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- Review -

The Structural and Functional Role of p53 as a Cancer Therapeutic Target

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The p53 gene plays a critical role in the transcriptional regulation of cellular response to stress, DNA damage, hypoxia, and tumor development. Keeping in mind the recently discovered manifold physiological functions of p53, its involvement in the regulation of cancer is not surprising. In about 50% of all human cancers, inactivation of p53's protein function occurs either through mutations in the gene itself or defects in the mechanisms that activate it. This disorder plays a crucial role in tumor evolution by allowing the evasion of a p53-dependent response. Many recent studies have focused on directly targeting p53 mutants by identifying selective, small molecular compounds to deplete them or to restore their tumor-suppressive function. These small molecules should effectively regulate various interactions while maintaining good drug-like properties. Among them, the discovery of the key p53-negative regulator, MDM2, has led to the design of new small molecule inhibitors that block the interaction between p53 and MDM2. Some of these small molecule compounds have now moved from proof-of-concept studies into clinical trials, with prospects for further, more personalized anti-carcinogenic medicines. Here, we review the structural and functional consequences of wild type and mutant p53 as well as the development of therapeutic agents that directly target this gene, and compounds that inhibit the interaction between it and MDM2.

Key words : MDM2 interaction, p53, p53 mutant, tumor suppression

Introduction

Numerous biological functions of the p53 gene, which has been identified as a tumor suppressor gene [51], have been discovered, including modulation of apoptosis, cell cycle arrest, senescence, metabolism, and DNA repair in response to various cellular stresses such as DNA damage, oncogene expression, hypoxia, and viral infection (Fig. 1) [30, 41]. Furthermore, p53 plays an essential role in preventing the development of cancer [18, 49]. Early on, p53 was classified as an oncogene and identified as the most frequently mutated gene in a variety of human cancers [21]. Indeed, p53 is inactivated by mutations or loss in about 50% of all human cancers. Unlike other tumor suppressor genes, p53 is primarily altered in human cancers by missense mutations, which are located in the DNA-binding domain (DBD) and caused by a single amino acid change at various sites. Missense mutations in hot-spot sites (Arg^{175}, Tyr^{220}, Gly^{245}, Arg^{248}, Arg^{249}, Arg^{273}, and Arg^{282}) are most frequently observed in human cancers. Additionally, nonsense or frame-shift mutations, which are less frequent than missense mutations, constitute 10% of the p53 gene mutations in cancers [36, 40]. Mutations can result in loss of DNA binding, disruption of structural stability, and loss of transcriptional transactivation function [4, 20, 52, 56]. Several recent studies have shown that p53 reactivation leads to tumor regression, which is considered a very promising anticancer strategy. Therefore, many research groups are searching for small molecules or peptides that can reactivate mutant p53 [9].

Under normal conditions, the expression of p53 protein is tightly controlled to remain low by the E3 ubiquitin protein ligase MDM2 (Mouse double minute 2), which binds to the N-terminus transactivation domain of p53 [34]. Mouse double minute 2 regulates p53 stability through mono- or poly-ubiquitination and proteasome-mediated degradation [6]. Mouse double minute X (also known as MDM4) is a structural homologue of MDM2 that plays a critical role in down-regulation of p53 activity [22]. The presence of cellular stress inhibits the interaction between p53 and MDM2 by the post-translational modification of both proteins, leading to p53 accumulation and activation [45]. Thus, the interaction of p53 with MDM2 is an attractive target for develop-

