Objectives

- Distinguish between sublethal and lethal cell damage
- Differentiate between necrosis and apoptosis as forms of cell death
- Understand the major mechanisms of cell injury and how they are involved in selected clinicopathologic examples
- Recognise various adaptive cell responses and other cellular changes, and identify appropriate examples of each response

Outline

I. General Features of Cell Injury

a. **Causes of cell injury**
b. **Progression of cell injury and death**

II. Reversible Cell Injury vs Cell Death

a. **Reversible cell injury**
b. **Cell death:** *Necrosis vs Apoptosis, Other mechanisms, Autophagy*

III. Mechanisms and Selected Clinicopathologic Examples of Cell Injury

a. **Cellular targets of injurious stimuli:** *Mitochondrial damage, Membrane damage, DNA damage*
b. **Biochemical alterations in pathways involved:** *Oxidative stress, Calcium homeostasis disturbances, ER stress*
c. **Clinicopathologic examples of cell injury and death:** *Hypoxia and ischemia, Ischemic-reperfusion injury, Chemical (toxic) injury*

IV. Adaptations of Cellular Growth and Differentiation

a. **Hypertrophy:** *Physiologic vs Pathologic*
b. **Hyperplasia:** *Physiologic vs Pathologic*
c. **Atrophy:** *Physiologic vs Pathologic*
d. **Metaplasia**

V. Other Cellular Changes

a. **Intracellular accumulations:** *Lipids, Proteins, Glycogen, Pigments*
b. **Pathologic calcifications:** *Dystrophic vs Metastatic*

VI. Cellular Aging

a. **Accumulation of DNA damage**
b. **Replicative senescence:** *Telomere attrition, Activation of tumour suppressor genes*
c. **Defective protein homeostasis:** *Chaperone, proteasome functions*
d. **Dysregulated nutrient sensing:** *IGF-1 pathway, Sirtuins*

References

Kumar V, Abbas A, Aster J. *Robbins & Cotran Pathologic Basis of Disease*. 10th ed.

***Note**: Pathweb Study Notes are based on the key topics covered in the lectures in the Yong Loo Lin School of Medicine, as well as additional topics covered in major texts. For more comprehensive discussion on specific pathology topics, readers are advised to refer to the recommended texts in your respective courses.*

- Normal cells can handle most physiological demands, maintaining a healthy steady state (**homeostasis**)
- When cells experience stress (e.g. changes in physiologic states like pregnancy, or some pathologic stimuli), they undergo **adaptations**, which are reversible functional and structural responses to achieve a new but altered steady state to allow continual cell survival and function. Once the stress is eliminated, the cell can return to its original state without harmful consequences
- If the limits of adaptive responses are exceeded, essential cell functions are compromised or the stress experienced by the cell is too injurious, **cell injury** occurs. Cell injury is reversible up to a point, but if the stimulus is too severe or persistent, irreversible injury leads to **cell death**
- Cell death is a normal and essential process in certain situations e.g. embryogenesis, maintenance of homeostasis. However, excessive cell death due to irreversible injury causes disease. Cell death occurs mainly either via **necrosis** or **apoptosis**
- Cell stresses can also result in **other cellular changes** e.g. intracellular accumulations and pathologic calcification. **Cellular aging** also demonstrates characteristic morphologic and functional changes

I. GENERAL FEATURES OF CELL INJURY

Causes of cell injury

- **Oxygen deprivation (hypoxia):** Causes cell injury via reduction in aerobic oxidative respiration. Depending on severity, cells can adapt, undergo injury or death. May be due to ischaemia (reduced blood flow), inadequate blood oxygenation due to cardiorespiratory failure, decreased oxygen-carrying capacity of blood (e.g. in anaemia, carbon monoxide poisoning)
- **Physical agents:** E.g. mechanical trauma, temperature extremes (burns, freezing), barometric pressure injury, radiation, electric shock
- **Chemical agents and drugs:** Includes simple chemicals like salt or glucose in hypertonic concentrations, poisons like arsenic, environmental pollutants, occupational hazards, recreational and therapeutic drugs
- **Infectious agents:** Viruses, bacteria, fungi, parasites, helminths etc. via diverse mechanisms
- **Immunologic reactions:** Tissue injury occurs during appropriate immune reactions as collateral damage, but also during inappropriate immune reactions (hypersensitivity / autoimmune diseases)
- **Genetic abnormalities:** May cause cell injury due to deficient protein function, or accumulation of damaged DNA or misfolded proteins. Gene polymorphisms can also influence the susceptibility of cells to injury by chemicals and other environmental insults
- **Nutritional imbalances:** Nutritional shortages (protein-calorie deficits, vitamin deficiency) as well as nutritional excess (leading to obesity, or excess of a certain diet component like lipids)

Progression of cell injury and death

- All stresses and noxious influences first affect the molecular or biochemical level (affecting cellular function), before morphologic changes are seen (ultrastructural level → light microscopy → gross)
- Morphologic changes of reversible cell injury also appear earlier than changes from irreversible cell injury, to which it may progress

