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Molecular Mechanism of Cross-regulation between DNA Repair and Apoptosis

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Abstract:

DNA damage is an inevitable event in cell life activities, and its repair and coping mechanism is the core of maintaining genome stability. When DNA damage occurs, cells activate a complex network of signals that restore genomic integrity through repair mechanisms or initiate programmed apoptosis when the damage is irreversible in order to eliminate potentially harmful cells. The regulation of homeostasis involves several key molecules. As the core molecules regulating these two major processes, tumor suppressor P53 and polyadenosine diphosphoribose polymerase (PARP) play an important role in maintaining genome homeostasis and cell fate determination. In recent years, studies have shown that P53 and PARP form a dynamic balance in DNA damage response through a complex interaction network, and the dysregulation of its regulatory mechanism is closely related to disease progression, especially cancer. This paper aims to systematically elucidate the molecular interaction mechanism between P53 and PARP, explore how they balance cell fate through dynamic synergism or antagonism, and analyze their potential targeting value in tumor therapy. Future studies are needed to further reveal tissue-specific regulatory differences, the effects of epigenetic modifications, and the effects of microenvironmental signals on cross-regulation in order to promote the optimization of individualized treatment programs.

Keywords: DNA repair, apoptosis, cross-regulation.

1. Introduction

DNA is always at risk of damage from DNA replication, telomere shortening, UV exposure, chemical toxins, and reactive oxygen species produced during metabolism and inflammation. Mammalian cells have evolved complex networks of signaling pathways and repair mechanisms to respond to various forms of DNA damage, protecting genomic stability. There are many ways to repair DNA damage, including base excision repair (BER), nucleotide excision repair(NER), mismatch repair(MMR), non-homolo-

gous end joining (NHEJ) and homologous recombination (HR) [1,2].

Apoptosis is a universal phenomenon in the biological world. It is an important factor for the body to eliminate senescent and damaged cells, resist the interference of external factors and maintain the stability of the organism environment. Apoptosis is an induced behavior with many causes, such as DNA damage, growth factor withdrawal, FasL and TNF action, glucocorticoid action, and intercellular contact. When DNA damage exceeds the ability to repair, cells eliminate potentially harmful cells through the apoptotic pathway, a process that involves a complex network of molecular signals [3]. The cross-regulation of DNA repair and apoptosis is a core biological process for maintaining genome stability and cell fate decision-making. However, at present, there are few systematic studies and analyses on this aspect. Therefore, this paper discusses the cross-mechanism of DNA damage repair and apoptosis signaling pathways. It includes the dynamic regulatory network of key molecules (e.g. p53, PARP family, etc.), the synergistic effect of epigenetic and metabolic reprogramming, and the pathological significance of these mechanisms in disease

2. Basic Features of DNA Repair and Apoptosis

2.1 DNA Repair

DNA repair system, as the „molecular restorer“ of life, plays a decisive role in maintaining genome stability and species continuity.

Direct repair is the simplest way, without cutting out the base or nucleotide chain, leaving the DNA structure intact. ER mainly responds to a small range of base damage (such as oxidation, alkylation, or deamination damage), NA glycosylase specifically recognizes abnormal bases, hydrolyzes n-glycoside bonds to form a purine-free/pyrimidine-free site (AP site), and then the AP endonucliase cuts the phosphodiester bond, and DNA polymerase β fills the gap and is blocked by the ligase. NER involves a variety of DNA substrate species, including UV damage, protein-DNA cross-linking, and mismatched DNA. In NER repair, identification of DNA damage sites is performed by repair proteins UvrA and UvrB [4]. Double-strand break repair (DSB) is the deadliest type of DNA damage and is mainly done by HR and NHEJ. HR relies on sister chromatid homologous sequences, and RAD51 mediates single-strand intrusion into homologous templates to form Hawley ligands for high-fidelity repair. This pathway mainly occurs in S and G2 phases. NHEJ is a type of repair that can occur at any cell stage and is designed to directly connect the broken ends to avoid the more serious consequences of prolonged exposure to DSB, which is prone to introducing fragment insertion/deletion at the DNA break. After exposure, DSB ends are quickly recognized by KU70-KU80 heterodimers and bind DNA-PKCs to form DNA-PK complexes, which narrow the two broken DNA ends [5]. MMR corrects DNA replication errors such as A-C mismatches or insertion/deletion loops. MutS protein recognizes the mismatch site, MutL recruits the endonuclide MutH to cut near the methylation marker of the neonatal GATC, and the exonuclide degrades the unmethylated chain, followed by polymerase III to fill the gap [6].

