

Introduction

- Good evening everyone, I am Dr. Patricia Berg and I will be talking about cell death.
- I thank associate professor Manjari Dimiri who will join this lecture, My lab student Hafsah Mughal who is most responsible- I am deeply grateful- for helping draft it, My home office technical assistant Anjela Bachman, IT guru Jay Wind for loading the graphics from Hafsah, my husband Bob Weiner, over all graduate student program director Jack Vanderhoek for very helpful guidance, and his teaching assistant Juntian Wei for tonight's course webex direction.

One place you can see all the lecture notes for tonight, and all the graphs and pictures, is at weinerpublic.com/berg1 and for a summary of all of our lab's work go to weinerpublic.com/berg.html

- Over the course of the semester you all have learned about the relationship between protein structure and functions, as well as the connection between metabolic pathways and human diseases.
- In this lecture, I will summarize the topic of cell death and how it is related to other parts of our course.
- Cell death includes apoptosis, necrosis, and autophagy, which each of these have specific and unique properties.
- Understanding each one of them can help assess treatments for diseases such as cancer.
- I would like to start off with an interesting fact, all cells in the human body do not regenerate themselves, this is an internet myth.
- Cells such as neurons and cells of organs such as the heart, intestines, or muscles do not replace themselves (neurons) and if damaged they will be replaced by a scar.
 - Skin cells and red blood cells and liver cells do regenerate and replace themselves.
 - This will be further explained in the causes of cell injury.

Causes of Cell Injury

- Before learning the specifics of cell death, it is essential to understand the biological events and causes that lead to this.
- The majority of cell injury is brought about by hypoxia, which is a decrease in the supply of oxygen, and anoxia, which is a complete block in the oxygen supply.
- Hypoxia and anoxia occur when there is an inadequate oxygen supply (i.e. low concentration of oxygen in air at high altitudes, drowning, or lung disease), a failure in oxygen transport in blood (anemia), a disruption in blood flow (ischemia, caused by heart failure), blood vessel obstruction (thrombosis or embolism), disruption in blood supply (rupture of an aneurysm) or a consequence of inhibition of cellular respiration (cyanide poisoning).

Normal Cell Division

- In normal cell division, cells will divide and reproduce in two different ways through either mitosis (somatic) and meiosis (gametes)
- Cell differentiation happens when stem cells become specialized and distinct from one another, giving rise to blood cells, fats cells, neurons, and muscle cells to name a few.
- All multicellular organisms regulate the total number of cells in their organism by cell division and cell death.
- Cellular death is essential for organisms to grow and survive.
- Cells can undergo death by either injury, being exposed to harmful environments, or a programmed process of disintegration.
- When cells are no longer needed they will undergo a pathway called programmed cell death, otherwise known as apoptosis.

Apoptosis

- Apoptosis is one of the pathways of cell death which activates enzymes that work to degrade the cell's own nuclear and cytoplasmic proteins.
- Under normal physiological conditions, cells deprived of survival factors, damaged, or senescent commit suicide through an orderly regulated cell death program called apoptosis.
- Apoptosis (“apo” = away, “ptosis” = fall, aka “cell suicide”) is also known as programmed cell death and is brought about by a protease enzyme called caspase.
- It is one of the main modalities of cell death.
- Apoptosis is a natural process in which the body has actively programmed a way for cell death
- It occurs in many normal situations and works to eliminate harmful cells that have out-lived their usefulness.
- It is carried out in response to intracellular or extracellular signals.
 - Intracellular signals can originate from DNA lesions, mitosis defects, oxidative stress, or other stressors.
 - Extracellular signals respond to death messages emitted by other cells. Apoptosis leads to the activation of proteases, called caspases, which are able to hydrolyse the cell contents independently from the proteasome.
- Cell death can be pathological, a sign of disease and damage, or physiological, a process that is essential for normal health.
- Apoptosis has many roles in tissue homeostasis and during embryology.¹

¹ Robert, J. (2015). CH 18 Apoptosis Induction and Regulation. In 1033813467 792230942 J. Robert (Author), *Textbook of cell signalling in cancer: An educational approach* (pp. 221-240). Cham, France: Springer.