II. REVERSIBLE CELL INJURY VS CELL DEATH

Reversible cell injury

Characterized by **functional** and **structural alterations** in early stages or mild forms of injury, which are correctable if the damaging stimulus is removed. Reversibly injured cells often show:

- **Generalised cellular swelling (hydropic change / vacuolar degeneration)**: Earliest manifestation of almost all forms of cell injury. Results from influx of water due to failure of the ATP-dependent Na^+-K^+ plasma membrane pump *(see "III. Mechanisms of cell injury")*
  Ultrastructural changes: swelling of cell organelles, plasma membrane blebs, detachment of ribosomes from the endoplasmic reticulum (ER), accumulation of 'myelin figures' in the cytoplasm composed of phospholipids from damaged cellular membranes, clumping of nuclear chromatin
  Light microscopic changes: small cytoplasmic vacuoles, cytoplasmic eosinophilia (due to loss of RNA)
- **Fatty change**: occurs in organs actively involved in lipid metabolism e.g. liver, when the toxic injury disrupts metabolic pathways and leads to rapid accumulation of triglyceride-filled lipid vacuoles

Cell death

Although the 'point of no return' at which cell damage becomes irreversible is still largely undefined, 2 phenomena consistently characterize **irreversibility, leading to cell death**:

1. Inability to reverse mitochondrial dysfunction (lack of oxidative phosphorylation and ATP generation) even after resolution of the original injury
2. Profound disturbances in membrane function

There are 2 principal types of cell death, each with their own mechanisms, morphologic features and physiologic/pathologic roles:

Feature	Necrosis	Apoptosis
Morphology	- Cellular swelling - Increased cytoplasmic eosinophilia (with loss of cytoplasmic RNA) or cytoplasmic vacuolation (with digestion of cell organelles) - Nuclear pyknosis *(nuclear shrinkage and basophilia)*, karyorrhexis *(fragmentation of the pyknotic nucleus)*, karyolysis *(loss of DNA resulting in fading basophilia)* - Disrupted plasma membrane	- Cellular shrinkage - Increased cytoplasmic eosinophilia: organelles are relatively normal but more tightly packed - Chromatin condensation (often aggregating peripherally under the nuclear membrane) and nuclear fragmentation into nucleosome-size fragments - Intact plasma membrane, although with extensive surface membrane blebbing that eventually form apoptotic bodies
Cellular contents	Undergo enzymatic digestion and may leak out of the cells	Remain intact and may be released in apoptotic bodies
Association	Adjacent inflammation frequently present	Not usually associated with inflammation → apoptotic bodies usually rapidly phagocytosed
Situation	Usually pathologic (irreversible cell injury)	Usually physiologic (to eliminate unwanted cells), but may be pathologic after some forms of cell injury (esp. DNA damage)

Necrosis

- **Pathologic process** that is the consequence of severe cellular injury, characterized by damaged membranes resulting in denaturation of cellular proteins by released lysosomal enzymes, as well as leakage of cellular contents into the extracellular space, inciting local inflammation
- Damage-associated molecular patterns (DAMPs) released from injured cells (e.g. ATP, uric acid) are recognized by macrophage receptors, triggering phagocytosis as well as cytokine production to attract/ activate other inflammatory cells which release proteolytic enzymes to facilitate enzymatic digestion of the lethally injured cell. This eventually results in clearance of the necrotic cells
- Blood tests can detect tissue-specific cellular injury by measuring levels of these necrosis-associated leakage of intracellular proteins, which act as serum biomarkers e.g. Troponin I from cardiac muscle cells, transaminases from hepatocytes
- When large numbers of cells die, the tissue is said to be necrotic. Tissue necrosis has several morphologically distinct patterns which may provide a clue about the underlying cause:
    - **Coagulative necrosis:** Preserved architecture of dead tissue with intensely eosinophilic cells showing loss of / reddish nuclei for days to weeks. Presumably, the injury denatures intracellular enzymes therefore initially preventing proteolysis of dead cells, but eventually the dead cells will be broken down by infiltrating leukocytes
        - **Cause:** Ischemia (in all organs except brain, which manifests instead as liquefactive necrosis)
    - **Liquefactive necrosis:** Dissolution of tissue into a viscous liquid (often creamy yellow due to presence of leukocytes i.e. pus) due to digestion of dead cells
        - **Cause:** Focal bacterial (or sometimes fungal) infections; hypoxia/ischemia in the brain
    - **Gangrenous necrosis:** Not a really specific pattern of cell death – extensive coagulative necrosis +/- liquefactive necrosis due to superimposed bacterial infection usually in the context of the limbs (dry / wet gangrene)
    - **Caseous ('cheese-like') necrosis:** Friable white gross appearance; microscopically amorphous granular debris surrounded by inflammation, often in granulomas
        - **Cause:** Tuberculous infection
    - **Suppurative necrosis:** Often refers to abscess formation (aggregates of neutrophils)
    - **Haemorrhagic necrosis:** Necrosis associated with haemorrhage, often in organs with dual blood supply (lung, liver) or necrosis due to venous congestion (e.g. bowel ischemic necrosis from volvulus)
    - **Fat necrosis:** Focal area of lipid breakdown. The fatty acids generated can combine with calcium to form grossly chalky-white areas (fat saponification); microscopically the necrotic fat cells have 'ghost' outlines, loss of nuclei +/- associated basophilic calcium deposits and inflammation
        - **Cause:** Due to release of enzymes (classically, activated pancreatic lipases in acute pancreatitis)

