

Research Article

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Understanding P53-Mdm2 Interactions: Future Prospect of Anti Cancer Therapy

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Abstract

MDM2-P53 interaction plays a vital role in regulating P53 cellular functions. With the discovery of Nutlin, MDM2-P53 inhibitor; there is a search for similar compound that potentially modifies the MDM2-P53 interactions. Design of non-peptide, small-molecule inhibitors that block the MDM2-P53 interaction has been an attractive alternative strategy for the treatment of cancer. There has been significant development in the design of small-molecule capable of inhibiting MDM2-P53 interaction recently. Nonetheless, further investigations, including explorations into the specificity of inhibitors and in-vivo models of the identified compounds to analyze the pharmaceutical value of design of non-peptide, small-molecule inhibitors that block the MDM2-P53 interaction is needed for future developments of such compounds.

Introduction

Our cells face many dangers, from chemicals, viruses, and ionizing radiation. If cells are damaged in sensitive places by these attackers, the effects can be disastrous, leading to cancers. Cancer can be correctly defined as a group of cells that have undergone unregulated growth, and will often form a mass or lump, but may be distributed diffusely [1]. Most of the cancers result as a consequence of genetic mutations, as such mutations having direct influence and quantity and quality of protein production for smooth cellular functioning with DNA repair. There are two major categories of mutated genes; these are "oncogenes and tumor suppressor genes". Oncogenes are deviation from the normal genes, having direct influence in cell growth. The mutation in oncogenes may result in direct and continuous stimulation of the pathways such as cell surface growth factor receptors, intracellular signal transduction pathways, transcription factors, secreted growth factors that control cellular growth and division. Tumor suppressor genes are normal genes that control cell cycle, cell division and programmed cell death, commonly known as apoptosis. An important difference between oncogenes and tumor suppressor

genes is that oncogenes result from the activation of proto-oncogenes and is liable to cause cancer, but tumor suppressor genes cause cancer when they are inactivated (Figure 1).

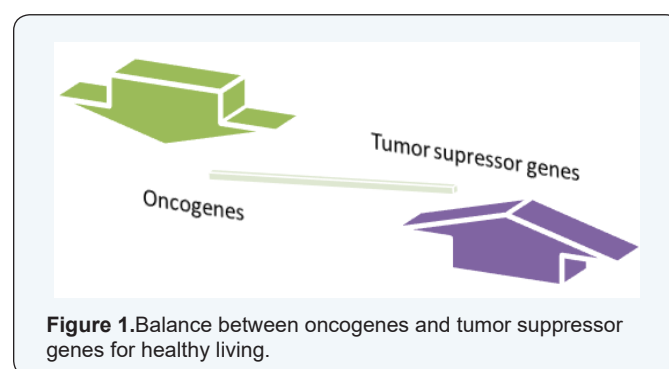


Figure 1. Balance between oncogenes and tumor suppressor genes for healthy living.

Tumor suppressor genes such as P53 gene, which codes of P53 tumor suppressor protein, plays a significant role in normal cell division and DNA repair; and are plays an important role in detecting impaired growth signals (or) DNA damage in cells. Acquired mutations of these genes appear in a wide range of

cancers [2]. If these genes as a result of inherited (or) acquired mutations becomes unable to function, DNA division and repair becomes impaired increasing the likelihood of mutative changes, and cell proliferate unconditionally resulting in a tumor. In this scenario the P53 has become the focus of intensive cancer based research in laboratories around the world. Hence the P53 is considered as a critical tumor suppressor gene. There are many tumor suppressor genes that are associated with well-defined clinical syndromes, with many more tumor suppressor genes still remains to be discovered. In this review article we have mainly focused in P53 and its interaction with MDM2, for future prospect to develop substances able to inhibit P53-MDM2 interaction, potentially as anti-cancer agents.

