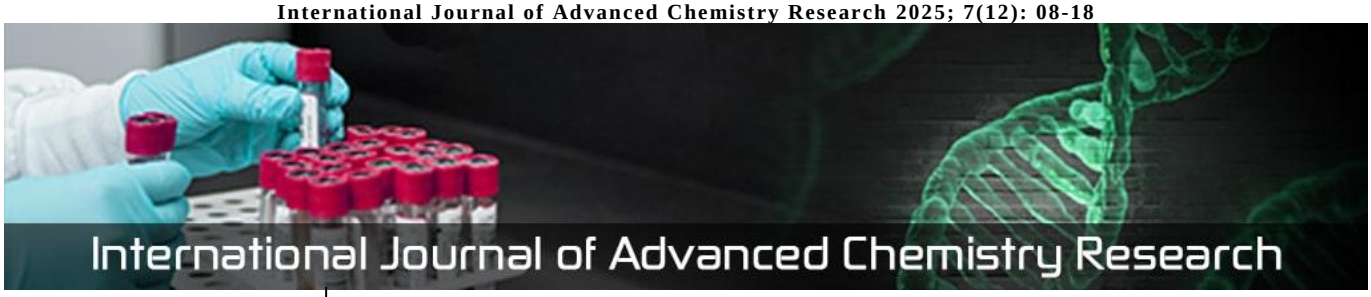

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Review Article:

Role of ubiquitin proteasome pathway in cancer development

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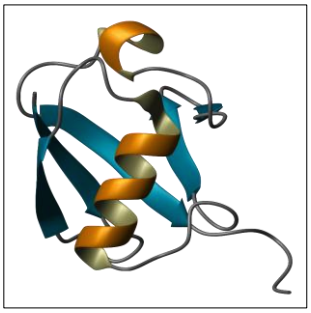
Abstract
The ubiquitin-proteasome pathway (UPP) is a highly regulated protein degradation system essential for maintaining cellular homeostasis. Its dysregulation plays a central role in cancer development by altering the stability of key proteins involved in cell-cycle control, apoptosis, DNA repair, and hypoxia signaling. Ubiquitination, mediated by E1, E2, and E3 enzymes, determines whether proteins undergo proteasomal degradation or participate in non-proteolytic signaling. Aberrant activity of E3 ligases and deubiquitinating enzymes leads to uncontrolled degradation or accumulation of oncogenes and tumor-suppressor proteins such as p53, cyclins, p27, and Bcl-2. The pathway also regulates apoptosis through modulation of Bcl-2 family proteins, caspases, and IAPs, enabling cancer cells to evade programmed cell death. Additionally, hypoxia-inducible factors (HIFs) are tightly controlled by VHL-mediated ubiquitination, and their dysregulation promotes angiogenesis and tumor progression. Understanding UPP biology has enabled development of novel anticancer therapies, including proteasome inhibitors and E3-targeted drugs.

Keywords: Ubiquitin-proteasome pathway, ubiquitination, protein degradation, cellular homeostasis

Introduction
The unexpected "bottom-to-top" discovery of the ubiquitin-proteasome system (UPS) has changed contemporary cell biology and shown that protein degradation is a carefully controlled, selective process crucial for maintaining cellular homeostasis and preventing cancer. Ubiquitination was identified as the molecular signal that targets proteins for proteolysis after early research by Hershko, Ciechanover, and Rose showed that cytosolic protein degradation requires ATP [36, 10]. Over time, the UPS has emerged as a master regulator of cellular processes, including cell cycle progression, apoptosis, DNA repair, transcription, immune surveillance and stress responses [66]. According to [40], dysregulation of UPS components, specifically E3 ubiquitin ligases and deubiquitinating enzymes, can result in aberrant stabilization or degradation of oncogenes and tumor-suppressor proteins, which can drive carcinogenesis, tumor growth, and therapeutic resistance. Therefore, comprehending UPS biology is essential to knowing how cancer develops and has resulted in new therapeutic approaches including E3 ligase-targeting medicines and proteasome inhibitors.

What is Ubiquitin?



Ubiquitin is a highly conserved protein with 76 amino acids that is essential for controlling many cellular functions and designating intracellular proteins for destruction. Ubiquitin is a compact, densely folded globular protein that is highly heat-stable and resistant to proteolysis due to its hydrophobic core and vast network of hydrogen bonds [91]. Its C-terminal glycine residue (Gly76), which protrudes from the protein surface and forms covalent isopeptide bonds with target protein lysine residues or with lysines on other ubiquitin molecules to form polyubiquitin chains, is largely responsible for its functional activity [94, 66]. These polyubiquitin chains—particularly those linked through Lys48 or Lys29—serve as signals for 26S proteasome-mediated degradation, whereas other linkages such as Lys63 regulate non-proteolytic functions including DNA repair, signaling and transcriptional activation [9, 93]. Additionally, ubiquitin can bind as a single unit (mono-ubiquitination), which aids in viral budding, endocytosis, and histone modification [37]. Ubiquitin is regarded as one of the most important regulatory proteins in cellular homeostasis because of its widespread engagement in vital biological pathways and universal presence in eukaryotic cells.