- **Fibrinoid necrosis:** Special type of vascular damage when immune complexes are deposited in the walls of arteries, which together with exuding plasma proteins, manifest as a bright pink amorphous appearance on H&E
  - **Cause:** Immunologically mediated vasculitis syndromes

Apoptosis

- Type of cell death induced by a **tightly regulated** suicide program in which cells destined to die activate intrinsic enzymes that degrade the cells' genomic DNA, nuclear and cytoplasmic proteins, causing the cell to break up into **apoptotic bodies** (plasma membrane-bound fragments that have altered surface components acting as signals to phagocytes) that are rapidly phagocytosed
- **Causes**: Can be physiologic or pathologic
  - **Normal physiologic situations**: Serves to eliminate cells no longer needed or as a mechanism to maintain a constant number of various cell populations in tissues. These cells undergo apoptosis after they are deprived of necessary survival signals or receive pro-apoptotic signals from other cells/ environment. Examples of such situations:
    - **Removal of excess cells during development ('programmed cell death')**: Involution of primordial structures and remodeling of maturing tissues
    - **Involution of hormone-dependent tissues on hormone withdrawal**: e.g. endometrial breakdown during menstruation
    - **Cell turnover in proliferating cell populations ('homeostasis')**: e.g. intestinal epithelial cells, B lymphocytes in germinal centers that fail to express useful antigen receptors
    - **Elimination of potentially harmful self-reactive lymphocytes**: Prevent autoimmune reactions
    - **Death of host inflammatory cells after they have served their purpose**
  - **Pathologic conditions**: Eliminates cells injured beyond repair without eliciting a host reaction to limit collateral tissue damage e.g.
    - **DNA damage**: The cell triggers intrinsic mechanisms to induce apoptosis when DNA damage caused by radiation and cytotoxic drugs is not repairable. This is protective by preventing survival of cells with DNA mutations that can lead to malignant transformation
    - **Accumulation of misfolded proteins**: This induces the endoplasmic reticulum (ER) stress response that initiates apoptosis
    - **Infections and other situations causing a cytotoxic T-cell response**: The virus itself (e.g. adenoviruses) or the host immune response (e.g. cytotoxic T-cell response in viral hepatitis) can induce apoptosis. The cytotoxic T-cell response is also involved in killing of tumour cells, cellular rejection in transplants and tissue damage in graft-vs-host disease
    - **Pathologic organ atrophy**: e.g. pancreatic atrophy after duct obstruction

- **Mechanisms of apoptosis**: Activation of enzymes called **caspases** is central. During the **initiation phase**, some caspases become catalytically active and unleash a cascade of other caspases; in the **execution phase**, terminal caspases then trigger cellular fragmentation. This entire process is regulated by a balance between pro-apoptotic and anti-apoptotic proteins. The initiation phase has two pathways, which are distinct but can intersect:
    1. **Mitochondrial (intrinsic) pathway:** Responsible in most physiologic and pathologic situations
        - **Increased permeability of the mitochondrial outer membrane** causes release of pro-apoptotic molecules from the mitochondrial intermembrane space into the cytoplasm (e.g. cytochrome c – essential for ATP production but when released into the cytoplasm, indicates that cell is not healthy and is pro-apoptotic)
        - The integrity of the outer mitochondrial membrane is tightly controlled by the BCL2 family of proteins (can be divided into 3 groups based on their functions):
            - **Anti-apoptotic**: BCL2, BCL-X_L, MCL1. Help keep the mitochondrial outer membrane impermeable
            - **Pro-apoptotic**: BAX, BAK. When activated, oligomerize to enhance permeability of mitochrondrial outer membrane
            - **Regulated apoptosis initiators**: BAD, BIM, BID (BH3-only proteins). Can initiate apoptosis when upregulated and activated by sensors of cellular stress and damage
        - Growth factors and other survival signals stimulate production of anti-apoptotic proteins. Conversely, when cells are deprived of survival signals, suffer DNA damage or develop ER stress, BH3-only proteins are upregulated through increased transcription and/or post-translational modifications, and in turn directly activate the BAX and BAK as well as bind and block the function of BCL2 and BCL-X_L, resulting in release of mitochondrial proteins into the cytoplasm
        - In the cytosol, cytochrome c binds to **APAF-1** (apoptosis-activating factor-1 protein) to form the apoptosome, a multimeric complex that binds to **caspase-9** and promotes its autocatalytic cleavage to produce active forms. Active caspase 9 is the critical initiator caspase of the mitochondrial pathway which acts by cleaving and activating other pro-caspases in the execution phase of apoptosis
        - Other released mitochondrial proteins bind to and neutralize cytoplasmic proteins that function as physiologic inhibitors of apoptosis (i.e. they block inappropriate activation of caspases in the cell)
    2. **Death receptor-initiated (extrinsic) pathway:**
        - Initiated by engagement of plasma membrane death receptors (members of the tumour necrosis factor (TNF) receptor family with a cytoplasmic domain involved in protein-protein interactions) e.g. TNFR1, Fas (CD95), which then delivers apoptotic signals
        - Fas is expressed on many cell types. **Fas binds to Fas ligand (FasL)**, which is expressed on T-cells that recognize self-antigens, and on some cytotoxic T-cells that

kill virus-infected and tumour cells. When FasL binds to Fas, 3 or more molecules of Fas are brought together, and their cytoplasmic death domains form a binding site for an adaptor protein called **FADD** (Fas-associated death domain).