2.2 Apoptosis

In 1972, Kerr et al. first used the term apoptosis to describe the physiological death process of cells [7]. Currently, apoptosis is considered as a complex biochemical process involving multiple signaling pathways, such as the mitochondrial apoptosis pathway, the endoplasmic reticulum apoptosis pathway and the death receptor-mediated apoptosis pathway [8].

Under the induction of the apoptosis signal, the permeability transfer pore between the inner and outer membranes of mitochondria opens, the membrane permeability increases, the mitochondrial transmembrane potential decreases, the apoptosis initiation factor cytochrome C (Cyt-C) and the apoptosis inducing factor are released, and the cascade reaction of caspases is activated to break DNA and produce apoptotic bodies. Causing cell apoptosis. Bcl-2 family proteins are widely distributed in the outer membrane of mitochondria and regulate the release of Cyt-C [9]. Endoplasmic reticulum (ER) stress is caused by dysregulation of the unfolded protein response (UPR). When misfolded proteins accumulate in ER, transmembrane sensors IRE1 and PERK are activated. IRE1 activates JNK pathway, phosphorylates Bcl-2 to inactivate it, and releases Bax/Bak. PERK phosphorylates eIF2α, inhibits translation, induces ATF4, up-regulates CHOP protein, and promotes Bim expression. If stress persists, pro-apoptotic signals dominate [10]. The death receptor pathway is triggered by extracellular death ligands (such as FasL, TNF-α, TRAIL) and transmits signals through transmembrane receptors (Fas, TNFR1, DR4/5) [11].

3. Cross-regulation Mechanism of P53 in DNA Repair and Apoptosis

P53 is the most commonly mutated tumor suppressor in human cancer, which establishes a dynamic balance be-

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tween DNA repair and apoptosis by activating or inhibiting the expression of specific target genes.

When DNA damage occurs, ATM completes the phosphorylation of p53 Ser15 within 15 minutes after DNA damage, and deactivates MDM2-mediated ubiquitination degradation. Subsequent acetylation modifications (Lys373/382) enhance its affinity to DNA, form a positive feedback regulatory loop, stabilize and activate p53, and initiate non-homologous end junction (NHEJ) or homologous recombination repair (HR). In addition, p53 activates the expression of p21 (CDKN1A), blocks the cell cycle process (G1/S phase arrest), and buys a time window for repair mechanisms such as HR. p53 can also directly bind to gene promoters such as GADD45A and XPC to enhance the efficiency of nucleotide excise repair (NER) and mismatch repair (MMR). For example, the binding of p53 to the MSH2 promoter enhances the recruitment efficiency of repair proteins through chromatin remodeling complexes such as SWI/SNF [12]. P53 can also activate OGG1, APE1 and other base excise repair (BER) genes, bind the OGG1 promoter region, promote the recognition and excisions of oxidation-damaged bases, or enhance the activity of APE1 promoter to ensure the efficient cleavage of phosphodiester bonds at the damage site. At the same time, ROS generating genes (such as SCO2) are inhibited, resulting in negative feedback regulation [13,14].

When the damage exceeds the repair ability, p53 up-regulates the expression of FAS, BAX, PUMA and other genes, promotes the change of mitochondrial membrane permeability, releases cytochrome C, and promotes cell apoptosis. p53 induces the Fas mRNA expression by binding to elements in Fas gene promoter and the first intron. This induction occurs after γ irradiation and appears to be strictly tissue-specific. In addition to stimulating Fas transcription, overexpressed p53 may also increase the level of Fas on the cell surface by facilitating the transport of Fas receptors from the Golgi apparatus to the cell surface. This may allow p53 to rapidly increase cell sensitivity to Fas induced apoptosis before transcription-dependent effects can take effect [15,16]. Upon activation by stress, BAX forms homeodimers and releases CytoC in mitochondria. CytoC, apoptotic protease activator-1 (APAF1), and caspase 9 then form a complex called an apoptotic body, in which caspase 9 is activated and promotes the activation of caspase 3, 6, and 7, inducing cell death after cutting the critical death substrate. The expression of PUMA promotes the mitochondrial translocation and polymerization of BAX and finally induces apoptosis. Single-cell RNA sequencing showed that the expression level of PUMA was positively correlated with the activation time of p53, and its threshold effect determined cell fate [17,18]. It is worth noting that p53 achieves a fine balance between pro-apoptotic protein (Bax) and anti-apoptotic protein (BCL-2) expression by binding the inhibitory elements of Bcl-2 and BCL-XL promotors, reducing their transcription levels. Cell type specificity determines the apoptotic tendency of p53. p53 mainly activated PUMA in neural progenitor cells, while intestinal epithelial cells preferentially induced NOXA expression. This difference is due to the regulation of the p53 transcriptional complex by tissue-specific cofactors such as ASPPL. In highly differentiated cells, p53 is more likely to initiate apoptosis procedures, while stem cells show a stronger propensity to repair.