Ubiquitin Enzymatic Cascade

In order to control the stability and destiny of particular substrate proteins, ubiquitin is systematically activated, transferred, and conjugated to them by a highly coordinated enzyme cascade that powers the ubiquitin-proteasome system. The E1 (ubiquitin-activating enzyme) initiates the process by adenylating ubiquitin with ATP to create a high-energy ubiquitin-E1 thioester intermediate [9, 66]. An E2 (ubiquitin-conjugating enzyme) receives activated ubiquitin and acts as the carrier, interacting with several E3 ligases via its conserved core domain [93]. Lastly, E3 (ubiquitin ligases) facilitate the ubiquitin transfer from E2 to the target protein and offer substrate selectivity. In certain instances, extra enzymes called E4 ligases (like Ufd2) lengthen ubiquitin chains to guarantee the production of polyubiquitin signals necessary for effective degradation [41]. Deubiquitinating enzymes (DUBs) balance this process by removing ubiquitin from substrates at different phases, either by regenerating free monomeric ubiquitin or editing polyubiquitin chains. The choice between protein degradation and non-proteolytic functions is regulated by the two main DUB families, UBPs/USPs and UBHs [46, 93].

26S Proteasome

According to [38], the 26S proteasome is a highly conserved, ATP-dependent complex that breaks down proteins that have been tagged with ubiquitin. It is present in the nucleus and cytoplasm and is made up of one or two 19S regulatory particles and a 20S catalytic core that are formed in an ATP-dependent manner [65]. Trypsin-like, chymotrypsin-like, and caspase-like proteolytic sites are found in the cylindrical α7β7β7α7 structure of the 20S core [31]. The α-subunit gate controls access to this chamber. Poly-ubiquitinated substrates are identified by the 19S regulator, which then routes them into the 20S core for destruction after unfolding them via its ATPases [29, 67]. The 26S proteasome works in concert to break down damaged or misfolded proteins into tiny peptides, which are then transformed into amino acids [87].

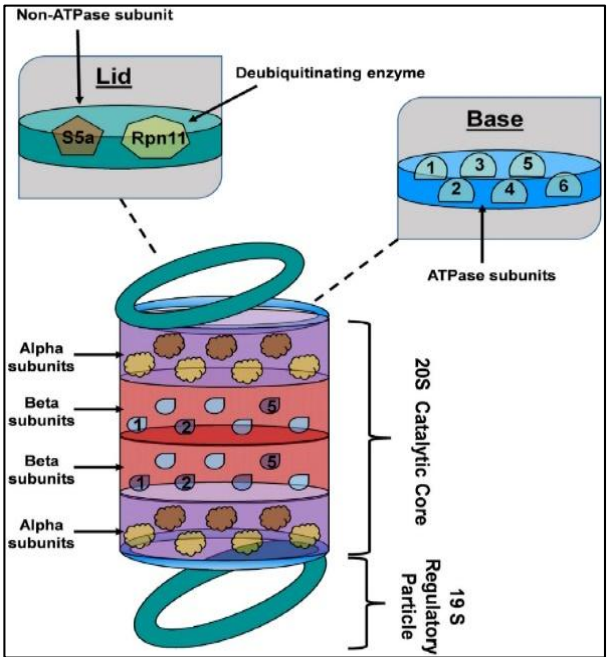


Fig 2: 26S proteasomes are composed of four heptameric stacked rings (α7β7β7α7) and the outer rings are made up of α-type subunits whereas the inner two rings are made up of β-type subunits.

Ubiquitination

According to [9], ubiquitination is a post-translational modification in which the tiny protein ubiquitin is covalently bound to a target protein via an isopeptide bond between the C-terminal glycine of ubiquitin and the ε-amino group of a lysine residue on the substrate. Polyubiquitin chains, which often mark proteins for proteasome breakdown or function as signaling tags that control different physiological processes, can be formed by additional ubiquitin molecules attaching in the same manner.

