

Contributed Mini Review

Structural insights into the transcription-independent apoptotic pathway of p53

Seung-Wook Chi^{1,2,*}¹Medical Proteomics Research Center, KRIBB, Daejeon 305-806, ²Department of Bio-Analytical Science, University of Science and Technology, Daejeon 305-350, Korea

Reactivating the p53 pathway in tumors is an important strategy for anticancer therapy. In response to diverse cellular stresses, the tumor suppressor p53 mediates apoptosis in a transcription-independent and transcription-dependent manner. Although extensive studies have focused on the transcription-dependent apoptotic pathway of p53, the transcription-independent apoptotic pathway of p53 has only recently been discovered. Molecular interactions between p53 and Bcl-2 family proteins in the mitochondria play an essential role in the transcription-independent apoptosis of p53. This review describes the structural basis for the transcription-independent apoptotic pathway of p53 and discusses its potential application to anticancer therapy. [BMB Reports 2014; 47(3): 167-172]

INTRODUCTION

p53 is a tumor suppressor protein that triggers cell cycle arrest, apoptosis, and senescence in response to diverse stresses such as DNA damage, hypoxia, ultraviolet light, and oncogene activation (1-4). In many human cancers, p53 function is abrogated by p53 gene mutation (5). However, approximately 50% of human cancers retain wild type p53, but impairment in p53 signaling or regulation inactivates its function and thus serves a critical role in cancer progression by protecting cancer cells from p53-dependent responses. Thus, restoring the function of inactivated p53 in tumors retaining wild type p53 is an important strategy for cancer treatment. Previously, several mouse model studies supported this therapeutic concept by showing that the reactivation of wild type p53 function induces cancer regression in vivo through apoptosis and/or senescence (6-8). Using this therapeutic strategy, pharmaceutical

and academic research has identified many lead compounds for cancer drug discovery. Here, this review provides structural insights into targeting the transcription-independent p53 apoptotic pathway for anticancer therapy.

TRANSCRIPTION-DEPENDENT APOPTOTIC PATHWAY OF P53

Within the nucleus, p53 induces transcription-dependent apoptosis via the transactivation of target genes such as *Noxa*, *Bax*, and *Puma* (Fig. 1). Transcription-dependent apoptosis induced by p53 is mediated via interactions of the p53 transactivation domain (p53TAD) with the components of the basal transcriptional machinery. Under normal conditions, the p53 level is precisely controlled by mouse double minute 2 (MDM2). MDM2 is an E3 ubiquitin ligase that negatively regulates p53 stability through ubiquitination and inhibits the transactivation ability of p53 by interacting with the p53 transactivation domain (p53TAD). In many cancers, p53 function is impaired by overexpression of MDM2. Interaction of the p53TAD with transcriptional machinery components such as the transcriptional coactivator p300/CBP is neutralized by MDM2 in cancer cells. This neutralization by MDM2 could be inhibited by p53-mimetic compounds for cancer treatment. Thus, blocking the interaction of p53 with MDM2 is a promising anticancer strategy to reactivate the p53 pathway. The α -helical structure of the 15-residue p53TAD peptide fragment (residues 15-29) in complex with an N-terminal domain of MDM2 provides a useful template for the structure-based rational design of MDM2-inhibiting anticancer drugs (9) (Fig. 2A and B).

TRANSCRIPTION-INDEPENDENT APOPTOTIC PATHWAY OF P53

Recent studies have provided evidences supporting the pro-apoptotic effects of cytoplasmic p53 independent of its transcription ability (10-14). This non-transcriptional apoptosis of p53 is mainly achieved by the interaction between p53 and anti-apoptotic as well as pro-apoptotic B-cell lymphoma 2 (Bcl-2) family proteins. In an immediate response to apoptotic stresses, the cytoplasmic p53 moves rapidly to the mitochon-

*Corresponding author. Tel: +82-42-860-4277; Fax: +82-42-879-8596; E-mail: swchi@kribb.re.kr