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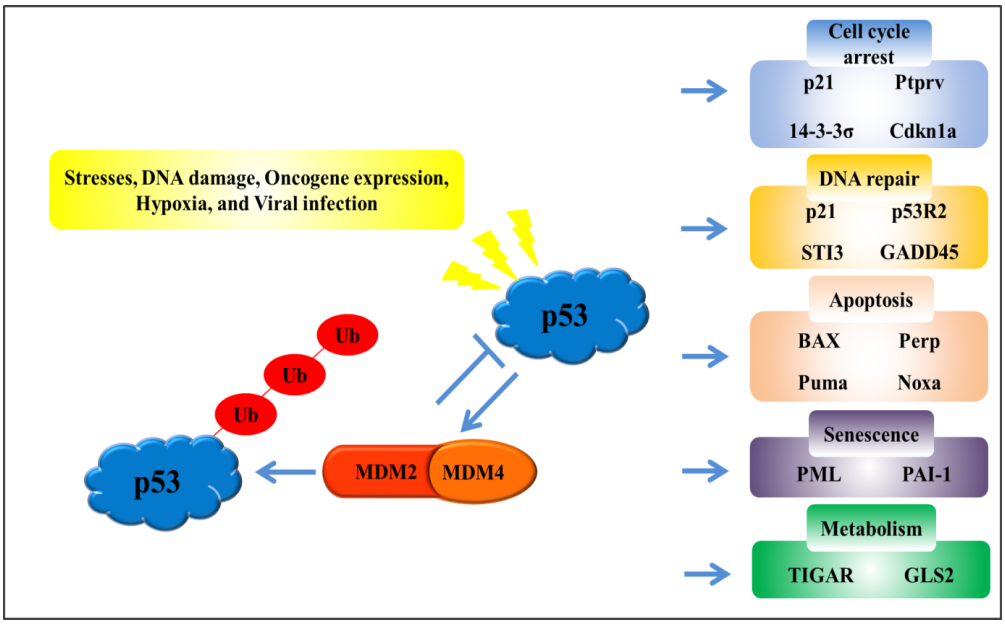

Fig. 1. The p53 signaling pathway. The p53 tumor suppressor gene acts as the center of a complex network of numerous biological functions that translates in response to various cellular stresses, DNA damage, oncogene expression, hypoxia, and viral infection. Activation of p53 acts as a transcription factor that induces the transcription of target genes involved in regulating cell cycle arrest, DNA repair, apoptosis, senescence, and metabolism.

ment of anticancer therapy, and various small molecules have been developed as protein-protein interaction inhibitors based the structure of the p53-MDM2 complex. Several compounds, including nutlins, are known to prevent MDM2/MDM4 from binding to wild-type p53 and thereby block its degradation. Nutlins derived from imidazole scaffold were first reported, and Nutlin3a, the most potent nutlin inhibitor, selectively activates p53 and induces apoptosis in selected cancer cell lines. However, most drug discovery efforts have focused on small molecule inhibitors that interfere with the p53-MDM2/MDM4 interaction [55].

In this review, we summarize the structures of wild-type and mutant p53 and their diverse structural and functional consequences. In addition, we review therapeutic agents that reactivate mutant p53 and the compounds that directly inhibit the interaction between p53 and MDM2, and we look into the prospect of future development of therapeutic agents targeting p53.

Structure and function of p53

Human p53, a nuclear phosphoprotein of MW of 53 kDa, contains 393 amino acids and is composed of structural and functional main domains: an amino-terminal transactivation domain (TAD1; amino acids 1-40, TAD2; aa 41-61), a proline-rich domain with multiple copies of the PXXP sequence (PRD; aa 61-94, where X is any amino acid), the central DNA-binding domain (DBD; aa 94-292), a tetramerization domain (TD; aa 318-355) and a carboxyl terminal regulatory domain (CTD; aa 363-393) (Fig. 2). N-terminal TAD, which is required for p53 transcriptional activity, interacts with many proteins including CREB-binding protein (CBP)/p300, p300/CBP-associated factor (PCAF), MDM2, transcription factor II D (TFIID), transcription factor IIH (TFIIH), and TATA-binding protein associated factors (TAFs) [27, 48]. These partner proteins overlap with the binding site of TAD. Few studies of the structure and function of the N-terminal domain of p53 have been conducted, and NMR studies have shown this region was natively unstructured. In 1996, the structure of the transactivation domain peptide of p53 bound to the N-terminal domain of MDM2 was first solved by X-ray crystallography. Amino acids 17-28 of TAD form a random coil to helix that interacts with a hydrophobic cleft in the N-terminal domain of MDM2 [32]. In NMR structural studies of the Taz2/$p53_{2-39}$ complex, the region of p53 has been shown to be highly flexible, forming a short α-helical conformation within amino acids 15-27 of TAD in complex with CBP Taz2 [14]. The TAD1 region corresponds to conserved hydrophobic residues essential for the p53 transactivation function, while residues 18-25 form an amphi-