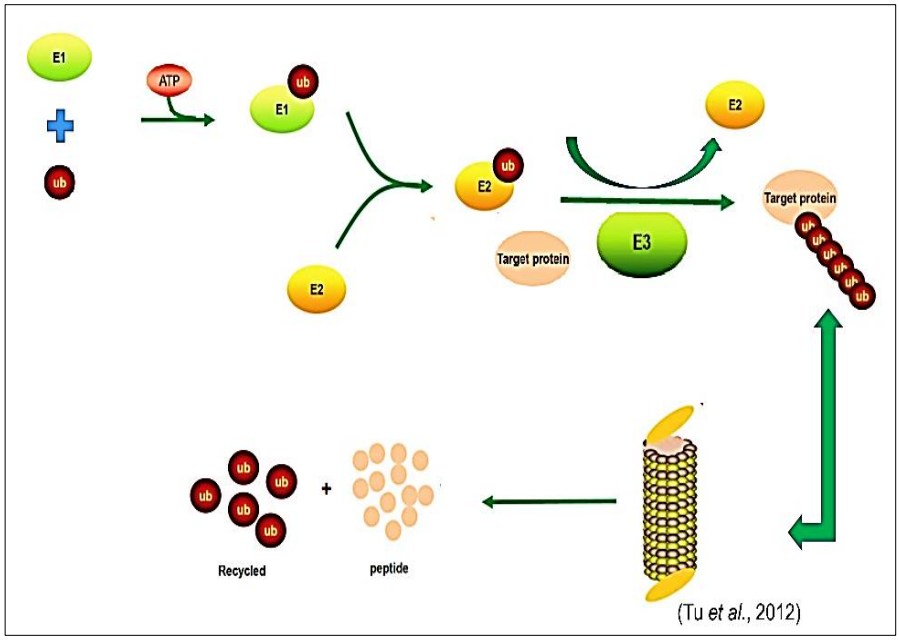


Fig 3: Ubiquitin is activated by the E1 enzyme in the presence of ATP. The E1 enzyme forms a thiol ester bond with ubiquitin, resulting in the formation of ubiquitin-E1 thiol ester. The ubiquitin-E1 thiol ester is recognized by multiple E2 enzymes, which transfer ubiquitin to their active site cysteines. E2 enzymes carry the activated ubiquitin to the substrate protein. E3 enzymes are responsible for transfer of ubi to target protein. Here, polyubiquitination occur. The target protein is degraded by the proteasome Proteasome cleaves proteins into 6-10 amino acid peptidesAfter the binding of ubiquitine with target protein the complex is degraded by the proteasome

Function of UPP

The ubiquitination system functions in a wide variety of cellular processes, including:

- Antigen processing
- Apoptosis
- Biogenesis of organelles
- Cell cycle and division
- DNA transcription and repair
- Differentiation and development
- Immune response and inflammation
- Neural and muscular degeneration
- Maintenance of pluripotency [77]
- Morphogenesis of neural networks
- Modulation of cell surface receptors, ion channels and the secretory pathway
- Response to stress and extracellular modulators
- Ribosome biogenesis
- Viral infection

Regulation of cell growth

Cell growth is controlled by several groups of genes that work together to maintain normal proliferation and survival. Growth-promoting genes, including growth factors, their receptors, intracellular signaling proteins, and nuclear regulators such as MYC, along with cell-cycle regulators like cyclins and CDKs, stimulate cell division. Tumor suppressor genes including Rb, p53, APC, VHL, and PTEN act as brakes to prevent uncontrolled growth. Apoptosis-regulating genes also play a crucial role, with pro-apoptotic proteins such as BID, Puma, and Noxa promoting cell death, while anti-apoptotic proteins like BCL-2, BCL-XL, and MCL-1 support cell survival. Additionally, transcription factors such as HIF-α and NF-κB modulate gene expression in response to environmental and cellular signals, further influencing cell growth and homeostasis.

2.1 Role of Ubiquitine Proteasome Pathway in Cell Cycle Control and Cancer Development

The cell cycle is a tightly regulated series of events in which DNA and cellular components are duplicated and accurately segregated into daughter cells. Cyclin-dependent kinases (CDKs), whose activity depends on periodic association with cyclins and inhibition by CDK inhibitors (CKIs), are the main drivers of progression through the four major phases—G1, S, G2, and M [3, 54]. The unidirectional, irreversible advancement of the cell cycle depends on the timely production and degradation of cyclins, CKIs, and other regulators due to their fluctuating amounts.

Ubiquitination, a post-translational modification that controls protein stability, location, and function, is a key mechanism governing these variations. Key cell-cycle regulators, such as cyclins, CKIs, securin, and mitotic cyclins, are selectively broken down by the ubiquitin-proteasome system (UPS) to guarantee appropriate transitions, especially the G1-S transition and mitotic exit [62, 69, 82]. The time and accuracy of DNA replication, chromosomal segregation, and cell-cycle advancement are thus determined by the exact synchronization of phosphorylation and ubiquitination.