<http://dx.doi.org/10.5483/BMBRep.2014.47.3.261>

Received 26 November 2013, Revised 16 December 2013,
Accepted 30 December 2013

Keywords: Anticancer therapy, Apoptosis, Bcl-2 family proteins, p53, Structure

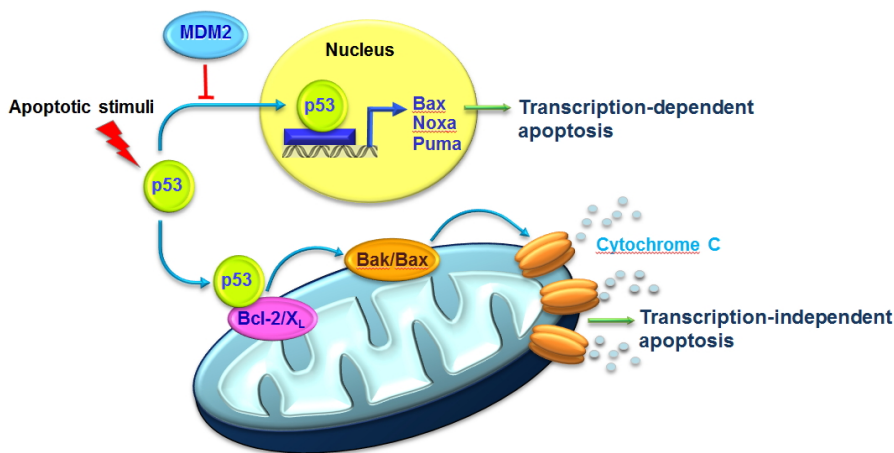


Fig. 1. Dual role of p53 in different cellular compartments to promote apoptosis. Interaction between p53 and the transcriptional coactivator CBP/p300 induces transcription-dependent apoptosis within the nucleus. This interaction can be neutralized by the interaction of p53 with MDM2. At the same time, binding of p53 to anti-apoptotic Bcl-2 and Bcl-X_L can occur in the mitochondria, releasing pro-apoptotic Bak/Bax from complexes and triggering transcription-independent apoptosis.

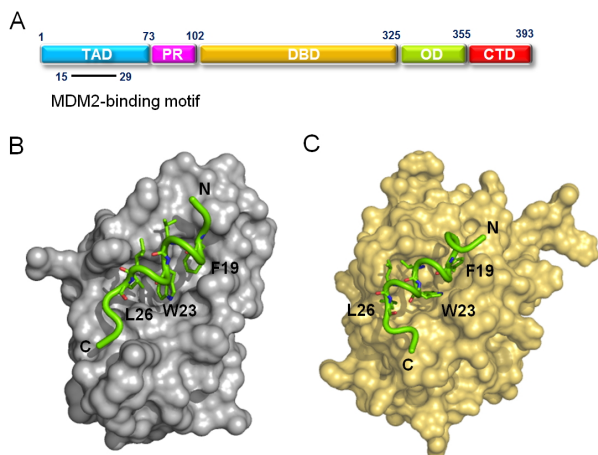


Fig. 2. Structural similarity between MDM2/p53TAD and Bcl-2/p53TAD complexes. (A) Domain organization of p53. p53 consists of a transactivation domain (TAD), proline-rich domain (PR), DNA-binding domain (DBD), oligomerization domain (OD), and C-terminal domain (CTD). The residues 15-29 of p53TAD are indicated as MDM2-binding motif. (B) Crystal structure of the MDM2/p53TAD peptide (residues 15-29) complex (PDB code: 1YCR) (9). (C) A refined structural model for the Bcl-2/p53TAD peptide (residues 15-29) complex generated from an NMR data-driven structure calculation (21). The p53TAD peptide is shown in green.

dria (14). In the mitochondria, p53 binds to anti-apoptotic Bcl-2 family proteins Bcl-X_L and Bcl-2 and, releasing the pro-apoptotic effectors Bak/Bax from the complex with the anti-apoptotic proteins. Subsequently, the released Bak and Bax induce the lipid pore formation in the outer mitochondrial membrane, which elicits cytochrome c release (15). In addition, p53 can directly activate Bak and/or Bax through a “hit and run” mechanism to trigger the permeabilization of outer mitochondrial membrane (11).