In the field of tumor therapy, the regulation of p53 decision balance has become a new direction. Novel MDM2 inhibitors selectively enhanced p53 repair function, while PRIMA-1 analogues restored the pro-apoptotic activity of mutant p53. It is noteworthy that the nano drug delivery system can realize the spatio-temporal specific delivery of p53 regulated drugs, which may increase the apoptosis rate of tumor cells by 40% in animal models while protecting normal tissues. Future research will focus on the development of microenvironment-responsive regulation strategies, which can sense the degree of local damage in real time through smart materials and dynamically adjust the functional state of p53 and ultimately achieve accurate tumor therapy.

4. Cross-regulation Mechanism of PARP in DNA Repair and Apoptosis

Poly(ADP-ribose) (PAR) polymerase (PARP) is a family of NAD+ dependent enzymes that are involved in key biological processes such as DNA damage repair, chromatin remodeling, transcriptional regulation and cell death by catalyzing ADP-ribosylation to modify target proteins or themselves. There are 17 known members of the PARP family, among which PARP1, PARP2 and PARP3 play the most significant roles in DNA damage response, while PARP1 plays 90% to 95% of the functions in DNA damage repair, and its function is the most in-depth research. The PARP family plays a pivotal role in maintaining genomic stability and determining cell fate by dynamically regulating the balance between DNA repair and apoptosis [19].

When cells encounter DNA SSB induced by ionizing radiation, alkylating agents, or oxidative stress, PARP1 rapidly recognizes and binds to the damage site through its N-terminal zinc finger domain, and the C-terminal catalytic domain is activated to synthesize polyADP ribose chains (PAR chains) using NAD+ as substrates. This dynamic modification process activates the BER pathway

through two mechanisms: On the one hand, the PAR chain acts as a molecular scaffold to recruit repair proteins such as XRCC1 and DNA ligase III; On the other hand, the chromatin structure is changed by charge repulsion effect and the damaged area is exposed. It is worth noting that the self-modification of PARP1 can trigger its dissociation from DNA, which not only ensures the effective recruitment of repair proteins, but also avoids the depletion of NAD+ caused by over-PARization. If the damage is not repaired in time, a replication fork collision may result in a single strand break transformed into a DSB, where PARP1 and PARP2 work together to promote HR or NHEJ. Notably, the functions of PARP family members are spatio-temporal specific and substrate selective, for example, PARP2 compensates for PARP1 function in BER, while PARP3 is specifically involved in NHEJ repair of DNA double-strand breaks [20,21].

However, when DNA damage exceeds its ability to repair, the PARP family‘s function shifts from pro-repair to pro-apoptotic, with its core mechanism involved in the energy crisis triggered by NAD+/ATP depletion. PARP1 consumes one NAD+ molecule for each PAR unit synthesis, and its overactivation under severe injury can lead to a decrease of NAD+ levels by more than 70%, thereby inhibiting key enzymes of glycolysis and oxidative phosphorylation (OXPHOS) (such as GAPDH, complex I), and eventually leading to ATP depletion and mitochondrial membrane potential collapse. This process forms a positive- feedback with mitochondrial release of apoptosis-inducing factor (AIF): activation of PARP1 leads to translocation of AIF from the mitochondrial membrane space to the nucleus, inducing chromatin agglutination independent of caspase. At the same time, NAD+ deficiency up-regulated the acetylation level of p53 by inhibiting the deacetylation activity of SIRT1 and enhanced the expression of its pro-apoptotic target genes (such as BAX and PUMA). In addition, PARP1-mediated PAR chain can serve as a signaling platform to recruit apoptosis-related proteins (such as Apaf-1) and co-activate caspase-9 with cytochrome c to form a Caspase-dependent apoptotic pathway [22,23].

In cancer therapy, PARP inhibitors (PARPi) have revolutionized treatment strategies for BRCA-mutated cancers through a „synthetic lethal“ effect. However, with the deepening of research, the complexity of PARP-mediated cross-regulatory network has gradually emerged, and drug resistance, off-target effect and tissue-specific regulation have become key challenges restricting its clinical application. Studies have found that about 40-70% of ovarian cancer patients develop resistance after initial treatment. Future breakthroughs need to rely on the integration of interdisciplinary technologies, and advance in three levels of molecular mechanism analysis, dynamic monitoring technology and innovative therapy development. By accurately analyzing the cross-dialogue between DNA repair and apoptosis, PARP targeted therapy is expected to break through the existing bottleneck, not only providing a better solution for tumor treatment, but also opening up a new paradigm for intervention in other diseases [24].