- In the pathological process, conditions such as tumors, viral infections (HIV/AIDS), and graft versus host disease can occur.
- Viral infections can induce apoptosis by preventing viral replication, viral dissemination, or persistent viral infection of the cell. One therapeutic strategy is the use of anticancer drugs to induce apoptosis of cancer cells.
- Apoptosis counterbalances cell proliferation to regulate tissue homeostasis and cell numbers.
- It is distinct from necrosis and involves shrinkage and fragmentation of both the nucleus and the cell without rupture of the cellular membrane.
- This prevents inflammation of the surrounding tissue.
- Cells that undergo apoptosis will lose intercellular adhesion, fragment the chromatin, and break down into small blebs called apoptotic bodies.
- Apoptotic bodies are then phagocytosed by macrophages and inflammation does not occur.
- Apoptotic cell death is observed during normal fetal development.
 - The formation of fingers and toes of the fetus requires the elimination by apoptosis of the tissue between them.
 - During fetal development of the central nervous system, an excess of neurons is required to establish appropriate connections (synapses) between them is later eliminated by apoptosis.
 - Another example of apoptosis is the mature granulocytes in peripheral blood that have a life span of 1 to 2 days before undergoing apoptosis.
 - The clonal selection of T cells in the thymus (which eliminate self-reactive lymphocytes to prevent autoimmune diseases) and cellular immune responses involve apoptosis.

NOTE: Apoptosis occurs in four steps: (1) extracellular and intracellular factors induce cell death, (2) cell killing is activated by caspases (intracellular proteases), (3) phagocytosis of apoptotic bodies by macrophages, (4) lysosomes degrade apoptotic bodies.

Caspases: Initiators and Executioners of Cell Death²

- Caspases are a family of 12 different enzymes.
- Caspases stands for cysteine-dependent aspartate-directed protease.
- Many enzymes are activated by specific proteolytic cleavage, including caspases.
- Apoptosis results from the activation of enzymes called caspases, which are a cysteine proteases that cleave proteins after aspartic residues

² Kierszenbaum, Abraham L, and Laura L. Tres. *Histology and Cell Biology: An Introduction to Pathology*. Philadelphia, PA: Elsevier Saunders, 2012. Book.

- The precursor form, procaspases, is the inactive form of caspase. The procaspases require cleavage to produce the active form, caspase.
- Activation of caspases depends on a balanced production of both proapoptotic and antiapoptotic proteins
- Caspases exist as inactive precursors (procaspases), which are activated to produce directly or indirectly cellular morphologic changes during apoptosis.
- Procaspases consist of two subunits (p10 and p20) and an N-terminal recruitment domain.
- Activated caspases are heterotetramers consisting of two p10 subunits and two p20 subunits derived from two procaspases.
- Caspases can be upstream initiators and downstream executioners.
 - Upstream initiators are activated by the cell-death signal (Fas ligand or TNFL).
 - Upstream initiator caspases activate downstream caspases, which regulates cell destruction.
 - Completion of the cell death process occurs when executioner caspases activate the DNA degradation machinery.
 - Caspases cleave two DNA repair enzymes (poly-ADP-ribose polymerase [PARP] and DNA protein kinase), and unrestricted fragmentation of chromatin occurs.
 - Upstream (initiator) procaspases include procaspases 8, 9, and 10 with a long N-terminal prodomain called CARD (caspase-recruitment domain).
 - Downstream (executioner) procaspases comprise procaspases 3, 6, and 7 with a short N-terminal prodomain called DED (death-effector domain).
- Caspase activation takes place when a caspase-specific regulatory molecule (FADD) binds to the CARD/DED domain.
- Caspase activation may become out of control and destroy the cell.
 - An uncontrolled event is prevented by the inhibitors of apoptosis that are available to interact with modulators of cell death, thus preventing unregulated caspase activation.