A DUAL ROLE OF P53 IN APOPTOSIS PATHWAY

Based on results from recent studies, it is proposed that, under pro-apoptotic conditions, p53 can play a dual role in apoptosis in different cellular compartments (Fig. 1). Within the nucleus, p53 acts as a transcriptional activator and induces target gene expression through its interaction with the basic transcriptional machinery components, such as transcriptional coactivator CBP/p300, eventually leading to transcription-dependent apoptosis. This transcription-dependent interaction of p53 can be inhibited by binding with MDM2. In the mitochondria, p53 interacts with Bcl-2 and Bcl-X_L, thereby triggering transcription-independent apoptosis. It should be noted that these binding events with different partners, occurring in distinct cellular compartments, are governed by the same binding motif of p53TAD (residues 15-29).

Previous mutational studies have shown that Leu22/Trp23 and Trp53/Phe54 mutations in the p53TAD render p53 completely incapable of mediating transcription-dependent apoptosis (16) because they are involved in interactions with the transcription machinery components such as TATA box-binding protein (TBP) (17), CREB-binding protein (CBP)/p300 (18), and hTAF_{II}31 (19). This suggests that the p53TAD is crucial for transcription-dependent apoptosis induced by p53. Mutational and structural researches showed that the p53TAD also serves a critical role in the transcription-independent interaction of p53 with the anti-apoptotic Bcl-2 family proteins in the mitochondria (20, 21). Thus, the p53TAD may be important in both the transcription-dependent and transcription-independent activation of apoptotic pathways by p53. Furthermore, a noticeable similarity was identified in the binding mode and binding site of the p53TAD with CBP/p300, MDM2, and Bcl-2/Bcl-X_L (20). This finding revealed a highly conserved molecular mechanism underlying the interaction of the p53TAD with CBP/p300 and MDM2 in transcription-dependent p53 apoptotic pathway, and Bcl-X_L and Bcl-2 and in transcription-independent p53 apoptotic pathway.

BCL-2 PROTEIN FAMILY

The Bcl-2 family proteins regulate mitochondrial apoptosis by modulating outer mitochondrial membrane permeability and cytochrome c release (11, 13, 14, 22-26). According to the structure of Bcl-2 homology (BH) domains, the Bcl-2 protein family is classified into anti-apoptotic and pro-apoptotic subfamilies. Pro-apoptotic Bcl-2 family proteins have three BH domains called BH1, BH2, and BH3 (e.g. Bax and Bak) or a singular BH3 domain (BH3-only proteins), while anti-apoptotic Bcl-2 family proteins (e.g. Bcl-X_L, Bcl-2, Mcl-1, A1, and Bcl-w) have four BH domains called BH1, BH2, BH3, and BH4. The Bcl-2 protein family maintains the balance between cell survival and death via sophisticated interactions between pro-apoptotic and anti-apoptotic subfamily members (26). The pro-apoptotic Bcl-2 family effectors, Bax and Bak, undergo oligomerization to form lipid pores, which induces the outer mitochondrial membrane permeabilization and cytochrome c release. Anti-apoptotic Bcl-2 family proteins perform their pro-survival function by binding and sequestering the pro-apoptotic effector proteins Bax and Bak and inhibiting membrane pore formation.

STRUCTURAL BASIS FOR THE INTERACTION BETWEEN P53 AND BCL-2 FAMILY PROTEINS

Interactions of p53 with Bcl-X_L and Bcl-2 have been observed within the cell (14). Previously, the p53 DNA-binding domain (p53DBD) was suggested to mediate the interactions with Bcl-X_L and Bcl-2 (15, 28-30). Recently, it was shown that the binding of the p53DBD induces an allosteric conformational change in the BH3 peptide-binding region of Bcl-X_L to facilitate binding with pro-apoptotic BH3-only proteins (29, 31). However, previous cell biology studies showed that the p53 N-terminal domain (p53NTD), encompassing the p53TAD and proline-rich (PR) domain, is sufficiently able to trigger mitochondrial apoptosis in a transcription-independent manner, while the p53DBD is dispensable (32). In addition, the caspase-cleaved p53 N-terminal fragment corresponding to residues 1-186, which includes the p53NTD and the truncated p53DBD, triggered transcription-independent apoptosis (33). These accumulated evidences indicated that the p53NTD alone, without the p53DBD, can mediate transcription-independent apoptosis by binding to anti-apoptotic Bcl-2 family proteins.