Cancer is directly caused by this system's dysregulation. Cancer cells multiply because of flaws in proliferation-inhibitory pathways and the disruption of negative feedback mechanisms, in contrast to normal cells, which only divide in response to suitable growth signals [67]. Early genetic research showed that tumor suppressors that limit cell-cycle progression, like RB and p53, are often deleted or altered in malignancies [4]. Additional changes pertain to the UPS components themselves. For example, increased degradation of the CKI p27 in cancer is caused by the E3 ligase component SKP2, which is frequently overexpressed in malignancies [63, 80, 61].

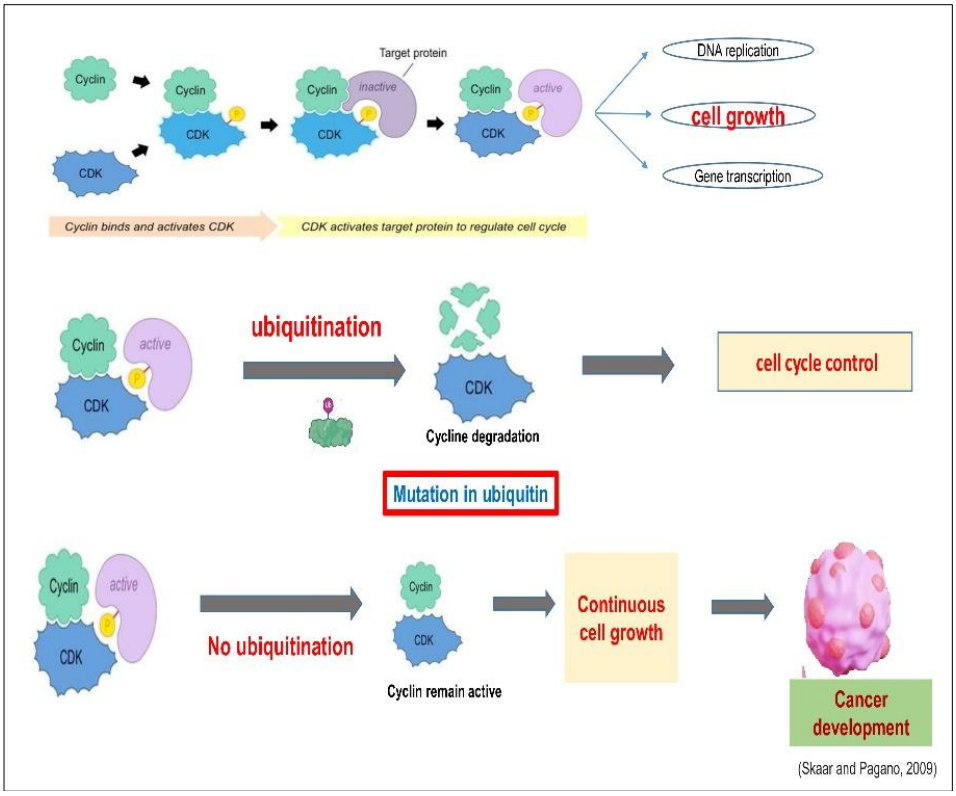


Fig 4: Cyclin-dependent kinases (CDKs) become active only after binding with cyclins, forming cyclin-CDK complexes that drive DNA replication, cell growth, and gene transcription. Proper cell cycle control requires timely cyclin degradation through the ubiquitin-proteasome system (UPS). When ubiquitination is intact, cyclins are degraded, CDKs become inactive, and cell proliferation is regulated. However, defects in ubiquitination prevent cyclin degradation, allowing CDKs to remain continuously active, leading to uncontrolled cell growth and cancer development.

On the other hand, FBW7, another E3 ligase component that targets several oncogenic proteins, is often altered or deleted, which supports its function as a tumor suppressor [61]. Together, these results show that the UPS is essential for controlling the cell cycle and that its dysregulation—caused by aberrant ubiquitination or proteasomal degradation—promotes unchecked cell division and the emergence of cancer.

Role of ubiquitine proteasome pathway in p53 gene and cancer development

p53, widely known as the "guardian of the genome," plays a central role in coordinating cellular responses such as DNA repair, cell-cycle arrest, senescence, and apoptosis [50]. p53-deficient animals exhibit a considerably increased sensitivity to spontaneous tumor formation, demonstrating the significance of p53 in tumor suppression [17]. In addition to eliminating its tumor-suppressive properties, mutations in p53 can result in gain-of-function and dominant-negative activities that actively encourage the development and spread of tumors [6, 30, 96]. Wild-type p53 is a highly unstable protein with a brief half-life of 5-20 minutes under normal, unstressed conditions. It is kept at low cellular levels by ongoing ubiquitin-mediated degradation [49, 27]. On the other hand, mutant p53 can cause oncogenic gain-of-function consequences since it is often stable and accumulates in cancer cells [6, 23].