Signaling Pathways^{3,4}

- There are two main apoptotic pathways: intrinsic (mitochondrial) and extrinsic (death receptor) pathways. Extrinsic and intrinsic signals determine cell apoptosis.
- Each pathway requires specific triggering signals to begin an energy-dependent cascade of molecular events. Each activates its own initiator caspase (8, 9, 10) which will then activate the executioner caspase-3.
- Extrinsic signals bind to cell surface receptors (Fas ligand and granzyme B/perforin).
- Intrinsic signals in which the release of cytochrome c from mitochondria, can trigger cell death.
- The Fas receptor (APO-1 or CD95) is a cell membrane protein that belongs to the TNF (tumor necrosis factor) receptor family and has an intracellular cell death domain.
- The Fas ligand will bind to the Fas receptor and cause trimerization.
- Then the Fas ligand will initiate programmed cell death by binding to the Fas receptor and will trigger a cell signaling cascade consisting of the sequential activation of procaspases into active caspases.
- The trimerized cell death domain recruits procaspase 8 through the FADD (Fas-associated protein with death domain) adaptor and forms a DISC (death-inducing signaling complex). DISC consists of Fas receptor, FADD, and procaspase 8. Procaspase 8 autoactivated at DISC becomes active caspase 8.
- Active caspase 8 can do two things:
 1. It can process procaspase 3 to active caspase 3, which can cleave several cellular proteins, including ICAD (inhibitor of CAD) giving rise to CAD. CAD (caspase-activated DNase) is released from ICAD, translocates to the cell nucleus, and breaks down chromosomal DNA.
 2. Caspase 8 can cleave Bid, a proapoptotic member of the Bcl-2 family. The truncated Bid translocates to mitochondria to release cytochrome c into the cytoplasm. A cytotoxic T cell destroys a target cell (i.e., a virus-infected cell) by the activation of procaspase 8 by the combined Fas/Fas ligand and granzyme B/perforin pathways. Caspase activation is the key event of apoptosis and involves two extrinsic pathways: the Fas/ Fas ligand and the granzyme B/perforin pathways and an intrinsic pathway, the mitochondrial cytochrome c pathway.

³ Kierszenbaum, Abraham L, and Laura L. Tres. *Histology and Cell Biology: An Introduction to Pathology*. Philadelphia, PA: Elsevier Saunders, 2012. Book.

⁴ Ayllón, Nieves et al. "Systems biology of tissue-specific response to *Anaplasma phagocytophilum* reveals differentiated apoptosis in the tick vector *Ixodes scapularis*." *PLoS genetics* vol. 11,3 e1005120. 27 Mar. 2015, doi:10.1371/journal.pgen.1005120

Intrinsic Pathway: Mitochondrial Cytochrome c⁵

- The mitochondria contains cytochrome c and other proteins that neutralize endogenous inhibitors of apoptosis.
- Cell survival and death is determined by the permeability of mitochondria which is controlled by the Bcl-2 family, which is a family of more than 20 proteins.
- In the intrinsic pathway
 - Cells that are deprived of growth factors and other survival signals
- The mitochondria plays a key role in activating apoptosis in mammalian cells.
- Cytochrome c is a component of the mitochondria electron-transporting chain involved in the production of ATP, and also a trigger of the caspase cascade.
- “In the intrinsic pathway, the event of mitochondrial outer membrane permeabilization (MOMP) is what causes the cytochrome c to be released into the cytosol.
- This pathway can be activated by DNA damage, ER stress, metabolic stress, UV radiation or growth-factor deprivation (non-receptors).
- Bcl-2 family members regulate the release of proteins from the space between the mitochondrial inner and outer membrane that, once in the cytosol, activate caspase proteases that dismantle cells and signal efficient phagocytosis of cell corpses”.⁶
- The cell death pathway is activated when cytochrome c is released from the mitochondria into the cytoplasm.
- Bcl-2 family members can have proapoptotic or antiapoptotic activities and include Bax, Bak, Bid, and Bad which are proapoptotic proteins.
- Bcl-2 is associated with the outer mitochondrial membrane of viable cells and prevents Bax from creating holes in the outer mitochondrial membrane, causing cytochrome c to leak out.
- The balance between proapoptotic Bax and antiapoptotic Bcl-2 proteins controls the release of cytochrome c.
- In the cytoplasm, leaking cytochrome c, in the presence of ATP, soluble internal membrane proteins (SIMPs), and procaspase 9, binds to Apaf-1 to form a complex called an apoptosome.
- The apoptosome determines the activation of caspase 9, an upstream initiator of apoptosis. Caspase 9 activates caspase 3 and caspase 7, leading to cell death.
- Exogenous activators, such as Fas ligand and Granzyme B and the endogenous mitochondrial permeability transition, lead to an abrupt release of cytochrome c, are three key triggers of apoptosis.