5. Conclusion

The cross-regulation of DNA repair and apoptosis is the core biological process to maintain genome stability and cell fate determination, the analysis of its molecular mechanism is of key significance for understanding important biomedical problems such as tumorigenesis. Existing studies have shown that these two systems achieve dynamic dialogue through a multi-level molecular network: When DNA damage occurs, cells first activate the DNA damage response (DDR) signaling pathway with ATM/ATR kinase as the core and regulate cell cycle arrest through a phosphorylation cascade to gain a time window for DNA repair. At this time, repair mechanisms such as NHEJ, HR, BER, MMR and other pathways are specifically activated, and their repair efficiency directly determines the direction of cell fate. When the damage exceeds the threshold of repair ability, the apoptotic pathway is activated via p53-dependent/independent pathway, in which p53 regulates the expression of pro-apoptotic genes (e.g. BAX, PUMA) by transcription, while inhibiting the function of anti-apoptotic proteins (e.g. Bcl-2), forming precise molecular switches. PARP family also proteins play a dual role in this process: not only does PARP1 participate in EBR, but the accumulation of PAR polymers resulting from its overactivation induces mitochondrial release of apoptosis-inducing factor (AIF), initiating Caspase-independent apoptosis. At the same time, the caspase family proteases achieve programmed shutdown of the repair mechanism by cutting DNA repair related proteins (such as XRCC1 and DNA ligase III), and this bidirectional regulation constitutes a molecular timer for cell fate determination.

The breakthrough direction of future research will focus on the spatio-temporal analysis of multi-level regulatory networks. In spatial dimension, cryo-electron microscopy was used to analyze the instantaneous interaction interface between restorations and apoptotic bodies, and super-resolution imaging was used to trace the dynamic correlation between γH2AX lesions and mitochondrial membrane potential changes. In the temporal dimension, a gene editing tracer system was developed to monitor the temporal relationship between homologous recombination repair and caspase activation in real time. Ai-driven multi-omics integration analysis will establish predictive models

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of DDR-apoptosis regulation, in particular by decoding quantitative relationships between DNA damage patterns (such as double-strand breaks versus oxidative damage) and apoptosis pathway selection (endogenous/exogenous) through deep learning. The construction of artificial regulatory loops based on synthetic biology and the design of programmable „molecular switches“ (such as the photocontrolled PARP1 allosteric body) to achieve precise spatio-temporal manipulation of repair and apoptosis may break through the accuracy bottleneck of existing gene therapy technologies. Targeting drug resistance in tumor therapy, the development of dual-targeted inhibitors can synergistically block repair escape and apoptosis resistance pathways. In addition, the establishment of organoid models to simulate the effects of mechanical signals (such as matrix stiffness) in the tissue microenvironment on the DDR-apoptotic balance will advance the development of individualized treatment strategies. With the fusion application of spatial metabolomics and epigenome editing technology, it is expected to achieve precision medicine solutions that selectively activate specific repair pathways and regulate apoptosis sensitivity through metabolic microenvironment remodeling (such as regulating alpha-ketoglutaric acid levels) in the future. These breakthroughs will improve the basic theoretical system and also give rise to a new generation of treatment paradigms, such as the development of apoptosis-inducing nanomedical drugs based on dynamic assessment of DNA repair ability, or the creation of individualized damage response prediction systems based on artificial intelligence and ultimately achieve the whole chain of innovation from molecular mechanism to clinical transformation.

References

[1] Chatterjee N, Walker GC. Mechanisms of DNA damage, repair, and mutagenesis. Environ Mol Mutagen. 2017 Jun;58(5):235-263.

[2] Kang TH. DNA Damage, Repair, and Cancer Metabolism. Int J Mol Sci. 2023 Nov 17;24(22):16430.

[3] Kaina B. DNA damage-triggered apoptosis: critical role of DNA repair, double-strand breaks, cell proliferation and signaling. Biochem Pharmacol. 2003 Oct 15;66(8):1547-54.

[4] Jiang Ting, Zhai Fanfan, Zhong Shanshan, et al. Research progress of DNA damage repair at single molecule level [J]. Nature Journal,2023,45(01):22-32.

[5] Wu Shuheng, Liang Yu, Wu Wei. Advances in high-throughput sequencing of DNA double-strand breaks [J]. Chinese Journal of Cell Biology, 2019,46(08):1488-1496.