Recently, the biochemical and structural studies showed that the MDM2-binding motif (ΦXXΦΦ sequence motif, Φ; bulky hydrophobic residue, X; any amino acid) in the p53TAD binds to the BH3 peptide-binding groove in diverse anti-apoptotic Bcl-2 family proteins with a similar mode of binding as to MDM2 (20, 21, 34-37). These results revealed that the MDM2-binding peptide motif in the p53TAD can act as an universal site for binding diverse anti-apoptotic Bcl-2 family proteins. This is in conflict with the previous observation that anti-apoptotic Bcl-2 family proteins have unique specificity for

pro-apoptotic Bcl-2 family proteins. For instance, Bcl-2, Bcl-X_L, and Bcl-w bind to Bax whereas Mcl-1 and A1 bind to Noxa. However, the p53TAD was shown to bind to various anti-apoptotic members of Bcl-2 protein family such as Bcl-2, Bcl-X_L, Bcl-w, and Mcl-1. Indiscriminate binders of Bcl-2 family proteins such as Bim, tBid, and Puma are known to be more potent inducers of apoptotic cell death.

Refined structural models of Bcl-X_L/p53TAD and Bcl-2/p53TAD complexes unveiled a molecular basis for the mechanism underlying the interaction between the p53TAD and anti-apoptotic Bcl-2 family proteins (Fig. 2C), which explains how the p53TAD could block the function of the anti-apoptotic Bcl-2 family proteins. The sites filled by the p53TAD peptide in Bcl-2 and Bcl-X_L are significantly overlapped with those occupied by the BH3 peptide of the pro-apoptotic effectors Bak and Bax, indicating that binding of both peptides to Bcl-2 and Bcl-X_L is mutually exclusive. Hence, the p53TAD would competitively block the binding of Bak and Bax to Bcl-2 and Bcl-X_L, which consequently liberates Bak and Bax (Fig. 1). Based on these results, it was proposed that the p53TAD can lead to mitochondrial apoptosis through competitive inhibition of the interaction between pro-apoptotic Bcl-2 family proteins and anti-apoptotic Bcl-2 family proteins. This multifunctional role of p53 might provide an alternative mechanism of regulation for Bcl-2 family proteins.

APPLICATION TO ANTICANCER THERAPY

Since the interaction of p53 with MDM2 is a promising target in anticancer therapy, various peptidomimetic small molecules have been developed as protein-protein interaction blockers using the structure of the p53TAD peptide bound to MDM2 (9, 38, 39). Nutlin-3 is an imidazoline-based MDM2 antagonist that potently inhibits the interaction between p53TAD and MDM2 (40, 41) (Fig. 2A). Currently, Nutlin derivatives are in early clinical development as anticancer therapeutics. Previously, anticancer apoptotic pathway activation by Nutlin was thought to stem only from its inhibition of the p53-MDM2 interaction. However, recent studies showed that Nutlin exerts its pharmacological action via transcription-independent as well as transcription-dependent apoptosis program of p53 (42). Recently, it was also shown that the transcription-independent p53 apoptosis largely contributes to Nutlin-triggered apoptosis in cancer cells (43, 44).

Anti-apoptotic Bcl-2 family proteins are anticancer therapeutic targets since they are overexpressed in many tumor cells and are associated with cancer progression and resistance against anticancer chemotherapy (45, 46). Although the Bcl-2 family protein inhibitor ABT-737 induces the regression of solid tumors (47), it can bind only to Bcl-2, Bcl-w, and Bcl-X_L, but not to Mcl-1, which results in resistance to apoptosis induced by ABT-737 (48, 49). Therefore, concomitant inhibition of various anti-apoptotic Bcl-2 family proteins is more effective in anticancer therapy.