⁵ Kierszenbaum, Abraham L, and Laura L. Tres. *Histology and Cell Biology: An Introduction to Pathology*. Philadelphia, PA: Elsevier Saunders, 2012. Book.

⁶ Wang, Chunxin, and Richard J Youle. “The role of mitochondria in apoptosis*.” *Annual review of genetics* vol. 43 (2009): 95-118. doi:10.1146/annurev-genet-102108-134850

- Apoptosis-inducing factor (AIF) is a protein of the intermitochondrial membrane space that can be released into the cytoplasm, migrate to the nucleus, bind to DNA, and trigger cell destruction without participation of caspases.

Extrinsic Pathway⁸

- Many cells express death receptors, which are surface molecules that trigger apoptosis.
- Most are members of the tumor necrosis factor (TNF) receptor family, which regulate the interaction between other proteins involved in cell death.
- “The extrinsic pathway involves stimulation of members of the tumor necrosis factor (TNF) receptor subfamily, which includes TNFR1, CD95/Fas or TRAILR (death receptors), located at the cell surface, by their specific ligands, such as TNF- α , FasL or TRAIL, respectively”.⁷
- The extrinsic pathway is regulated by death receptors which include Fas receptors, tumor necrosis factor (TNF) receptors, and TNF-related apoptosis-inducing ligand (TRAIL) receptors.
- The surface receptor, TNF receptor-1 (TNF-R1), will interact with TNF to induce the recruitment of adaptor proteins such as Fas-associated protein with death domain (FADD) and tumor necrosis factor receptor type 1-associated death domain protein (TRADD), which recruits a series of downstream factors, including Caspase-8, which is an important regulator of the extrinsic pathway that leads to cell apoptosis.
- The extrinsic pathway that initiates apoptosis is triggered by a death ligand binding to a death receptor, such as TNF- α to TNFR1.
- The TNFR family is a large family consisting of 29 transmembrane receptor proteins, organized in homotrimers and activated by binding of the respective ligand(s).⁸
- They share similar cysteine-rich extracellular domains and have a cytoplasmic domain of about 80 amino acids called the "death domain" (DD).⁸
- This death domain plays a critical role in transmitting the death signal from the cell surface to the intracellular signaling pathways.⁹
- There are 19 members of the TNF ligand family and binding may result in a number of responses, including proliferation, inflammation, and apoptosis, depending on the adaptor proteins associated with the activated receptor.
- TNFR can stimulate pro-inflammatory pathways leading to activation of NF κ B, via recruitment of RIP.
- The death domain kinase RIP is essential for TRAIL-induced I κ B kinase (IKK) activation.