- Once bound, FADD binds inactive **caspase-8 or -10**, bringing multiple molecules together leading to autocatalytic cleavage and generation of active caspase-8, which then initiates the execution phase of apoptosis
- FLIP inhibits the extrinsic apoptosis pathway by binding to pro caspase-8, thereby blocking FADD binding

Execution phase of apoptosis: The intrinsic and extrinsic pathways (via caspase-9 and caspase-8 and -10, respectively) converge to trigger the rapid sequential activation of the executioner caspases e.g. caspase-3 and -6, which then proteolyze cellular components such as inhibitors of DNase (thereby allowing DNA degradation to occur) and nuclear structures.

Removal of dead cells with least host inflammatory response: The apoptotic cells and the smaller apoptotic bodies undergo membrane changes and may also be coated by antibodies and complement proteins (e.g. C1q) that actively promote their phagocytosis. Dying cells also secrete soluble factors that recruit phagocytes, and macrophages themselves may also produce proteins that bind selectively to apoptotic cells. The process of apoptotic cell phagocytosis (efferocytosis) is thus highly efficient, occurring within minutes. Macrophages that have ingested apoptotic cells also produce less pro-inflammatory cytokines, limiting the inflammatory response in apoptosis

Other mechanisms of cell death

- **Necroptosis**: Hybrid form of cell death that shares aspects of both necrosis and apoptosis ('programmed necrosis' / 'caspase-independent programmed cell death') i.e. triggered by signal transduction pathways but results in loss of ATP, cellular and organelle swelling, generation of reactive oxygen species (ROS) and release of lysosomal enzymes with plasma membrane rupture
- **Pyroptosis**: Form of apoptosis associated with release of the fever-inducing cytokine IL-1 via activation of caspase-1, often in some microbial infections
- **Ferroptosis**: Cell death triggered when excessive intracellular levels of iron or ROS overwhelm glutathione-dependent antioxidant defenses, resulting in lipid peroxidation

Autophagy

- **Adaptive response** whereby the cell cannibalizes its own contents to survive, enhanced during nutrient deprivation as well as other physiologic situations to maintain cellular integrity by recycling essential metabolites and clearing intracellular debris e.g. during aging and exercise. Autophagy has been implicated in pathologic conditions such as cancer, neurodegenerative disorders, inflammatory bowel diseases, and also in host defense against certain microbes
- **Mechanism**: Initiation, nucleation and elongation phases involve formation of a **phagophore** (isolation membrane), that eventually forms the **autophagosome**, an enclosed double-membrane-bound vacuole in which intracellular organelles are sequestered. The autophagosome matures and **fuses with lysosomes** which then digest and degrade the enclosed material. The digested material is finally released for recycling of metabolites

III. MECHANISMS AND SELECTED CLINICOPATHOLOGIC EXAMPLES OF CELL INJURY

The cellular response to injurious stimuli depends on the (i) nature, (ii) duration and (iii) severity of injury, as well as the type, state and adaptability of the injured cell. Multiple mechanisms of cell injury may be triggered simultaneously by a single injurious stimulus

Cellular targets of injurious stimuli:

1. Mitochondrial damage

Mitochondria are critical in all pathways leading to cell injury and death, and can be damaged by increases of cytosolic Ca^{2+}, ROS and oxygen deprivation. Some inherited diseases are caused by mutations in mitochondrial genes.

Consequences of mitochondrial damage:

- **ATP depletion**: Decreased ATP synthesis and ATP depletion occur due to the reduced supply of oxygen and nutrients (in hypoxic injury) and actions of some toxins (e.g. cyanide). Mitochondrial damage also results in the formation of the mitochondrial permeability transition pore in the mitochondrial membrane, leading to loss of mitochondrial membrane potential, failure of oxidative phosphorylation and progressive ATP depletion.
  Depletion of ATP to 5-10% of normal levels results in failure of energy-dependent functions e.g. the plasma membrane energy-dependent Na^+,K^+-ATPase pump, protein synthesis, as well as alteration of cellular metabolic activities (e.g. increased glycolysis under anaerobic conditions causing lactic acid accumulation). Ultimately, irreversible damage to cellular organelles results in necrosis
- **Formation of ROS**: Due to incomplete oxidative phosphorylation
- **Leakage of mitochondrial proteins**: By action of BAX and BAK on mitochondrial membranes, leading to activation of the intrinsic pathway of apoptosis

2. Membrane damage

Membrane damage affects the integrity and functions of all cellular membranes, and can be caused indirectly by ischemia or directly by bacterial toxins, viral proteins, complement proteins and various physical and chemical agents. Most forms of cell injury (except apoptosis) manifest early loss of selective membrane permeability, leading eventually to overt membrane damage.