P53 tumor suppressor genes

P53 gene is located in chromosome 17 and was first identified as a transformation-related cellular protein which accumulates in the nuclei of cancer cells and binds tightly to the simian virus 40 (SV40) large T antigens [3]. It was further noted that the P53 protein was overexpressed not only in SV-40 transformed cells but also in carcinoma cell lines. The three compact, globular portions in P53 are termed as "domains". The tetramerization domain, at the center of P53, ties the four chains together. A long flexible region in each chain then connects to the second stable domain: a large DNA-binding domain that is rich in arginine residues, and interacts with DNA. This domain recognizes specific regulatory sites on the DNA. The third stable domain studied thus far is the trans-activation domain; found near the end of each arm that activates the DNA-reading machinery [4].

Normal function of the P53 gene

The P53 gene codes for the protein P53, which acts as a tumor suppressor, regulating cell division by keeping cells from growing and dividing too fast or in an uncontrolled way. Tumor protein P53 is found in the nucleus of the cells, where it binds directly to DNA. P53 determines the fate of the cell after DNA damage by inciting stimulus. So it plays a major role in repair or destruction of damaged DNA, preventing the formation of tumors in the future. Because tumor protein P53 is essential for regulating cell division and preventing tumor formation, it has been nicknamed as the "guardian of the genome" [5,6].

P53 as a positive cell regulator

P53 has been demonstrated to have positive implication in cell growth and proliferation. In an experiment with mouse, the mouse 3T3 cell growth arrested by serum deprivation, showed very low levels of P53 mRNA and protein. In contrast cell growth with serum stimulation; there were high level of P53 mRNA with higher the rate of P53 protein synthesis increased markedly, mostly in the G1/S boundary just prior to initiation of DNA replication [7]. The experiments performed with normal resting T lymphocytes and normal diploid fibroblasts showed that P53 expression is always concomitant with induction of cell

growth [8,9]. The level of P53 mRNA and protein is somewhat constant throughout the cell cycle when the cells are growing exponentially [10].

The idea was further strengthened by demonstrating that microinjection of P53 antibody after serum stimulation into the nucleus of 3T3 mouse cells, which inhibited the progression of the cell into the S phase. This inhibition was effective only when microinjection was performed at or around the time of growth stimulation, suggesting that P53 is critical for G0/G1 transition [9]. Antisense experiment which showed the inhibition of P53 expression prevented cell proliferation in both non-transformed NIH3T3 cells and transformed cells is also consistent with this result leading to the notion that wild type P53 is a positive regulator of cell proliferation [11]. Murine P53 could immortalize normal rat chondrocytes leading to cells sensitive to ras transformation. With all these findings we can effectively classify the P53 as a nuclear dominant oncogene. Immuno cytochemical and immune histochemical analysis proves that the P53 protein accumulates in the nucleus of transformed or tumor cells.

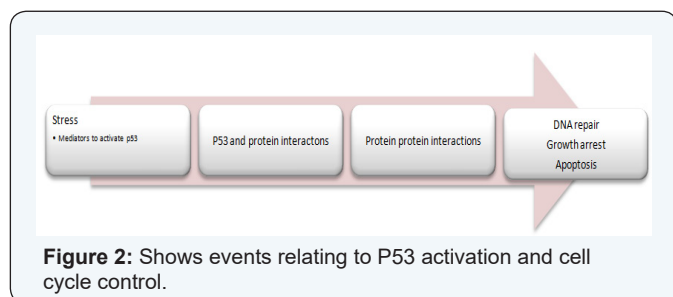
Cancers - associated with the P53 gene

Mutations in the TP53 genes are the most common implicated factors responsible for about 50% of all the human cancers. TP53 mutations have been identified in different brain tumors, colorectal carcinoma, osteosarcoma, rhabdomyosarcoma and adrenocortical tumors. Most of these P53 mutations alter the single protein in tumor protein P53, leading to point mutation, resulting in formation of impaired proteins unable to bind DNA effectively. This impaired protein accumulates in the nucleus and halts the progression for self destruction in response to DNA damage. The damaged cells continue to grow and divide in an unregulated way, which can lead to cancerous tumors [12].