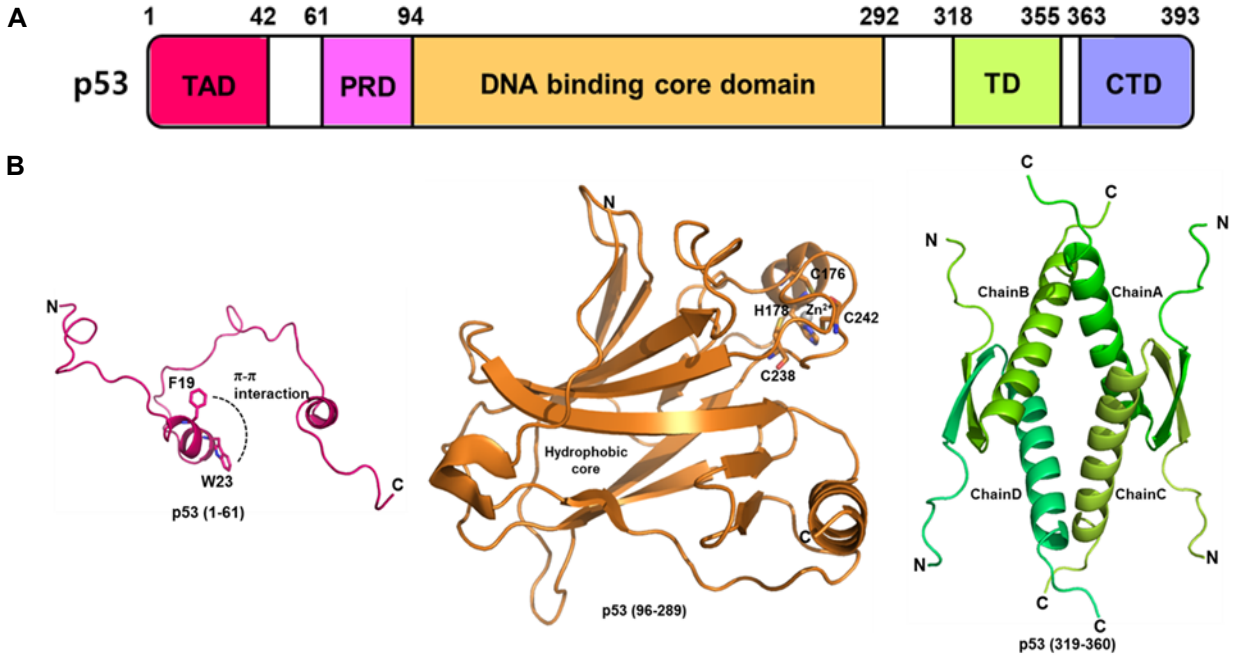

Fig. 2. Overall structure of the p53 gene. (A) Structure of the p53 domain (TAD: Transactivation Domain, PRD: Proline-Rich Domain, TD: Tetramerization Domain, CTD: C-Terminal Domain). (B) Schematic ribbon diagrams of the human p53 TAD domain (PDB ID, 5HOU), p53 DBD domain in the absence of DNA (PDB ID, 2OCJ), and p53 TD domain (PDB ID, 1SAL).

pathic helix when coupled with the partner proteins [26]. Similar to TAD1, the TAD2 region folds into an amphipathic alpha-helix when it binds to replication protein A and TFIIH [1, 10]. Investigations of the helical characteristics of the N-terminal region using molecular dynamics (MD) simulations have shown that the region of p53 (aa 17-29) contains a stable helical form and depicts aromatic stacking (π-π interaction) between Phe^{19} and Trp^{23} [13]. This region is considered to have an important influence on disturbing the secondary structure of the TAD, and these two amino acids also play important roles in maintenance of the functional and structural stabilities of p53 [35]. The PRD contributes to regulation of the p53 stability, transcription activity and induction of transcription independent apoptosis. This domain is required for interactions with the co-repressor mSin3A [61], prolyl isomerase Pin1 [59] and p300/CBP [11].