Mechanisms of membrane damage:

- **ROS**: Via lipid peroxidation
- **Decreased phospholipid synthesis**: Due to ATP depletion e.g. from mitochondrial dysfunction or hypoxia, affecting energy-dependent biosynthetic pathways
- **Increased phospholipid breakdown**: Likely due to activation of calcium-dependent phospholipases by increased cytosolic and mitochondrial Ca^{2+}. The accumulation of phospholipid breakdown products also has a detergent effect on membranes, affecting permeability and electrophysiology

- **Cytoskeletal abnormalities**: Likely due to proteases activated by increased cytosolic Ca^{2+}

Consequences of membrane damage:

- **Mitochondrial membrane damage**: Opening of the mitochondrial permeability transition pore, decreased ATP generation, leakage of mitochondrial proteins triggering apoptosis
- **Plasma membrane damage**: Loss of osmotic balance, cellular contents and metabolites important for ATP generation
- **Lysosomal membrane damage**: Leakage of lysosomal enzymes into cytoplasm and activation of acid hydrolases that degrade cellular contents e.g. DNA, RNA

3. DNA damage

DNA damage activates sensors that trigger p53-dependent pathways: activated p53 arrests cells in the G1 phase of the cell cycle and activates DNA repair mechanisms. If these fail, p53 triggers apoptosis via the mitochondrial pathway

Biochemical alterations in pathways involved in cell injury:

1. Oxidative stress due to accumulation of oxygen-derived free radicals

Involved in many pathologic conditions e.g. chemical and radiation injury, ischaemia-reperfusion injury, cellular aging, microbial killing.

Free radicals = chemical species that have a single unpaired electron in an outer orbit, which is highly reactive and can cause covalent modification of cellular proteins, lipids and nucleic acids

Reactive oxygen species (ROS) = type of oxygen-derived free radical e.g. superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$)

Oxidative stress = excess of free radicals

Mechanisms of generation of free radicals:

- **Reduction-oxygen reactions during normal metabolic processes**: e.g. during normal respiration when molecular O_2 is reduced to generate two water molecules
- **Absorption of radiant energy**: ionizing radiation can hydrolyze water into $^{\cdot}OH$ and H free radicals
- **During inflammation**: by activated leukocytes (e.g. using NADPH oxidase)
- **Enzymatic metabolism of exogenous chemicals or drugs**: Can generate free radicals e.g. $^{\cdot}CCl_3$
- **Transition metals**: e.g. iron in the Fenton reaction, and copper
- **Nitric oxide (NO)**: although an important chemical mediator, it can act as a free radical and also be converted to the highly reactive peroxynitrite anion ($ONOO^-$) and other nitrogen species

Mechanisms of removal of free radicals:

- Free radicals are inherently stable and generally decay spontaneously
- **Antioxidants**: e.g. glutathione, lipid-soluble vitamins E and A, either block free radical formation or inactivate (scavenge) free radicals
- **Binding of free iron and copper to storage and transport proteins**
- **Enzymes that act as free radical-scavenging systems**: usually located near sites of generation of oxidants e.g. catalase, superoxide dismutases (SODs), glutathione peroxidase

Consequences of free radicals:

- **Lipid peroxidation in membranes**: ROS attack double bonds in unsaturated fatty acids of membrane lipids, yielding peroxides that are themselves reactive and causing an autocatalytic chain reaction (propagation), resulting in extensive membrane damage
- **Oxidative modification of proteins**: Oxidative modification can damage active sites of enzymes, cause protein misfolding and thereby enhance proteasomal degradation of damaged proteins
- **DNA damage**: Free radicals can cause single- and double-stranded breaks in DNA, cross-linking and adduct formation

2. Disturbance in calcium homeostasis

Calcium ions are important second messengers in several signaling pathways, but cause cell injury if present in excessive amounts in the cytoplasm. Usually maintained at low cytosolic concentrations by sequestration within the mitochondria and ER. Insults such as ischemia and toxins cause excessive increase in cytosolic Ca^{2+} because of release from these intracellular stores as well as increased influx across the plasma membrane

Consequences of excessive intracellular Ca^{2+}:

- Accumulation of Ca^{2+} in mitochondria results in opening of the mitochondrial permeability transition pore and ATP depletion
- Activates enzymes e.g. phospholipases, proteases, endonucleases and ATPases, causing damage

3. Endoplasmic reticulum (ER) stress (unfolded protein response)

ER stress = presence of excess misfolded proteins, which may be due to an increased rate of misfolding or reduction in the cell's ability to remove them. This could be due to genetic mutations, aging, viral infections, metabolic alterations (e.g. increased demand for secretory proteins like insulin, changes in redox state), ischemia and hypoxia that result in decreased ATP needed for "foldases" to function

