL-2 family was first discovered because of a chromosomal translocation in follicular lymphomas that juxtaposed the immunoglobulin enhancer to the gene that became known as BCL-2 (B-cell lymphoma/leukemia associated gene 2). BCL-2 localizes to the same membranes as BAX and BAK, but its job is to directly and indirectly oppose the ability of BAX and BAK to kill a cell. It binds up BH3 proteins, which share a homology in a single domain with a BCL-2 protein, and it directly inhibits the function of BAX and BAK.

The morphologic features of programmed cell death are carried out by enzymes called caspases, which are cysteine proteases that cleave after aspartic acid residues. Caspases affect the order dissolution of the cell while maintaining the plasma membrane integrity. Thus, the cell initiates its own destruction by degrading its DNA, a vast majority of its protein, and most of its intracellular organelles. The dying cells expose phosphatidylserine ("eat me" signals) that allow macrophages in the environment to recognize them as undergoing this programmed phase of cell death and to essentially engulf and clear them from the body in a noninflammatory and effective manner that does not disrupt normal physiology. There is a growing appreciation that apoptotic signals not only put "eat me" signals on their surface, but that some of the molecules that are created during caspase degradation act as chemical gradients to recruit phagocytic cells into the environment in order to clear cells that have not been cleared rapidly enough by their neighbors.

The intrinsic pathway allows the cell to recognize various perturbations in its intracellular integrity, and modify a series of proteins—at the transcriptional, post-transcriptional, and translational levels—to enact activation of BAX and BAK. Their activation functions to disrupt mitochondria and the endoplasmic reticulum. The one surprising feature of this form of cell death is that the point of no return for the cell is when the endoplasmic reticulum or the mitochondria loses its integrity. Whether or not caspases act downstream of those 2 events determines if the death is orderly, but regardless, the cell always dies as a result of that loss of integrity.

It has been known from the first discovery of BCL-2 that cancer cells can avoid apoptosis by acquiring the gains of function of the anti-apoptotic BCL-2 family members, and therefore they are able to avoid apoptotic decisions that might otherwise lead to their elimination before they

accumulate in excess and are harmful to a cancer patient. This overexpression of anti-apoptotic BCL-2 related proteins is a potential target, which might be manipulated in cancer therapy.

The other mechanism by which apoptosis is enacted is based on an extrinsic pathway. This pathway can be activated in 2 independent ways. First, immune cells inject proteins that activate the caspases directly and thereby affect the apoptotic program that is carried out by caspases as a result of their immunologic function. Second, immune cells and other cells regulate communications through signaling pathways, which initiates cell death through receptors that are on the surface of most, but not all, cells. To date, all of the receptors that can initiate programmed cell death through apoptosis are members of the tumor necrosis factor (TNF) family. The first that was described was the protein FAS, which, when activated by its ligand—which can be initiated by its expression by neighboring cells in the environment—instigates a signal transduction pathway that can directly activate caspases to prompt programmed cell death. In many forms of TNF receptor–initiated cell death, the death of the cell can be avoided by inhibiting the activation of caspases by the receptor signal transduction pathway.

The discovery of this pathway, and of the variety of levels at which the extrinsic and intrinsic pathways cross-talk to reinforce cell death decisions based on information from outside and inside the cell, has lead to the finding of several levels of regulation of apoptosis that can also be dysregulated in cancer. One such level is a series of proteins called the inhibitor of apoptosis proteins (IAP), which act to prevent the amplification of signal transduction downstream of either the intrinsic or extrinsic pathways of cell death. IAPs act as E3 ligases to clear the proteins that are necessary to enact programmed cell death. The best characterized of these is a gene now known as X-linked inhibitor of apoptosis protein (XIAP), which prevents activation of caspases and functions to raise the threshold of activation of the pathway that is needed to affect apoptosis. Therefore, cancer cells can upregulate their expression of IAP proteins to protect themselves from cell death.

H&O What are some concerns in developing drugs that target the BCL-2 family?