In addition to its traditional functions in apoptosis and cell-cycle regulation, p53 also affects metabolism, stem-cell dynamics, differentiation, and aging, highlighting the need for strict control over its activation and stability [48, 92]. Numerous post-translational changes, such as phosphorylation, acetylation, methylation, neddylation, sumoylation, and ubiquitination, control protein stability, cellular localization, and transcriptional output. These modifications also affect the amount and activity of p53. In addition to its non-transcriptional roles, such as the direct control of mitochondrial apoptosis, which further contribute to tumor suppression, p53 is a transcription factor that either activates or represses a large number of target genes necessary for genome integrity [89, 92]. Following DNA damage, p53 quickly stabilizes, enabling strong activation of its target genes and stopping the growth of injured cells through senescence or apoptosis [33].

The ubiquitin-proteasome system, which regulates p53 turnover through dynamic and reversible ubiquitination, is essential to p53 regulation [89]. The stability, localization, and activity of p53 are all influenced differently by mono- and polyubiquitination. The finding that HPV E6 causes proteasomal degradation of p53 was the first indication of the importance of ubiquitin-mediated degradation in p53 regulation [21]. All things considered, ubiquitination offers a precise, adaptable method for regulating p53 activity and guaranteeing proper cellular reactions to stress [34, 60].

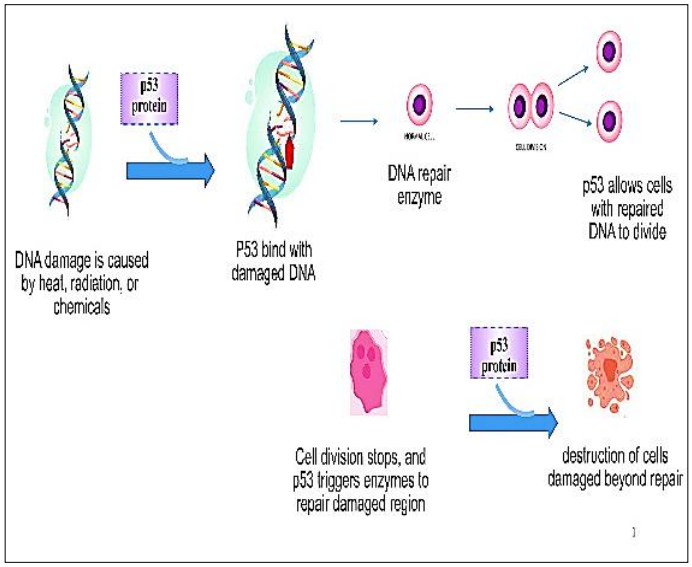


Figure 5: When DNA damage occurs, p53 becomes activated and initiates the cellular damage-response pathway. Activated p53 stimulates the expression of DNA repair enzymes. If the damaged DNA is successfully repaired, p53 permits the cell to resume division. However, if the damage is irreparable, p53 halts cell division and triggers programmed cell death, preventing the propagation of genetically unstable cells.

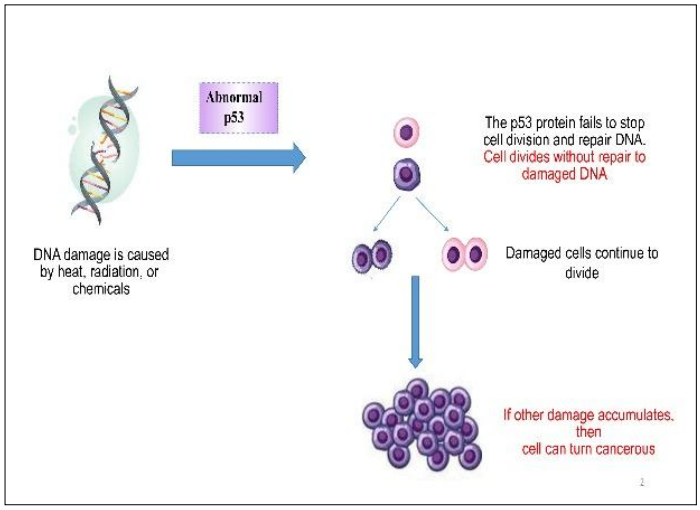


Fig 6: If p53 becomes damaged or mutated, it loses its ability to halt the cell cycle and repair DNA. As a result, cells with unrepaired DNA continue to divide. With ongoing cell division, additional genetic abnormalities accumulate, eventually leading to uncontrolled proliferation and cancer development.