Understanding P53 Pathway

The P53 pathway consists of multiple genes, each gene products responding to different stress levels. There are many queries to be uncovered how these genes play a role against cancers and their effective understanding for our benefit [12]. In normal unstressed cells, the level of P53 protein is reduced by interaction with other proteins such as MDM2. These MDM2 effectively induce P53 breakdown through the ubiquitin/proteasome pathway. Most of these MDM2 genes are increased by P53, this lead to a regulation loop that will keep P53 level very low in normal cells [13,14]. After genotoxic or non-genotoxic stresses, activation of P53 is a two-step process. First P53 protein level is increased through the inhibition of its interaction with MDM2 and the other negative regulators. Second, a series of modulator (kinases, acetylases) will activate P53 transcriptional activity. Downstream signaling includes a large series of genes that are activated by the trans-activating properties of P53. It is possible by binding of the P53 protein to a P53 response element (P53 RE) in the DNA, either in the promoter or in the intron of

target genes [15]. The P53 activation leads to cell cycle arrest or apoptosis, if not repair (Figure 2).



Mouse double minute 2 homolog (MDM2)

Mouse double minute 2 homolog (MDM2), also known as E3 ubiquitin-protein ligase, is encoded by the MDM2 gene [16,17] in humans. The MDM2 genes were located on small, acentromeric extra chromosomal nuclear bodies, called double minutes, which were retained in cells only if they provided a growth advantage. MDM2 is not common in healthy individuals, and is only seen in tumors. MDM2 is an important negative regulator of the P53 tumor suppressor. MDM2 protein shows dual functions, both as an E3 ubiquitin ligase recognizing the N-terminal trans-activation domain (TAD) of the P53 tumor suppressor protein and an inhibitor of P53 transcriptional activation. Double minutes are composed of chromatin, and replicate in nucleus during cell division. They lodge in oncogenes and amplify them. The gene product of the MDM2 is responsible for the cell transformation, inactivating P53 in the process of transformation, their by effectively inhibiting over production of P53. Some tumors contain both high levels of MDM2 and mutations in the P53 gene. The reasons for activation of the two components of the same pathway are unclear but it possibly suggests that MDM2 may possess other growth related functions.

The MDM2 gene encodes a protein with several structural and central acidic domains, including an N-terminal P53 interaction domain. The MDM2 function is regulated by phosphorylation of residues within these domains. In many cancers, MDM2 oncoprotein binds to the trans-activation domain of P53 protein and down regulate its ability to activate transcription. The different domains of MDM2 are zinc finger domain and a c-terminal RING domain. Zinc binding is an important event, which is essential for proper folding of the RING domain. The RING domain of MDM2 is responsible for E3 ubiquitin ligase activity. Various human cancer-associated MDM2 alterations targeting the central acidic domain have been reported, yet the functional significance of these mutations in tumor development has remained unclear. MDM2 central zinc finger plays a significant role in MDM2's and ribosomal proteins interactions, leading to its ability to degrade P53. All of these MDM2 functions will be lost or impaired in human cancer-associated MDM2 mutations [18]. The biochemical basis of MDM2-mediated inhibition of P53 function was further elucidated by crystallographic data that showed that the amino terminal domain of MDM2 forms a

deep hydrophobic cleft into which the trans-activation domain of P53 binds, thereby concealing itself from interaction with the transcriptional machinery.

