Microreviews in Cell and Molecular Biology 2012

The Role of the Protein PAR-4 in the Apoptosis of Cancer Cells

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Key Words:
Cancer, cell, protein, apoptosis, degradation

Apoptosis is the self-programmed elimination of cells due to environmental factors. Apoptosis occurs when various signals are recognized by a cell and an intricate series of events takes place in which the cell degrades itself. This is a normal process in the life of multicellular eukaryotes. However, the process of apoptosis is often lacking in cancerous cells, allowing them to proliferate. Par-4 is a protein common to many eukaryotes which is rather unique in its ability to trigger apoptosis solely in cancer cells. It is signaled through a multitude of pathways and enacts cell death using various intriguing mechanisms. These mechanisms are currently being studied in order that PAR-4 might be used to selectively kill cancer cells without harming healthy cells.

Introduction

Apoptosis, the controlled method of systematic cell death, is a common part of everyday life among eukaryotes. In an average human adult 50 to 70 billion cells die every day due to apoptosis. Eukaryotes use apoptosis to eliminate cells which are too damaged or have too many mutations to remain viable. This is necessary in order to maintain healthy cell growth, organize certain cell structures during growth such as finger and toes, and, more importantly for our topic, to prevent mutated cells from surviving and passing on their genetic code. Apoptosis is an intricate process which has many steps and can be triggered in a variety of ways. Common players in the apoptotic pathways are caspases, cytokines, and various signal proteins. Caspases are the most important players in apoptosis. Certain caspase proteins are signaled initially and start a series of bindings until the effector caspases are triggered and degrade the cell and all its proteins. However, other proteins play key roles in this event as well.

PAR-4 is a more recently discovered protein which plays a role specifically in triggering apoptosis in cancerous cells. PAR-4 is 340 amino acids long and has many active sites. Among these are the leucine zipper, the SAC site, the CK2 and PKC site, the ATP/GTP binding site, and the NLS1/NLS2 site. Many of these sites perform functions which are not yet known and some are thought not to perform any function at all. However, the leucine zipper, SAC site, and NLS2 site have been well studied and have been found to play several important roles in apoptosis. The leucine zipper is an active site found on many different kinds of proteins and has been shown to facilitate protein-protein interactions. It is important to understand the leucine zipper's role in PAR-4 because apoptosis is based on cascading death signals in the cell via many protein-protein interactions. Uncovering which proteins interact with PAR-4 is important to understanding how it influences apoptosis. The NLS2 site is a nuclear translocation active site. This active site is thought to allow PAR-4 to move from cytoplasm, across the nuclear membrane, and into the nucleus. This is very important because PAR-4 must enter the nucleus in order to trigger apoptosis. The SAC site in PAR-4 is considered to be its most important site because it makes PAR-4 selective for apoptosis only in cancerous cells. It does this by interacting with protein kinase A (PKA). PKA triggers survival pathways in many cells and is often found in much higher amounts in cancerous cells. In order to begin apoptosis the SAC region of PAR-4 must be phosphorylated by PKA. However, this only happens when there is a high concentration of PKA, such as that found in cancer cells. Once this unique ability of PAR-4 to selectively kill cancer cells was discovered PAR-4 became the subject of intense study. PAR-4's unique attributes make it an ideal target for selective cancer therapies. However, more about PAR-4 must be

understood before it can be implemented in cancer research.