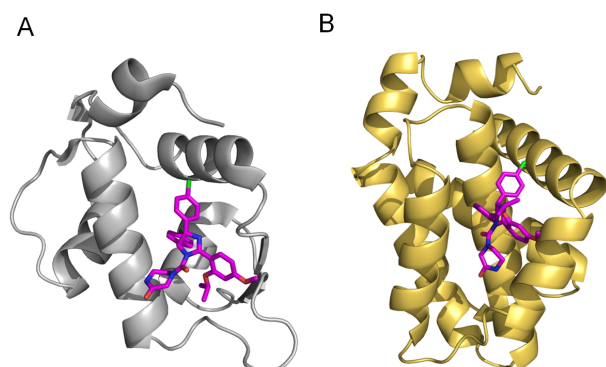


Fig. 3. Comparison between MDM2 and Bcl-X_L in the binding mode of Nutlin-3. (A) Crystal structure of Nutlin-3 in complex with MDM2 (PDB code: 4HG7). (B) A refined structural model of Nutlin-3 in complex with Bcl-X_L generated from an NMR data-driven structure calculation (50). Nutlin-3 is shown in purple.

A recent study provided a structural model of the Bcl-X_L/Nutlin-3 complex (Fig. 3B), which suggests the multi-target-based mechanism of Nutlin-3 binding to distinct target proteins, MDM2 and Bcl-X_L (50). MDM2 and Bcl-X_L display high similarity in their binding pockets, although their three-dimensional structures are quite different. Structural comparison between the Bcl-X_L/Nutlin-3 and the MDM2/Nutlin-3 complexes showed a highly conserved binding mechanism of Nutlin-3 to MDM2 and Bcl-X_L (Fig. 3). The imidazole-based scaffold structure of Nutlin-3 resembles the α -helical structure of both the pro-apoptotic BH3 and p53TAD peptide (residues 15-29) backbones (41, 51). In addition, the aromatic rings of Nutlin-3 bind to the binding sites surrounded by the bulky hydrophobic side-chains of MDM2 and Bcl-X_L. A chlorophenyl ring of Nutlin-3 makes the most important contribution to complex formation with both MDM2 and Bcl-X_L by fitting snugly into the hydrophobic grooves. Therefore, the overall mechanism underlying the binding of Nutlin-3 with Bcl-X_L is analogous to that of Nutlin-3 with MDM2.

MDM2 and Bcl-2 are promising anticancer target proteins that are related to cell cycle arrest and mitochondrial apoptosis, respectively (52). Concurrent suppression of MDM2 and Bcl-2 has a synergistic apoptotic effect in acute myeloid leukemia (AML) (53). In addition, co-treatment with Nutlin-3 and ABT-737 greatly enhanced the sensitivity of cells with high MDM2 levels to apoptosis (54). Thus, the combined inhibition of MDM2 and Bcl-2 could be a multi-target-based anticancer strategy to trigger tumor cell death. In combination with the previously reported results that the Bcl-2 inhibitor BH3I blocked the interaction between p53 and MDM2 (55), the Bcl-X_L/Nutlin-3 complex structure sheds lights on structure-based design of a multi-targeting anticancer agent that can simultaneously inhibit MDM2 and Bcl-X_L proteins. The three-dimensional structural information could also be utilized to optimize multi-target anticancer therapeutics.

CONCLUSION

To summarize, the molecular interaction of p53 with anti-apoptotic Bcl-2 family proteins serves a critical role in transcription-independent apoptotic pathway of p53. Recent studies showed that MDM2 and anti-apoptotic Bcl-2 family proteins have a highly conserved binding mechanism with the p53TAD. Based on this similarity, it was revealed that a potent MDM2 inhibitor Nutlin-3 can target the transcription-independent apoptotic pathway of p53 by binding anti-apoptotic Bcl-2 family proteins. These results provided structural insight into a multi-target-based therapeutic strategy, which have significant implications in the fields of cancer drug discovery.

ACKNOWLEDGEMENTS

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (NRF-2011-0016011).

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