⁷ https://www.genome.jp/dbget-bin/www_bget?ko04210

⁸ <https://www.creative-diagnostics.com/extrinsic-apoptosis-pathway.htm>

⁹ Elmore, Susan. “Apoptosis: a review of programmed cell death.” *Toxicologic pathology* vol. 35,4 (2007): 495-516. doi:10.1080/01926230701320337

- It has been identified that the binding of TNF- α and TNFR1 activates NF κ B pathway, which favors both cell survival and apoptosis, depending on the cell type and biological context.
- Besides TNFR1, the Fas and DR4/DR5 also involved the pathway as death receptors and bind CD95 and TRAIL, respectively.
- All of the ligand binding to receptors will lead, with the help of the adapter proteins (FADD/ TRADD) to recruitment, dimerization, and activation of a caspase cascade and eventually cleavage of both cytoplasmic and nuclear substrates.
- To date, the best-characterized ligands and corresponding death receptors include CD95/Fas, TNF- α /TNFR1, Apo2L/DR4 and Apo2L/DR5.
- Receptor trimerization results in recruitment of several death domains and eventually recruitment and activation of caspase-8 and caspase-10.
- Active caspase-8 and caspase-10 then either initiate apoptosis directly by cleaving and thereby activating executioner caspase-3/6/7), or activates the intrinsic apoptotic pathway through cleavage of the BID to induce efficient cell death.
- Immuno-blot analysis also revealed that the caspase-6 inhibitor blocked the cleavage of lamin A/C, whilst the caspase-3/7 inhibitor blocked the cleavage of poly (ADP-ribose) polymerase (PARP).
- Activation of caspase-8 may be prevented by FLICE inhibitory protein (FLIP).
- Taken together, these results suggest that activation of caspases, the subsequent cleavage of lamin A/C and PARP, and the NF κ B pathway are involved in the extrinsic pathway of cell apoptosis.

Necrosis¹⁰

- Necrosis is a nonphysiologic process that occurs after injury, such as an ischemic stroke. It involves a large group of cells or tissue (part of an organ).
- Necrosis (“unprogrammed”) is a cellular condition in which healthy cells are destroyed by external processes, like inflammation.
- In this case, cell death will cause a loss of functional tissue and premature cell death in living tissues by autolysis.
- Necrotic cells lyse and release cytoplasmic and nuclear contents into the environment, thus triggering an inflammatory reaction.
- One condition that is related to necrosis is hypoxia, which is a broad term that describes how the body's oxygen demand is greater than the oxygen supply. When this happens ATP levels drop and cellular functions can not be maintained and when this lasts long enough cells die.

¹⁰ Kierszenbaum, Abraham L, and Laura L. Tres. *Histology and Cell Biology: An Introduction to Pathology*. Philadelphia, PA: Elsevier Saunders, 2012. Book.

Types of Necrosis¹¹

- Necrosis can be recognized by specific changes at the microscopic and macroscopic level.
 - In addition to the breakdown of the cell membrane caused by the cell swelling, the nucleus displays **pyknosis** (Greek *pyknos*, crowded; *osis*, condition; condensation of chromatin), **karyolysis** (Greek *karyon*, nucleus; *lysis*, dissolution; breakdown of chromatin by endonucleases) and **karyorrhexis** (Greek *karyon* + *rhexis*, rupture; presence of fragmented chromatin in the cytoplasm).
- a. Coagulative necrosis
Accidental cell death in the tissues (multiple cells) caused by a lack of blood flow. Can be observed in the kidney, heart, and adrenal glands.
The most common form of necrosis is a result of vascular occlusion, characterized by a paler than normal tissue area that retains its overall shape but all cell functions have stopped. The initial inflammatory response (infiltration of neutrophils during the first 24 and 48 hours) is followed days later by the eosinophilic staining of anucleated cell remnants.
 - i. Myocardial infarction = tissue death in the heart (“myo” = muscle, “cardio” = heart). Caused by ischemia associated with blockage of a branch of the coronary artery
 - ii. Gangrene (coagulation necrosis)¹² = insufficient blood supply or infection in deeper tissues
 - b. Liquefactive necrosis (colliquative necrosis)
Bacterial (pus-forming) or fungal infection, results in tissues becoming a liquid viscous mass.¹³ Recognized by the softening of the necrotic tissue caused by hydrolytic lysosomal enzymes released from dead cells and neutrophils.
 - i. Brain abscess = pus-filled swelling in the brain¹⁴
 - ii. Brain infarct = necrotic tissue is removed by macrophages and the remaining cavity is filled by fluid derived from the surrounding brain interstitial spaces
 - c. Caseous necrosis
Type of cell death in tissues where the cellular outline is lost and tissues appear cheese-like and crumbly, mimics cottage cheese.¹⁵
 - i. Tuberculosis

¹¹ Kierszenbaum, Abraham L, and Laura L. Tres. *Histology and Cell Biology: An Introduction to Pathology*. Philadelphia, PA: Elsevier Saunders, 2012. Book.