Consequence: Accumulation of misfolded proteins in the ER activates adaptive mechanisms (unfolded protein response i.e. signaling pathways that increase the production of chaperones, enhance proteasomal degradation of abnormal proteins via ubiquination and slow protein translation). However, this may be overwhelmed, triggering apoptosis

Clinicopathologic examples of cell injury and death

Hypoxia and ischemia

- **Hypoxia** = lack of oxygen but energy production by anaerobic glycolysis can continue as blood flow is still maintained
- **Ischemia** = hypoxia as well as compromised delivery of substrates for glycolysis / accumulation of toxic metabolites due to reduced blood flow (due to arterial obstruction and/or reduced venous drainage), resulting in more rapid and severe cell and tissue injury compared to hypoxia
- **Mechanism of ischemic cell injury**: Decreased intracellular oxygen causes failure of oxidative phosphorylation and ATP depletion (see *'Consequences of mitochondrial damage'* above)
- **Methods to decrease hypoxic stress**: Induction of hypoxia-inducible factor-1 (HIF-1), a transcription factor that promotes new blood vessel formation, stimulates cell survival pathways and enhances glycolysis. Transient induction of hypothermia can also decrease cell injury by reducing metabolic demands, decreasing cell swelling, suppressing formation of free radicals and inhibiting the host inflammatory response

Ischemic-reperfusion injury

- Paradoxical exacerbation of cell injury / cell death when blood flow is restored to ischemic tissues e.g. in myocardial and cerebral infarction following treatment that restores blood flow
- **Mechanisms of reperfusion injury**:
    - **Oxidative stress**: Reoxygenation may cause increased generation of reactive oxygen and nitrogen species due to incomplete reduction of oxygen in leukocytes, and in damaged endothelial and parenchymal cells. Tissues may be more sensitive to free radical damage due to the preceding ischemia compromising antioxidant defense mechanisms
    - **Intracellular calcium overload**: Initiated during acute ischemia and exacerbated during reperfusion due to cell membrane damage and ROS-mediated injury to sarcoplasmic reticulum
    - **Inflammation**: Presence of dead cells as well as cytokines from macrophages and increased expression of adhesion molecules by hypoxic parenchymal and endothelial cells recruit circulating neutrophils during reperfusion, causing additional tissue injury
    - **Activation of complement system**: During ischemia, IgM antibodies tend to deposit in ischemic tissue; during reperfusion, complement proteins can bind to these deposited antibodies, are activated and exacerbate cell injury and inflammation

Chemical (toxic) injury

- Frequently affects the liver, which metabolizes many drugs, and other organs involved in absorption or excretion of the chemical. 2 main mechanisms:
    - **Direct toxicity**: Some chemicals combine with critical molecular components (e.g. cyanide binds to and inhibits mitochondrial cytochrome oxidase, preventing oxidative phosphorylation)

- **Conversion to toxic metabolites**: Usually via cytochrome P-450 mixed-function oxidases in the smooth ER of liver and other organs. The toxic metabolites then cause membrane damage and cell injury via formation of free radicals, lipid peroxidation or direct binding to membrane proteins and lipids e.g. $\cdot CCl_3$ from CCl_4

IV. ADAPTATIONS OF CELLULAR GROWTH AND DIFFERENTIATION

Adaptations are reversible changes in the size, number, phenotype, metabolic activity or functions of cells in response to changes in their environment

Hypertrophy

- **Increase in size of cells** due to synthesis and assembly of additional intracellular structural components, resulting in increase in size of the affected organ. Occurs in non-dividing cells, and often co-exists with hyperplasia in cells capable of division
- **Physiologic hypertrophy**: Due to increased functional demand (e.g. skeletal muscle fibers in exercise) or stimulation by hormones and growth factors (e.g. uterine smooth muscle stimulated by estrogen during pregnancy)
- **Pathologic hypertrophy**: Due to pathologic stress (e.g. cardiac muscle hypertrophy in response to pressure overload from hypertension or valvular disease – initially improves cardiac function but eventually results in cardiac failure and other heart disease, when the enlargement of muscle mass can no longer cope with the increased workload)
- **Mechanisms of hypertrophy**: Action of growth factors and agonists, as well as direct mechanical effects on cellular proteins to activate signaling pathways (e.g. PI3K/AKT pathway and G-protein-coupled receptor-initiated pathway) to activate transcription factors that stimulate increased production of growth factors as well as increase / switch gene expression

Hyperplasia

- **Increase in number of cells**. Occurs in organs with cells capable of division, often occurs with hypertrophy and frequently triggered by the same stimuli
- **Physiologic hyperplasia**: Due to action of hormones or growth factors when there is a need to increase the functional capacity of the organ (e.g. female breast at puberty) or when compensatory increase after organ damage or resection is needed (e.g. liver regeneration after resection)
- **Pathologic hyperplasia**: Usually due to excessive or inappropriate actions of hormones or growth factors on target cells (e.g. endometrial hyperplasia from absolute or relative estrogen increase), or some viral infections (e.g. HPV causing skin warts). Although abnormal and increases the risk of acquiring genetic changes that can drive unrestrained proliferation, the process is still controlled and can regress or stabilize if the hormonal stimulation is eliminated, unlike cancer
- **Mechanisms of hyperplasia**: Due to growth factor-driven proliferation of mature cells and possibly increased new cells from tissue stem cells (see *Study Notes on Inflammation and Repair*).