The central DBD, which is known to be characterized by high evolutionary conservation, binds directly to sequence-specific DNA at promoter regions and initiates the transcription of target genes. The structure of DBD contains an immunoglobulin-like β-sandwich of two antiparallel β-sheets, which serves as a scaffold for two large loops (L2 and L3) and a loop-sheet-helix motif, forming the DNA binding surface. The loop-sheet-helix motif contains loop L1, a short β-sheet (S2-S2′ hairpin), the C-terminal residues of β-strand S10, and helix H2 [56]. The two loops (L2 and L3) are structurally stabilized by a Zn^{2+} ion, which is tetrahedrally coordinated by Cys^{176}, His^{179}, Cys^{238}, and Cys^{242}. Loss of the Zn^{2+} ion results in loss of DNA binding specificity because it leads to high structural fluctuations of adjacent loops and increases aggregation tendency [12]. Most cancer mutations are found in this area, and several frequent mutations are known as hot-spot mutations. A highly flexible L1 loop with secondary structural disorder contains a few amino acids with a low mutation rate and is known as an area of cold-spot mutations [42]. The DBD is intrinsically unstable and kinetically stable, rendering it susceptible to oncogenic mutations [5, 29]. The majority of oncogenic P53 mutations occur in the DBD and result in a loss of DNA binding, thereby affecting p53 function in cell cycle control. Thus, one of the goals of cancer therapies is to stabilize the DBD and reverse the effects of mutations. Many proteins, including simian virus 40 large T antigen (SV40Tag), ASPP1 (P53BP1), ASPP2 (P53BP2), HIF-1α, BCL-XL, BCL2, BAK and MDM2, are known as P53 DBD binding partners [8]. The complex structures of p53 DBD and P53BP1 (PDB ID, 1GZH), p53 DBD and P53BP2 (PDB ID, 1YCS), p53 core dimer bound to DNA (PDB ID, 2GEQ), p53 DBD and sv40 (PDB ID, 2H1L) have been revealed by X-ray crystallography [47].

The TD regulates the oligomer status of p53 and the tet-

ramer formation of p53 is essential to DNA binding, post-translational modification, and protein-protein interactions. The structure of TD has been solved by NMR and X-ray crystallography. Each monomer comprises a short β-strand, a sharp turn (Gly^{334}), and an α-helix. Two monomers form a dimer via an anti-parallel interaction of the β–strands, and two dimers interact as a four-helix bundle to form a tetramer [25]. The CTD is subject to alternative splicing and post-translational modifications. It is known that the CTD affects non-specific DNA binding and transcriptional activity. Additionally, the CTD is involved in binding to 14-3-3, GSK3 β, hGcn5, PARP-1, S100B (ββ), TAF, TAF1, TRRAP and many other proteins. These p53 domains interact with a variety of partner proteins that create highly complex signaling pathways involving post-translational modifications and these pathways can be used therapeutically.

Therapeutic targets

Many cancer-associated p53 mutants result in the expression of point-mutated p53 proteins that can exert negative effects and acquire novel oncogenic functions [16]. Mutations are clustered within seven hot-spot amino acids (Arg^{175}, Tyr^{220}, Gly^{245}, Arg^{248}, Arg^{249}, Arg^{273}, and Arg^{282}) in the DNA-binding domain of p53. Interestingly, these p53 mutations were found to be associated with new oncogenic functions (mutant p53 gain-of-function, GOF) that include cancer cell survival, invasion, and metastasis [39]. Recent studies have focused on the degradation of mutant p53 or the restoration of wild-type p53 function (Table 1) [60]. One of the mutant specific inhibitors reported, CP-31398, was targeted by either formation of wild-type or mutant p53. In the wild-type human rhabdomyosarcoma (RMS) cell line, CP-31398 increased the expression of p53, downstream target p21, and MDM2. In addition, CP-31398 induced mitochondrial translocation of mutant p53, resulting in cytochrome c release and ROS-dependent apoptosis [58]. In another study, interference with the interaction between mutant p53 and other proteins was targeted. The growth suppressive and pro-apoptotic activities of p53 require that its function be tightly regulated by the E3 ubiquitin protein ligase MDM2, which has been shown to be involved in that regulation [15]. Therefore, drug-discovery groups have targeted the p53 pathway, with the interaction between p53 and MDM2 being the most attractive target (Table 2). Attempts to regulate the p53-MDM2 interaction have included the generation of anti-MDM2 antisense oligonucleotides and scaffold-attached peptides [28, 54]. Additionally, the studies have revealed potent and selective small molecule inhibitors that block the MDM2–p53 interaction. From a structural standpoint, the p53-MDM2 interaction has been mapped to the N-terminal p53 binding domain (aa 18-101) of MDM2 and the N-terminal transactivation domain 1 (TAD1; aa 1-40) of p53 [7]. The first potent and selective small molecule p53-MDM2 binding inhibitors, the Nutlins (cis-imidazoline analogs), were reported to competitively bind to the p53-binding pockets of MDM2 [50]. Importantly, Nutlin-3α inhibits proliferation and promotes apoptosis in cancer cells *in vivo* and *in vitro* [53]. Therefore, these selective small-molecule inhibitors suggest that targeted inhibition of p53-MDM2 interaction in p53-mutant cancers inhibits oncogenic functions.