¹² <https://www.news-medical.net/health/Types-of-gangrene.aspx>

¹³ <https://www.sciencedirect.com/topics/medicine-and-dentistry/liquefactive-necrosis>

¹⁴ <https://www.nhs.uk/conditions/brain-abscess/>

¹⁵ <https://www.dictionary.com/browse/caseous-necrosis>

d. Fat Necrosis

Occurs after enzymatic and traumatic injury. Enzymatic fat necrosis involves adipose tissue within and around the pancreas. Release of lipases from exocrine pancreatic cells during acute pancreatitis destroys the plasma membrane of adipose cells followed by the breakdown of triglycerides into fatty acids. Fatty acids combine with interstitial calcium, giving the necrotic adipose tissue a chalky white appearance by a process called fat saponification (Latin *sapon*, soap). Traumatic fat necrosis is the consequence of traumatic injury (sports and accidents affecting adipose tissue of the breasts, thigh, etc).

- i. Acute pancreatitis
- ii. Breast injury

e. Fibrinoid necrosis blood vessels

Found in the smooth muscle wall of small arteries, arterioles and renal glomeruli affected by autoimmune diseases such as systemic lupus erythematosus. Fibrin-like eosinophilic material impregnates the vascular wall. It can be recognized under the microscope because it does not have distinct macroscopic features.

- i. Malignant hypertension
- ii. Vasculitis

Autophagy

- Autophagy (Greek “self-eating) is essential for cell survival and homeostasis.
- It is a natural and regulated cleansing mechanism of the body that removes unnecessary and dysfunctional components of the cell by causing cells to eat themselves.
- Autophagy and apoptosis can occur in the same cell, where autophagy occurs after apoptosis.

p53 Gene and its Role in Cancer^{16,17}

- The most famous molecule in cancer research is p53. It is mutated in about half of all tumors.
- This 53-kilodalton protein was discovered in 1979
- Today, it is called the guardian of the genome.
- The p53 gene (TP53) is a gene which is the most common gene mutation found in cancer cells. The p53 protein has an integral role in the cell and is present in all cell types.
- There are 3 different types of cancer genes:

¹⁶ <https://www.cancerquest.org/cancer-biology/cancer-genes>

¹⁷ Kierszenbaum, Abraham L, and Laura L. Tres. *Histology and Cell Biology: An Introduction to Pathology*. Philadelphia, PA: Elsevier Saunders, 2012. Book.

- Oncogenes: Under normal conditions oncogenes accelerate cell division and growth. When mutated they act like stuck gas pedals. P53 interacts with oncogenes.
- Tumor suppressor genes: This is the category p53 falls under. In normal conditions they act like brakes. When mutations occur they can cause these brakes to fail.
- DNA repair genes: Under normal conditions they fix minor damage to the DNA when it is replicated. When these genes are mutated, DNA damage can accumulate and lead to cancer.
- p53 regulates cell division and normally functions to shut down cell division when a cell is stressed (ex DNA damage).
- When DNA is damaged, p53 activates genes that will stop cell growth and can trigger the cell to die.
- It guards against changes to cells that might lead to tumor formation and cancer.
- Activators of p53 include low oxygen concentrations, DNA damage, chemotherapeutic agents, and other stressors.
- The p53 gene is a transcription factor that regulates genes controlling cell division and cell death and has an important role in the cellular response to DNA damage. It also aids in the decision between repair and induction of cell death.
- The p53 protein interacts directly with DNA and other proteins that direct cellular actions.
- When DNA damage or other cellular insults are detected, p53 has the power to trigger cell death or apoptosis.
- The crucial role of p53 in maintaining proper control of cellular processes is underscored by the fact that the *TP53* gene is found to be defective in about half of all tumors, regardless of their type or origin.
- The gene is a type of tumor suppressor gene that codes for a protein that inhibits the development and growth of tumors.
- The tumor-suppressor protein p53 protects the integrity of DNA in response to harmful stress, called genotoxic stress.
- This protective function depends on the ability of p53 to induce programmed cell death or apoptosis or arrest cell cycle activities, when a cell undergoes genotoxic stress.
- As a transcription factor, p53 controls the transcriptional activation of proapoptotic genes and the inactivation of antiapoptotic genes.
- In this mechanism, a cell affected by genotoxic stress is eliminated.
- A loss of p53 function may occur by a mutation of the *TP53* gene, which encodes p53, or by an abnormal signaling pathway controlling p53 function.
- Cancer cells are highly sensitive to apoptotic signals, but can survive if there is a loss of p53 function.
- p53 is an important transcriptional activator of numerous target genes.