Recent Progress

In many types of cancer cells in which apoptosis by PAR-4 has not occurred it is has been found that PAR-4 is either down regulated or is present in the cell in normal amounts but has been deactivated in some way. The studies of the leucine zipper and its interactions with the protein Akt have produced some of the most interesting results pertaining to the inactivation of PAR-4. In the paper "Binding and Phosphorylation of PAR-4 by Akt Is Essential for Cancer Cell Survival" researchers from several different nations presented how they explored this interaction. When this study initially began nothing was known of the interaction between PAR-4 and Akt. These researchers used anti-PAR-4 antibodies to precipitate proteins in the cell which were binding to PAR-4. They discovered that PAR-4 was a substrate of Akt and that Akt interacts with PAR-4 by phosphorylation. The researchers discovered this by putting the enzyme GTP in solution, some with PAR-4 attached and some without, and then adding Akt and ADP-P. They found that only the GTP enzymes which had PAR-4 attached to them were phosphorylated. Finally, in order to determine the manner in which phosphorylation affected PAR-4 dependent apoptosis researchers created a complex consisting of an identifiable protein and PAR-4 which were expressed in host cells. Akt was then put into a strain of cells and left out of others while they grew. Researchers observed the distribution of the PAR-4 in the cell using confocal microscopy as well as noting whether or not apoptosis occurred. They found that in cells with Akt PAR-4 was only located in the cytoplasm and no apoptosis occurred. However, in cells lacking Akt it was seen that PAR-4 moved into the nucleus of the cell and apoptosis occurred. From this they drew the conclusion that phosphorylation by Akt prevented PAR-4 from entering the nucleus and thus prevented apoptosis.

Understanding the protein-protein interactions of PAR-4 is important to being able to implement its apoptosis triggering abilities in cancer therapy. One of the most recent discoveries in this area has been how PAR-4 interacts with caspace-3. Researchers at the University of Quebec found that during apoptosis PAR-4 is cleaved into two fragments by caspace-3 and that these fragments seem to be an integral part of apoptosis. Initially researchers studied whether or not PAR-4 was present in a form of apoptosis induced by the pro-apoptotic molecule cisplatin. They found that PAR-4 was present in cell death triggered by cisplatin but that levels of PAR-4 became significantly decreased when apoptosis was triggered. After measuring PAR-4 mRNA levels they concluded that there was no decrease in mRNA and that the method of PAR-4 reduction must be post-translational. After several different tests the researchers noted that reduction of PAR-4 was followed by the appearance of an unknown 25KDa polypeptide fragment. Anti-PAR-4 antibodies were then used to determine that the fragment was in fact a fragment of PAR-4.. It was found that the SAC, leucine zipper, and NLS2 sites were all conserved and thus the fragment retained its apoptotic ability. Caspase molecules were then suggested as the most likely enzyme to cleave PAR-4. In order to test this lyses cells were incubated with different caspases added to them and then tested for the 25KDa fragment. It can thus be reasonably concluded that caspace-3 cleaves PAR-4. Finally, researchers wanted to find out if cleavage of PAR-4 was necessary for apoptosis and what role the cleavage played in it. To do this they first expressed PAR-4 mutants in cells which could not be cleaved and found that apoptosis was reduced. They then used a fluorescently tagged antibody to locate PAR-4 in the cell after cleavage and found that its presence in the nucleus was greatly increased.

PAR-4 was originally found in prostate cells in rats and subsequently in humans. Much research has been done on PAR-4 in prostate cells but recently an important step forward has been the study of PAR-4 in other cells types. Researchers at the University of Saint Paul in Brazil recently furthered this area of study by publishing a paper on their findings about the function of PAR-4 in mammary cells. One of the structures which they studied in the mammary gland consisted of groups of cell forming circular cavities which were created by apoptosis. In order to determine the activity of PAR-4 in the formation of these mammary structures researchers used 3D cell cultures, confocal microscopy, and quantitative PCR techniques. They used to 3D cell cultures and confocal microscopy to determine that PAR-4 was found in an increased amount in mammary cells during apoptosis. Then, quantitative PCR techniques were used to determine that the expression of PAR-4 increases over time in certain cells in a timeframe relative to apoptosis. The researchers then hypothesized that a lack of nutrients or hormones in cells causes an increase of PAR-4 and an increased sensitivity to apoptosis. This lack of nutrients or hormones is found in cells that need to go through apoptosis in order to form the appropriate structures in the mammary gland.