The 90-kDa protein that MDM2, which is found on chromosome 12q13-14, encodes serves as the main negative regulator of p53 by binding its N-terminal transactivation domain, preventing transcriptional activity, and encouraging ubiquitin-mediated degradation via its RING-finger E3 ligase domain [5]. MDM2's structure includes a zinc-finger motif, a central acidic domain, a p53-binding region, and a C-terminal RING domain that is necessary for ubiquitination. MDM2 overexpression or amplification lowers p53 activity and promotes carcinogenesis; this is commonly seen in cancers such lung, breast, liver, and colorectal malignancies [57, 58]. Under basal settings, MDM2 and p53 form a crucial negative feedback loop that keeps p53 levels low; stress signals break this loop, stabilizing p53 [43].

Although MDMX lacks intrinsic E3 ligase activity, it heterodimerizes with MDM2 to enhance its stability and ubiquitination function, together forming the major complex responsible for p53 suppression [42, 84]. DNA damage triggers degradation of MDM2 and MDMX, thereby releasing p53 and enabling an appropriate cellular stress response [85, 88].

Role of Ubiquitine Proteasome Pathway in Apoptosis and Cancer Development

During development and tissue homeostasis, apoptosis—a controlled cell death program—is crucial for getting rid of injured or undesirable cells (Kerr *et al*., 2000; Fuchs and Steller, 2011). Apoptosis dysregulation is linked to immunological, neurodegenerative, cancer, and developmental problems [35, 56]. Apoptosis is characterized by caspase-dependent cell death, which is carried out by caspases that are triggered from their dormant pro-caspase forms [18]. The intrinsic (mitochondrial) and extrinsic (death receptor-mediated) mechanisms control apoptosis. These pathways interact through molecules such as tBID, which facilitate apoptosome formation, cytochrome c release, mitochondrial outer membrane permeabilization (MOMP), and downstream caspase activation.

Inhibitors of apoptosis proteins (IAPs) like XIAP, which have E3 ligase activity and directly inhibit caspases, regulate caspase activity [16, 68, 72]. By interfering with these regulatory checkpoints, cancer cells frequently avoid apoptosis [13, 14].

Correlation Between Apoptosis and Ubiquitination:

1. Increased ubiquitination is frequently seen in cells going through apoptosis, indicating that ubiquitination plays a regulatory function in programmed cell death.
2. Elevated ubiquitin conjugates are seen to precede morphological apoptotic alterations in a variety of species, including:
3. In Manduca sexta, intersegmental muscle (ISM) programmed cell death exhibits elevated polyubiquitin expression during muscle atrophy [75].
4. Teniposide-treated murine lymphocytes [70].
5. Schlosseri Botryllus [58].
6. γ-irradiated human cells [15].
7. Apoptosis caused by thyroxine in the tail tips of Rana catesbeiana tadpoles [64].
8. Mice lacking dystrophin [73].

Myoblasts treated with cisplatinum without serum [37].

- While etoposide-induced apoptosis did not change ubiquitin transcription, proteasome inhibition in mouse RVC cells increased ubiquitin transcription during apoptosis [86].
- Additionally, Ewing's sarcoma cells treated with radiation or proteasome inhibitors, as well as rat cerebellar external granule cells exposed to radiation or toxins, showed increased ubiquitin-protein conjugates [22, 83].

2.3.2 Role of Bcl-2 Family Proteins in the Intrinsic Pathway

The Bcl-2 family modulates mitochondrial apoptosis and comprises both pro- and anti-apoptotic proteins, which determine cellular sensitivity to apoptotic stimuli [12, 32, 81].

- **Anti-apoptotic:** Bcl-2, Bcl-xL, Bcl-w, Mcl-1, A1, Bcl-B
- **Pro-apoptotic:** BAX, BAK
- **BH3-only pro-apoptotic:** Bim, Bad, tBID, Bmf, Bik, Noxa, Puma, Hrk