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- Its role, as a cellular stress sensor, is to respond to DNA damage, oxidative stress and ischemia by controlling apoptosis through transcription-dependent and transcription independent (mitochondrial dysfunction) mechanisms leading to cell cycle arrest or limit cell damage.
 - Autophagy, necrosis and apoptosis are three distinct forms of cell death following acute cell injury.
 - Under low levels of genotoxic stress, p53 induces the expression of antioxidants, thereby supporting cell survival.
 - Increasing levels of DNA damage stimulate the generation of increased reactive oxygen species (ROS) levels to eliminate cells that are not fit to survive or sustain too much damage.
 - Loss of p53 function by mutations in p53 or by a disruption of the p53 signaling pathway is frequently associated with human cancers.
 - This observation underscores the significant importance of p53 in tumor suppression.
 - As a tumor suppressor, the function of p53 is controlled by sequestration and inhibition of its negative regulator, the E3 ubiquitin ligase MDM2.
 - When MDM2 is inhibited, p53 remains stable and active to operate within the context of DNA damage or tumor suppression leading to apoptosis or cell cycle arrest.
 - If MDM2 is active, p53 is degraded and the tumor suppression effect is lost.
 - Mutations of the TP53 gene, which encodes the p53 protein, are observed in 50% of human cancers.
 - The loss of TP53 gene expression by an autosomal dominant mutation is responsible for a multi cancer phenotype known as Li-Fraumeni syndrome.
 - The inactivation of p53 tumor suppression role has important therapeutic implications in cancer patients receiving chemotherapy with a potential genotoxic effect.
 - A negative side effect of chemotherapy is the p53-driven apoptosis in sensitive tissues (for example, stem cells in bone marrow and intestinal epithelium) thus compromising effective tumor suppressor function.
 - Efforts are directed towards understanding the molecular mechanisms by which p53 can discriminate between acute DNA damage (genotoxic insult) and tumor suppression (oncogenic signaling).
 - The goal is to block p53-dependent side effects of chemotherapy without the risk of compromising p53 tumor suppression function.
 - Pharmacologic agents binding to MDM2 could stabilize and increase the levels of p53 in cancer cells to exert a tumor-suppressor activity through its death inducing functions.

- Loss of p53 function by mutations in p53 or by a disruption of the p53 signaling pathway is frequently associated with human cancers. This observation underscores the significant importance of p53 in tumor suppression.
- TP53 gene mutations are present in around 50% of cancers overall.

Lastly I would like to bring in BP1 which my lab and I discovered and cloned. We had 16 TV cameras and major press at the announcement. BP1 is especially related to cell death which it can prevent.

Conclusion

- The **four main points** of emphasis in this lecture are,
 - what is the mechanism and causes of cell injury,
 - the specific and unique properties of apoptosis and necrosis,
 - the differences between the cell signaling pathways found in apoptosis, and
 - the relationship of tumor oncogenes and suppressors regulated by BP1.