CT One of the big questions in research has been at what point does manipulation of BCL-2 proteins, caspases, IAP proteins, or TNF receptor families have the best benefit in terms of a therapeutic index between cancer cells and normal cells. The biggest concern in developing drugs against the BCL-2 family has been the thought that elimination of the function of these genes might eliminate normal cells as opposed to cancer cells. Because cancer cells do in fact express higher levels of the anti-apoptotic members of the BCL-2 or the IAP family, in many cases they tend to be more resistant to receptor activation through the TNF receptor family. This exact reasoning kept researchers from developing therapies that targeted apoptosis or initiated apoptosis in cancer cells in the early days of apoptosis research. However, it became evident that one of the things apoptosis protects against is cells that promiscuously decide to proliferate. How this process acts out in a biochemical sense has also become more clear: the oncogenes that are recognized as initiating cancer create an imbalance of intracellular signaling that ultimately results in the activation of the intrinsic pathway. This idea has become collectively known as oncogene-induced stress. One of the primary pathways that the intrinsic apoptotic program recognizes and is initiated by is stress created by oncogenes.

H&O What is the role of p53 in apoptosis?

CT It has become clear, through studies of the p53 gene, that in fact one of the major roles of tumor suppressors is to activate, directly or indirectly, the apoptotic pathway as part of their tumor suppressor function. The best example of that is p53, which transcriptionally activates 2 of the key BH3 proteins that can regulate programmed cell death. Thus, p53 is a very potent tumor suppressor at least at one level of mechanism because it initiates apoptosis in an oncogene-initiated cell. There is an appreciation that the loss of p53 may be a way for cancer cells to avoid apoptosis, and as a result, strategies that re-initiate p53-dependant signaling might play a critical role in getting a therapeutic handle on one of the most common genes lost in cancer.

H&O What are some agents that are currently being studied?

CT Researchers have been studying numerous agents targeting various proteins and receptors, including BCL-2 inhibitors, TNF receptor inhibitors, and IAP inhibitors.

BCL-2 Inhibitors

The first therapeutic interventions that were studied addressed the BCL-2 family and focused on ways to suppress the anti-apoptotic BCL-2 genes. There have been 3 major therapeutic strategies to inhibit the anti-apoptotic BCL-2 family function. The first that was investigated was the use of antisense oligonucleotides, which suppress tumors that have evidence of BCL-2 overexpression. Trials with these agents are still ongoing. Early studies have reported positive results, and there continues to be an interest in suppressing BCL-2 in tumors such as small cell lung carcinoma, which appears to be a tumor in which there is frequently a lot of oncogene-induced stress and dependency on the BCL-2 family.

The other 2 agents that are being studied and tested but are not yet approved target the other anti-apoptotic BCL-2 proteins in addition to BCL-2. Abbott Laboratories, with whom I have collaborated on these studies, has developed chemical inhibitors based on the new methodology of chemical screening known as SAR by NMR (structure-activity relationships obtained from nuclear magnetic resonance) to produce an inhibitor of BCL-XL, which is the major anti-apoptotic BCL-2 protein other than BCL-2, that is expressed widely in cancer cells. The studies looked at whether a drug that inhibited the ability of BCL-XL to bind and sequester BH3 proteins might therefore initiate a pro-apoptotic decision in tumor cells. The chemical inhibitors that were developed are ABT-737 and an oral bioavailable derivative, ABT-263; preclinical and in vitro biochemical studies suggest that they are equivalent chemical mimetics to BH3 proteins in activating apoptosis. These 2 compounds recognize the stable anti-apoptotic tumor BCL-2 proteins that most cells express equally—BCL-2, BCL-XL, and in some cases BCL-W—but do not inhibit the other most commonly expressed BCL-2 family member MCL-1, which, unlike BCL-2 and BCL-XL, is a very labile protein that is dynamically regulated. Currently, clinical trials with these agents are ongoing.

The major issue seen with the Abbott compounds is their on target side effects. The body's platelets depend on the combined functions of BCL-2, specifically BCL-XL, to maintain their long-term circulation in the body as hemostatic cell particles. The only reported clinical complication to date in patients receiving these agents is a dose-dependent suppression of platelet life span. This complication suggests that there are limitations as to how far one can push these BCL-2 protein inhibitors, because there are at least some platelets in the body that depend on the function of these proteins for their normal physiology. Despite this, some patients in phase I and II studies have had responses that correlate with tumors that we would have expected to be protected by overexpression of BCL-2 proteins. The best evidence has come from patients with chronic lymphocytic leukemia (CLL) in preclinical studies.