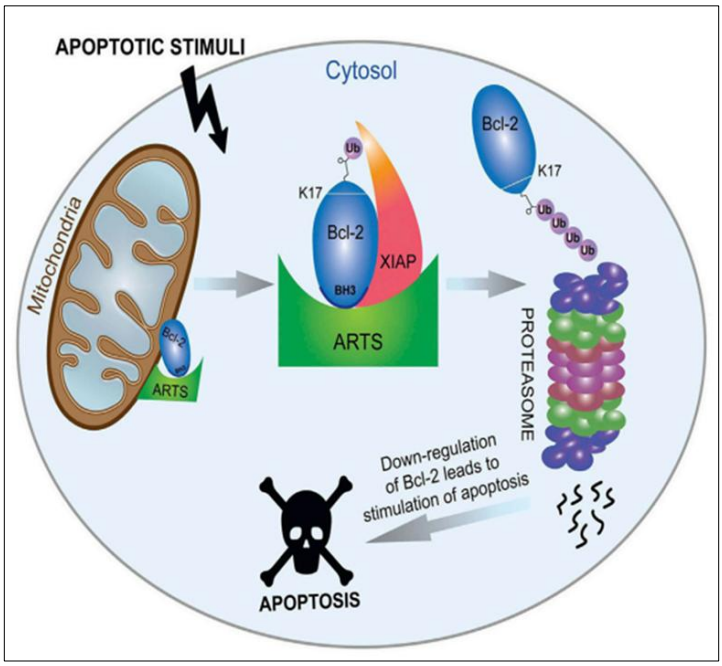


Figure 7: In response to apoptotic stimuli, ARTS is released from the mitochondria into the cytosol. ARTS binds to Bcl-2 and recruits XIAP, promoting the ubiquitination of Bcl-2 at lysine-17 (K17). The ubiquitinated Bcl-2 is then targeted to the proteasome for degradation. Down-regulation of Bcl-2 removes its anti-apoptotic effect, thereby facilitating activation of the apoptotic pathway.

Bcl-2 itself is a key regulator that inhibits apoptosis and is often overexpressed in cancer [12]. During apoptosis, Bcl-2 is degraded via the UPS, mediated by XIAP, with ARTS acting as a scaffold to facilitate this process [19, 45].

Regulation of Other Key Proteins by E3 Ligases

1. **Mcl-1:** Anti-apoptotic protein degraded via proteasome. E3 ligases include MULE/ARFBP1, TRIM17, Parkin, SCF^β-TrCP, and SCF^FBW7, ensuring precise apoptotic control [46].
2. **BID and BAX**

- BID is cleaved to tBID, linking extrinsic and intrinsic pathways. tBID is ubiquitinated by ITCH/AIP4 [2].
- Bax translocates to mitochondria to initiate MOMP and is tightly controlled by UPS. E3 ligases include IBRDC2 (specific to BAX) and Parkin [2, 53].

3. **IAPs and Antagonists**

- XIAP, cIAP1, cIAP2, ML-IAP, ILP2 inhibit caspases and possess E3 ligase activity through RING domains [68].
- ARTS antagonizes XIAP upon apoptotic stimuli, facilitating caspase activation and subsequent MOMP [19].

The UPS not only maintains apoptosis under physiological conditions but also contributes to cancer development when dysregulated. By modulating the degradation of key apoptotic regulators, the UPS enables cancer cells to evade apoptosis, promoting survival and proliferation [79].

2.4 Role of Ubiquitine Proteasome Pathway in HIF Alpha and Cancer Development

2.4.1 HIF and Oxygen Sensing

Through hypoxia-inducible factors (HIFs), which are master regulators of the cellular hypoxic response, oxygen tension controls genes related to angiogenesis, metabolism, and apoptosis [76, 77]. HIFs are heterodimers made up of a constitutive β-subunit (ARNT/HIF-1β) and an oxygen-sensitive α-subunit (HIF-1α, HIF-2α, and HIF-3α) [53]. Prolyl hydroxylases (PHDs) and factor-inhibiting HIF-1 (FIH-1) hydroxylate HIF-α under normoxia, which is then recognized by the von Hippel-Lindau (VHL) E3 ubiquitin ligase complex, ubiquitinated, and broken down by the 26S proteasome [28]. By inhibiting PHD and FIH-1 activity, hypoxia stabilizes HIF-α, which then translocates to the nucleus, dimerizes with HIF-1β, and triggers the transcription of genes such as erythropoietin, VEGF, and glycolytic enzymes [51]. By directing PHDs for degradation and increasing HIF activity, E3 ligases like SIAH-1 and SIAH-2 also control HIF stability during hypoxia [95].

2.4.2 Role of HIF in Cancer and Angiogenesis

Hypoxia brought on by rapid tumor growth forces a glycolytic metabolism (Warburg effect) and encourages an aggressive phenotype [47]. VEGF, PDGF-b, ANGPT2, SDF1, SCF, and TGF-α are among the proangiogenic factors that HIF-1α upregulates. These factors promote endothelial cell survival, proliferation, and vascular permeability and attract circulating angiogenic cells (CACs) to the tumor microenvironment [7, 8]. Additionally, VEGF supports tumor survival by promoting antiapoptotic signals through BCL-2 and A1 [26]. Tumor angiogenesis, progression, and metastasis are all influenced by these pathways.