Atrophy

- **Decrease in size of organ or tissue due to decrease in cell size and number**. Initially, decrease in cell size and organelles occurs to decrease the metabolic needs of the cell, in hopes of establishing a new equilibrium with the decreased blood supply/nutrition/trophic stimulation. However, if the cause is persistent, cells may become irreversibly injured and die, often by apoptosis
- **Physiologic atrophy**: Common during normal development (e.g. embryonic structures like thyroglossal duct)
- **Pathologic atrophy**: Can be local or generalized. Causes include:
    - **Decreased workload (disuse atrophy)**: e.g. skeletal muscle atrophy with immobilization / bed rest
    - **Loss of innervation (denervation atrophy)**: of muscle fibers supplied by those nerves
    - **Diminished blood supply**: e.g. senile atrophy of brain due to chronic ischemia
    - **Inadequate nutrition**: muscle wasting (cachexia)
    - **Loss of endocrine stimulation**: e.g. breast and endometrial atrophy after menopause
    - **Pressure**: e.g. surrounding tissue compression by enlarging tumours, probably due to ischemia from the mass effect
- **Mechanisms of atrophy**: Due to decreased protein synthesis (because of reduced trophic signals) and increased protein degradation in cells, mainly via the ubiquitin-proteasome pathway. Atrophy is also accompanied by increased autophagy

Metaplasia

- Reversible change in which one differentiated cell type (epithelial or mesenchymal) is **replaced by another cell type** e.g. columnar to squamous epithelium in the respiratory tract
- Often an adaptive response to chronic irritation, whereby the other cell type is better able to withstand the particular stress. However, this may reduce the primary cell functions e.g. when the respiratory tract epithelial lining undergoes squamous metaplasia, the protective mechanism of mucous secretion and ciliary action are lost, which is undesirable. Furthermore, persistent stressors that result in metaplasia can also initiate malignant transformation in the metaplastic epithelium e.g. squamous cell carcinoma in the lungs, gastric adenocarcinoma in Barrett oesophagus
- Connective tissue metaplasia (e.g. formation of cartilage/bone/adipocytes in tissues that do not normally contain these elements) is most likely due to cell/tissue injury rather than adaptive and is not associated with increased cancer risk
- **Mechanisms of metaplasia**: Due to either reprogramming of local tissue stem cells or colonization by differentiated cell populations from adjacent sites, rather than change of phenotype of an already differentiated cell. The metaplastic change is stimulated by cytokines, growth factors and extracellular matrix components in the cell environment

V. OTHER CELLULAR CHANGES

Intracellular accumulations

Manifestation of metabolic derangement. Substances accumulated can be intracytoplasmic, within organelles (usually lysosomes) or intranuclear, synthesized by the cell or produced elsewhere, and may be harmless or cause further injury. Usually reversible if the overload can be controlled/stopped; if progressive, can cause cellular injury and death

Mechanisms of abnormal intracellular accumulations:

1. **Abnormal metabolism** leading to inadequate removal of a normal substance e.g. liver steatosis
2. **Genetic or acquired defects in protein folding, packaging, transport or secretion** leading to accumulation of abnormal endogenous proteins e.g. α1-antitrypsin
3. **Failure to degrade metabolites due to inherited enzyme deficiencies** leading to accumulation of endogenous materials (typically affecting lysosomal enzymes in lysosomal storage diseases)
4. **Ingestion of indigestible exogenous substances** e.g. carbon pigment, silica particles

Substance	Manifestation / Associated condition
***Lipids**: lipid intracytoplasmic vacuoles*	
Triglycerides	**Steatosis (fatty change)** - Often in the liver (major organ involved in fat metabolism) but also heart, muscle, kidney - Causes: Alcoholic and non-alcoholic fatty liver disease, diabetes, obesity, toxins, protein malnutrition, anoxia
Cholesterol and cholesterol esters	**Atherosclerosis**: Aggregates of foam cells (macrophages and smooth muscle cells with numerous lipid vacuoles) in the intima of large vessels, forming the grossly yellow atheromas. These cells may ruptured, and the released cholesterol and cholesterol esters can crystallise, forming cholesterol clefts or activating the inflammasome **Xanthomas**: Aggregates of foamy macrophages in tissue. Can be seen in acquired and hereditary hyperlipidemic states **Cholesterolosis**: Aggregates of foamy macrophages in lamina propria of gallbladder **Niemann-Pick disease, type C**: Lysosomal storage disease (mutation in enzyme involved in cholesterol trafficking). Affects multiple organs
***Proteins**: rounded eosinophilic intracytoplasmic droplets, vacuoles or aggregates. Can also deposit extracellularly e.g. amyloid.* *Hyaline change: morphologic homogeneous glassy-pink appearance on H&E within cells (e.g. alcoholic hyaline) or extracellularly (e.g. hyaline arteriolosclerosis); not a specific pattern of accumulation*	
Normal proteins	**Reabsorption droplets in proximal renal tubules**: Due to increased reabsorption of the protein into vesicles by pinocytosis in renal diseases associated with proteinuria
	Accumulation of normal secreted proteins in the ER when produced in excessive amounts: e.g. Russell bodies in plasma cells actively synthesizing immunoglobulins
Abnormal endogenous proteins	**Defective intracellular transport and secretion of critical proteins**: e.g. α1-antitrypsin deficiency, in which protein mutations slow folding and cause accumulation of partially folded intermediates in the ER of hepatocytes. The proteins are not secreted and cause deficiency of the circulating enzyme. Unfolded protein response may also be activated causing apoptosis
	Deposition of abnormal or misfolded proteins that interferes with normal functions: e.g. amyloidosis, proteinopathies