Discussion

Each of these experiments show the importance of understanding the roles and mechanisms of PAR-4 and their results each expose a unique opportunity to develop PAR-4 as a therapy option. In the studies of the effect Akt has on PAR-4 it was found that Akt plays a significant role in preventing PAR-4 from inducing apoptosis. It was seen that Akt phosphorylates PAR-4 and this prevents

PAR-4 from entering the nucleus. It has been shown that PAR-4 must enter the nucleus in order to induce apoptosis and thus if PAR-4 is phosphorylated by Akt apoptosis is effectively shut down. This is an important connection because Akt has been found to be up regulated in many different kinds of cancer cells. Further studies could be done of ways in which Akt transcription and translation could be prevented or down regulated. Creating a molecule that more readily binds to Akt and is phosphorylated instead of PAR-4 would be another interesting avenue of research. However, in this study researchers only looked at one cell line when testing whether phosphorylation prevented the movement of PAR-4 into the nucleus. Further tests should be made into how many different kinds of cells are affected by the phosphorylation of PAR-4.

Research into effect caspase -3 has on PAR-4 has shown it to be a modifying enzyme for PAR-4 and revealed its part in PAR-4's mechanism for apoptosis. In this study it was found that PAR-4 acts as a substrate of caspase 3 during the triggering of apoptosis and in the process has a 25 KDa fragment cleaved off. This fragment contains all the important active sites for the continuation of apoptosis and is translocated to the nucleus after cleavage. Researchers hypothesize that the cleavage of PAR-4 allows the 25KDa segment to be separated from a possible active site which prevents translocation. Once inside the nucleus the fragment is able to carry apoptosis as designed. PAR-4 cleavage by caspase 3 has several implications for therapeutic research. First, it offers the possibility of inserting already cleaved PAR-4 fragments into cancerous cells in order to trigger apoptosis. If there is hindrance further up in the apoptosis signaling pathway this may allow apoptosis to be triggered by skipping one of these hindered steps. Second, this would lead to further study of the pathways by which caspace-3 is triggered to cleave PAR-4. In this study PAR-4 cleavage was only studied during a certain type of apoptosis induced by a chemical called cisplatin. It would be interesting to see if PAR-4 cleavage and the translocation of the cleaved fragment occurs in apoptosis induced through different pathways.

The study of the role of PAR-4 in cellular apoptosis in mammary tissue is a part of a larger area of research looking at the role of PAR-4 in cellular tissue other than the well studied prostate cells. This area of study will be one of the most important if PAR-4 is to be used as a cancer therapy option. It has been shown that PAR-4 can be found in many different types of cells. If the mechanism for PAR-4 is the same in all these different types of cells then PAR-4 presents itself as possible key to a therapy option for multiple types of cancer. In this particular study the way PAR-4 plays a role in the planned apoptosis of certain mammary gland structures was observed. PAR-4 does not cause the apoptosis of healthy cells. However, it can cause healthy cells to become more sensitized to apoptosis due to certain environmental signals such as lack of nutrients or changes in chemical levels in the cell's surroundings. During the formation of spheroid cavities called acinar PAR-4 was found to be expressed in the mammary cells. It was hypothesized that this was done in order to sensitize certain cells to a lack of nutrients or hormones so that they would perform apoptosis. The fact that PAR-4 is expressed in mammary cells opens the door to use PAR-4 in the fight against breast cancer. However, this research was performed in regard to planned apoptosis of cells by the body. It will be important to study the effect PAR-4 has on the apoptosis of cancer in mammary cells and to see if its mechanisms for apoptosis are similar to ones that have been previously studied.

Cancer is one of the most important areas of research in the field of cellular biology. It affects millions of people every year and is one of the most diverse and difficult physical ailments to treat. Par-4 offers a unique option for cancer therapy because of its selectivity for apoptosis of only cancerous cells. Even though the mechanisms of PAR-4 and its role in apoptosis are far from being fully understood, initial studies have proven to be fruitful and suggest that PAR-4 may have an important role in the treatment of cancer. Further research into PAR-4 and the apoptosis of cancerous cells will hopefully provide an effective method for providing therapy to cancer patients.

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