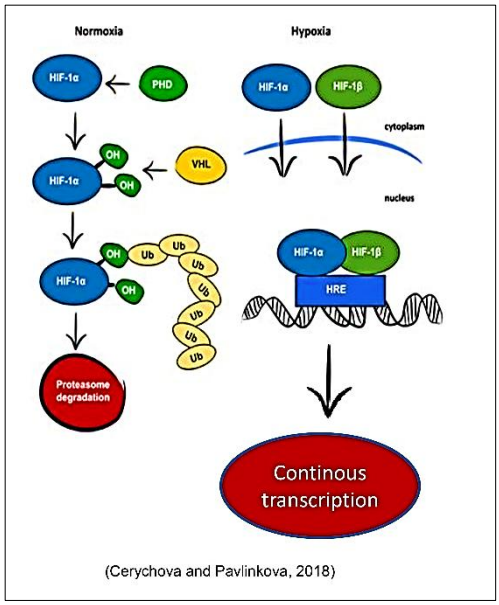


Fig 8: Regulation of HIF-1α under normoxic and hypoxic conditions.

2.5 Role of Ubiquitine Proteasome Pathway in NF Kappa Beta and Cancer Development

2.5.1 NF-κB and Cancer

NF-κB is a widely expressed transcription factor made up of heterodimers, mainly p50 and p65. It was first identified as a B-cell nuclear factor binding the κ immunoglobulin enhancer (Sen and Baltimore, 1986). Inhibitory proteins (IκBs) sequester NF-κB in the cytoplasm of resting cells, blocking nuclear translocation [90]. Extracellular stimuli, such as stress, cytokines, radiation, and oncogene signaling, cause IκB kinases (IKK) to phosphorylate IκB. This is followed by βTrCP-mediated ubiquitination and proteasomal degradation, which releases NF-κB to enter the nucleus [2, 44].

Numerous malignancies, including breast, lung, colon, prostate, myeloma, and leukemia, exhibit constitutive NF-κB activation, which is linked to oncogene activity, increased IKK/SCFβ-TrCP function, and chronic inflammation [24, 55]. Nuclear NF-κB promotes tumor growth and progression by driving the transcription of genes related to cell survival, proliferation, inflammation, and apoptosis inhibition [1, 70]. NF-κB-dependent oncogenesis is caused by changes in both kinase activity and ubiquitin-proteasome-mediated IκB degradation [24].

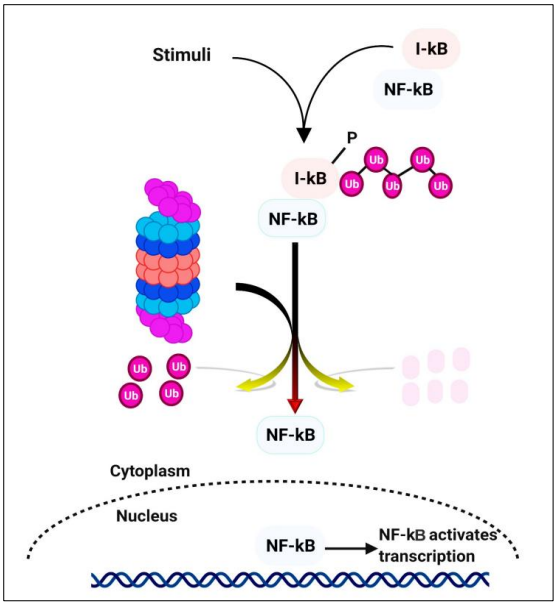


Fig 9: Stimuli cause phosphorylation and ubiquitination of I-κB, leading to its proteasomal degradation. Once I-κB is removed, NF-κB is released and moves into the nucleus, where it activates transcription of target genes.

Conclusion

The ubiquitin-proteasome pathway (UPP) is a cornerstone of cellular homeostasis, regulating protein turnover with remarkable specificity and precision. By orchestrating the controlled degradation of key regulatory proteins—including cyclins, CDK inhibitors, tumor suppressors like p53, apoptotic mediators, HIF-α, and transcription factors such as NF-κB—the UPP ensures proper cell cycle progression, apoptosis, stress responses, and adaptation to environmental changes. Dysregulation of this pathway, whether through aberrant ubiquitination, overactive E3 ligases, or impaired proteasomal degradation, disrupts these tightly regulated processes, enabling uncontrolled proliferation, evasion of apoptosis, sustained angiogenesis, and malignant transformation. Consequently, the UPP emerges not only as a pivotal driver of tumorigenesis but also as a promising therapeutic target, with proteasome inhibitors and E3 ligase modulators offering avenues for precise, mechanism-based cancer intervention. Understanding the nuanced interplay of ubiquitination and proteasomal degradation in normal and cancerous cells is therefore essential for developing effective strategies to prevent and treat malignancies.

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