The p53 is reported to be involved in energy, inflammation, proliferation and oxidative stress mechanisms which

Table 1. Compounds to target mutant p53

Compound	Mechanism of action	Target p53 mutants	References
CP-31398	Induction of apoptosis and stabilization of wild-type p53 protein	V173A, S241F, R249S, R273	[44, 46, 58]
PRIMA-1	Restoration of mutant p53 by covalent binding to the core domain	R175H, R273H	[33]
Chetomin	Increase of Hsp40 (DNAJB1) levels and Hsp40-p53R175H binding capacity, restoration of wild-type p53 conformation, reactivity, and MDM2- dependent degradation	R175H	[19]
RITA	Reactivation of p53 and induction of tumor cell apoptosis	R175H, R248W, R273H, R280K	[3, 23]
PK7088	Binding of p53 Y220C-specific surface cavity, increase of wild-type p53 conformation, restoration of transcriptional functions, and induction of p53 Y220C-dependent growth inhibition and apoptosis	Y220C	[37]

Table 2. p53-MDM2 interaction inhibitors

Compounds	Mechanism of action	Targets	References
Nutlin 3α	Binding MDM2, suppression of proliferation and promotion of apoptosis	MDM2 N terminal p53-binding domain	[48, 53]
MI-219	Inhibition of cell proliferation, induction of apoptosis, and completion of tumor growth inhibition	MDM2 N terminal p53-binding domain	[43]
Benzodiazepinediones	Hdm2 antagonists, Hdm2 antagonists-p53 complex suppression of tumor cell proliferation	p53-binding pocket of Hdm2	[17, 31]
sMTide02 and sMTide02A	Stapled peptides, arrest of cells resistant to p53, induction of apoptosis	Both MDM2 and MDMX N terminal p53-binding domain	[2]
AM-8553	Potent and selective disruption of p53-MDM2 interaction	MDM2 N terminal p53-binding domain	[38]

are also linked to tumor progression as well. Many cellular stimuli such as physical, physiological and oxidative stress result in the induction of crosstalk between NF-κB (Nuclear factor kappa B) and the p53 tumor suppressor [57]. In addition, p53 and NF-κB crosstalk participates in regulation of tumor cell metabolism [24]. Therefore, the understanding of these processes could contribute towards the design of new therapy for cancer.

In summary, this review suggests the need for a better understanding of the structural function of p53 and the development of more effective anticancer therapy to facilitate treatment of various cancers.

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초록 : 암 치료 표적으로서 p53의 구조적 및 기능적 역할

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p53 유전자는 스트레스, DNA 손상, 저산소증 및 종양 발생에 대한 세포 반응의 전사 조절에서 중요한 역할을 담당한다. 최근에 발견된 다양한 종류의 p53의 생리 활성을 생각한다면 p53이 암 조절에 관여한다는 것은 놀랄 만한 일이 아니다. 인간 암의 약 50%에는 p53 유전자의 돌연변이 또는 p53을 활성화시키는 기전의 결함을 통해 p53 단백질 기능의 불활성화가 나타난다. p53 기능의 이러한 장애는 p53 의존 반응으로부터 회피를 허용함으로써 종양의 진화에 결정적인 역할을 하게 된다. 최근의 많은 연구들은 p53의 돌연변이를 대폭 감소시키거나 p53의 종양 억제 기능을 복원하기 위하여 선택적인 저분자 화합물을 동정함으로써 p53의 돌연변이를 직접 표적하는 것에 초점을 두고 있다. 이들 저분자는 좋은 약물과 유사한 특성을 유지하면서 다양한 상호작용을 효과적으로 조절해야 한다. 이 중, p53의 음성조절인자 핵심인 MDM2의 발견은 p53과 MDM2 간의 상호작용을 차단하는 새로운 저분자 억제제의 설계를 제공하였다. 저분자 화합물 중 일부는 개념 증명 연구에서 임상 시험으로 옮겨졌으며 향후 맞춤형 항암제가 추가될 전망이다. 본 리뷰에서는 야생형 p53과 돌연변이 p53의 구조적 및 기능적 중요성과 p53을 직접 표적하는 치료제 개발, p53과 MDM2 간의 상호작용을 억제하는 화합물에 대하여 검토하였다.