Cytoskeletal proteins	**Associated with certain types of cell injury**: e.g. keratin intermediate filaments in alcoholic liver disease (alcoholic hyaline), neurofibrillary tangles in Alzheimer disease
***Glycogen**: clear vacuoles (glycogen dissolves in aqueous fixatives); PAS positive diastase sensitive*	
Glycogen	**Abnormalities in glucose or glycogen metabolism e.g. Diabetes mellitus**: Glycogen in renal tubular epithelial cells, liver cells, cardiac muscle **Glycogen storage diseases (glycogenoses)**: enzymatic defects in synthesis of breakdown of glycogen result in massive accumulation, cell injury and death
***Pigments**: coloured substances*	
Exogenous pigments	**Carbon (coal dust)**: Air pollutant in urban areas, which causes anthracosis of the lungs. Can cause pneumoconiosis in coal workers **Tattoo pigment**: Dermal macrophages phagocytose the inoculated pigment
Endogenous pigments	**Lipofuscin ('wear and tear' pigment)**: Yellowish-brown finely granular cytoplasmic insoluble inert pigment, indicator of lipid peroxidation and free radical injury
	Melanin: Brown-black pigment formed from oxidation of tyrosine in melanocytes
	Hemosiderin: Hemoglobin-derived golden-yellow to brown, granular/crystalline pigment. Major storage form of iron (aggregates of ferritin micelles) in local or systemic conditions of iron excess (e.g. haemorrhage, hemochromatosis, hemolytic anaemia, transfusions)

Pathologic calcification

Abnormal deposition of calcium salts in tissue (often with smaller amounts of iron, magnesium and other mineral salts). Microscopically, has a basophilic amorphous granular/clumped appearance which can be intracellular, extracellular or both. Can form psammoma bodies (lamellated concentric layers) e.g. in papillary thyroid carcinoma. Eventually, heterotopic bone can form in the focus of calcification

- **Dystrophic calcification**: Local deposition in necrotic tissues despite normal serum calcium e.g. atheromas, aging or damaged heart valves, and cause additional dysfunction
- **Metastatic calcification**: Systemic deposition in normal tissues in hypercalcemia (e.g. from hyperparathyroidism, increased bone resorption from various causes, vitamin D-related disorders and renal failure causing secondary hyperparathyroidism). Tissues with a predisposition are those with an internal alkaline component e.g. gastric mucosa, kidneys. Usually do not cause clinical dysfunction (exceptions: nephrocalcinosis causing renal damage)

VI. CELLULAR AGING

Cellular aging is the result of a **progressive decline in cellular function and viability**, caused by genetic abnormalities and the accumulation of cellular and molecular damage from exposure to exogenous influences. Mechanisms of cellular aging include:

1. Accumulation of DNA damage

Although most DNA damage is repaired by DNA repair enzymes, some persists and accumulates as cells age. Defective DNA repair mechanisms can be seen in syndromes that show premature aging

2. Replicative senescence

Cells become arrested in a terminally nondividing state after a fixed number of cell divisions. This is due to 2 mechanisms:

i. **Telomere attrition**: Telomeres are short repeated DNA sequences at the ends of linear chromosomes that are important for ensuing the complete replication of chromosome ends and preventing fusions and degradation of the ends. During somatic cell replication, a small section of the telomere is not duplicated and there is progressive shortening of telomeres. Eventually, the unprotected chromosome ends are recognized as broken DNA and signal cell cycle arrest. Telomerase can maintain telomere length; this enzyme is expressed in germ cells (not somatic cells), in low levels in stem cells and may be reactivated in immortalized cancer cells
ii. **Activation of tumour suppressor genes**: e.g. p16 encoded by *CDKN2A*, which controls G1 to S phase progression in the cell cycle and can push cells along the senescence pathway

3. Defective protein homeostasis

Impaired chaperone and proteasome functions affect normal folding and degradation of misfolded proteins, respectively

4. Dysregulated nutrient sensing

Caloric restriction increases longevity, which may occur through 2 major neurohormonal circuits: reducing the signaling intensity of the insulin-like growth factor 1 (IGF-1) signaling pathway, and increasing sirtuins, a family of NAD-dependent protein deacetylases thought to promote expression of genes to produce proteins with functions that include reducing apoptosis, stimulate protein folding, increasing insulin sensitivity etc.