The third drug, obatoclax, which was developed by Gemin X, is a pan-BCL-2 inhibitor specifically selective for MCL-1. As an MCL-1 inhibitor, it attacks what the Abbott compounds do not. Obatoclax has gone through some phase I and II clinical testing, which found that it has biologic activity and modest single-agent activity across numerous hematologic and solid tumor cancers. Currently, there are ongoing phase I and II studies looking at obatoclax in combination with other agents for the treatment of various cancers, including acute myelogenous leukemia, CLL, and small cell lung cancer.

At the moment, these drugs are relatively safe because the normal cells depend on the combined functions of the BCL-2 family, and tumor cells may be selectively dependent on specific family members that they have overexpressed because of oncogenic mutations. So, we are waiting to see the relative clinical efficacy: whether or not it is the short-lived members of the BCL-2 family or the long-lived members that will be providing protection against oncogenic stress in a meaningful way. Many researchers in the field believe that the drugs alone will not work as single agents, but that instead they will need to be combined with drugs that are already known to create oncogenic stress (traditional chemotherapeutic agents or targeted therapeutics) to get more selectivity on the tumor.

TNF Receptor Inhibitors

There are also ongoing clinical studies that are attacking some of the other core proteins involved in apoptosis. Some of the most exciting research has been in members of the TNF receptor family. Original screening for antibodies against the FAS receptor led people to believe that these antibodies were selectively toxic to tumor cells. However, preclinical studies in animals demonstrated that both the liver and heart had constitutive expression of FAS on the cell surface and that when antibodies directed against FAS were used, they were acutely toxic to the liver and secondarily toxic to the heart by initiating apoptosis. This finding has led to their discontinuation as clinical therapeutics for cancer despite the fact that many cancer cell lines are highly susceptible to FAS-induced apoptosis through the intrinsic pathway.

Screening of other TNF receptor familial members has resulted in the identification of an additional molecule known as Apo2L/TRAIL, a TNF homolog, which has led to drugs that directly target either the activation of the TRAIL receptor or that mimic the ligand. There are several clinical trials sponsored by Genentech using Apo2L/TRAIL; other pharmaceutical companies are researching the therapeutic efficacy of targeting these receptors as well. The in vitro data have suggested that there is good activity against tumors like CLL, and there are active clinical trials investigating the safety and efficacy of Apo2L/TRAIL. From all the available data, the TNF receptor family members appear to have at least some therapeutic efficacy, and clinical trials are ongoing to determine whether or not this single-agent efficacy

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is significant and whether or not combinations of these kinds of strategies with traditional chemotherapy will be beneficial.

Inhibitors of IAP

The other agents that are currently under investigation are inhibitors of IAP. Initially, it was thought that it would be difficult for these proteins to inhibit function in E3 ligase complexes because IAP proteins are E3 ligases. They work by changing the apoptotic threshold of the tumor cell, therefore potentially making it more susceptible to traditional agents. Peptidomimetics that mimic natural inhibitors of the IAPs that were discovered as part of apoptosis research gave credence to the idea that it was possible to directly inhibit IAP function. Several companies have reported efficacy in a number of compounds examined in preclinical studies. These compounds are undoubtedly going to be utilized in the clinic in the next few years. As such, IAP inhibitors do not appear to play a role in initiating apoptosis the way a TRAIL receptor activation would, but they may change the susceptibility window to traditional therapeutics in oncogene-stressed tumor cells and may therefore have efficacy.

I think that in the apoptotic community, we see these 3 classes of proteins as a test of whether the basic science of understanding apoptosis will play out to improve the more targeted therapies—the TNF receptor, inhibitors of the IAP protein, and inhibitors of the anti-apoptotic BCL-2 proteins—against that axis of cancer transformation.

Suggested Reading

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