[6] Ortega J, Lee GS, Gu L, Yang W, Li GM. Mispair-bound human MutS-MutL complex triggers DNA incisions and activates mismatch repair. Cell Res. 2021 May;31(5):542-553.

[7] Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer. 1972 Aug;26(4):239-57. doi: 10.1038/bjc.1972.33. PMID: 4561027; PMCID: PMC2008650.

[8] Mosadegh M, Noori Goodarzi N, Erfani Y. A Comprehensive Insight into Apoptosis: Molecular Mechanisms, Signaling Pathways, and Modulating Therapeutics. Cancer Invest. 2025 Jan;43(1):33-58.

[9] Sun Yan-Ting, Xin Guo-Song, Zhang Lu, et al. Research progress on mitochondrial pathways of apoptosis [J]. Heilongjiang Science and Technology Information,2015(8):30-30.

[10] Hetz C, Zhang K, Kaufman RJ. Mechanisms, regulation and functions of the unfolded protein response. Nat Rev Mol Cell Biol. 2020 Aug;21(8):421-438.

[11] Green DR. The Death Receptor Pathway of Apoptosis. Cold Spring Harb Perspect Biol. 2022 Feb 1;14(2):a041053.

[12] Menon V, Povirk L. Involvement of p53 in the repair of DNA double strand breaks: multifaceted Roles of p53 in homologous recombination repair (HRR) and non-homologous end joining (NHEJ). Subcell Biochem. 2014;85:321-36.

[13] Song J, Park C, Cabanting FEB, Jun YW. Therapeutic upregulation of DNA repair pathways: strategies and small molecule activators. RSC Med Chem. 2024 Oct 11;15(12):3970–7.

[14] Offer H, Milyavsky M, Erez N, Matas D, Zurer I, Harris CC, Rotter V. Structural and functional involvement of p53 in BER in vitro and in vivo. Oncogene. 2001 Feb 1;20(5):581-9.

[15] Cappello F, Bellafiore M, Palma A, Bucchieri F. Defective apoptosis and tumorigenesis: role of p53 mutation and Fas/FasL system dysregulation. Eur J Histochem. 2002;46(3):199-208.

[16] Follis AV, Llambi F, Merritt P, Chipuk JE, Green DR, Kriwacki RW. Pin1-Induced Proline Isomerization in Cytosolic p53 Mediates BAX Activation and Apoptosis. Mol Cell. 2015 Aug 20;59(4):677-84.

[17] Hikisz P, Kiliańska ZM. PUMA, a critical mediator of cell death--one decade on from its discovery. Cell Mol Biol Lett. 2012 Dec;17(4):646-69.

[18] Dirisina R, Katzman RB, Goretsky T, Managlia E, Mittal N, Williams DB, Qiu W, Yu J, Chandel NS, Zhang L, Barrett TA. p53 and PUMA independently regulate apoptosis of intestinal epithelial cells in patients and mice with colitis. Gastroenterology. 2011 Sep;141(3):1036-45.

[19] Zhou Qiyu, Zhang Nana, Lu Jiangyong, et al. Study on virtual screening, enzyme inhibition activity and metabolic stability of PARP inhibitors [J]. Chinese Chemical Bulletin, 2018,87(07):864-871+844.

[20] Shao Z, Lee BJ, Zhang H, Lin X, Li C, Jiang W, Chirathivat N, Gershik S, Shen MM, Baer R, Zha S. Inactive PARP1 causes embryonic lethality and genome instability in a dominant-negative manner. Proc Natl Acad Sci U S A. 2023 Aug;120(31):e2301972120.

[21] Kutuzov MM, Belousova EA, Kurgina TA, Ukraintsev AA, Vasil'eva IA, Khodyreva SN, Lavrik OI. The contribution of PARP1, PARP2 and poly(ADP-ribosyl)ation to base excision repair in the nucleosomal context. Sci Rep. 2021 Mar 1;11(1):4849.
[22] Jang KH, Jang T, Son E, Choi S, Kim E. Kinase-independent role of nuclear RIPK1 in regulating parthanatos through physical interaction with PARP1 upon oxidative stress. Biochim Biophys Acta Mol Cell Res. 2018 Jan;1865(1):132-141.
[23] Delavallée L, Cabon L, Galán-Malo P, Lorenzo HK, Susin SA. AIF-mediated caspase-independent necroptosis: a new chance for targeted therapeutics. IUBMB Life. 2011 Apr;63(4):221-32.
[24] Dibitetto D, Widmer CA, Rottenberg S. PARPi, BRCA, and gaps: controversies and future research. Trends Cancer. 2024 Sep;10(9):857-869.