MDM2-P53 interaction

MDM2 contains deep hydrophobic cleft as a P53 alpha helix peptide binding site, particularly a triad of P53 amino acids-Phe19, Trp23, and Leu26, which attaches deep into the MDM2 cleft. These P53 residues are also responsible for the trans-activation, to promote the finding that MDM2 possibly inactivates P53 by concealing its trans-activation domain. The structure also suggests that the amphipathic alpha helix may be a common structural motif in the binding of a diverse family of trans-activation factors to the TATA-binding protein-associated factors [19]. The interaction between P53 and MDM2 is regulated on many levels as any impairment of the P53-MDM2 complex can lead to premature P53 activation, leading to P53 induction and an unwanted biological response. Because the P53-MDM2 interaction is structurally and biologically well understood, the design of small lipophilic molecules that disrupt or prevent it has become an important target for cancer therapy [6].

MDM2 and P53 interactions inhibitors

Tumor suppressor P53 is an effective alternative cancer therapeutic target. There are two possible mechanisms for P53 application as chemotherapeutic agent: direct gene alterations in P53 or P53-MDM2 interaction. The onco-protein MDM2 binds to tumor suppressor protein P53 and inhibits its anticancer activity, which leads to promotion of tumor cell growth and tumor survival.

The nature of the interaction between P53 and MDM2 proteins has been firmly established. The atomic-level understanding of the MDM2-P53 interaction through X-ray crystallography provided the solid foundation for structure-based design of non-peptide, small-molecule antagonists of this interaction [20]. But, it was a challenging task due to wide binding interface of the proteins, making it difficult to break protein-protein interactions. MDM2-P53 small interface provides a rationale for the design of small-molecule MDM2-P53 interaction inhibitors. Even with available and well defined MDM2-P53 crystal structure, there were not significant progress made until recently for the development of potent P53-MDM2 inhibitors. But there have been several classes of small-molecule P53-MDM2 inhibitors with distinct chemical structures currently. These are the analogs of cis-imidazoline, spiro-oxindole, benzodiazepinedione, terphenyl, quinolinol, chalcone and sulfonamide.

Potent and specific MDM2 inhibitors such as Nutlins, have provided an opportunity to examine the details of their cellular mechanism of P53 activation. Consistent with in vitro biochemical binding assays, potent MDM2 inhibitors are capable of blocking the MDM2-P53 protein-protein interaction in cells. They induce accumulation of P53 protein but do not increase

the transcription of the P53 gene in either tumor or normal cells with wild type P53. Instead, they induce transcription of the P53 – targeted genes for p21 and MDM2, increasing their protein levels. As compared to conventional genotoxic anticancer agents and radiation, activation of P53 by MDM2 inhibitors does not require phosphorylation of P53.

Chalcones- based MDM2 inhibitors

Chalcones are known to have anticancer properties. Potency of some Chalcones to disrupt the MDM2-P53 interaction obtained by ELISA was confirmed by NMR titration experiments [21]. The first significant breakthrough for the design of small molecule to inhibit P53-MDM2 interactions was provided by the discovery of Nutlins. Nutlins contains cis-imidazoline in the core. The discovery of cis-imidazoline lead compound was achieved by screening of wide variety of synthetic compounds in the library using a Biacore surface Plasmon resonance assay. Through extensive chemical modifications of the lead compound, potent small-molecule MDM2 inhibitors were ultimately obtained [22].

Nutlin- Pharmacological MDM2 inhibitor

The progress in the design of non peptide small molecule inhibitors of the MDM2 – P53 interaction proceeded very slowly. The very first class of bonafide, potent, non peptide, small molecules MDM2 inhibitors, known as Nutlins, was reported in 2004. Nutlin is a potent and selective pharmacological MDM2 inhibitor that competitively binds to its P53, binding pocket, thereby leading to non-genotoxic P53 stabilization and activation of growth arrest and apoptosis pathways. Nutlin induced apoptosis is thought to be via induction of P53 transcription. Nutlins, represent the first group of potent P53-MDM2 interaction inhibitors; and there are wide reported study for the use of Nutlin-3 as a chemotherapeutic agents. [23] Nutlin -3 is a selective small molecule inhibitor of the P53-MDM2 interaction and inhibits growth in most tumor cells with wild type P53 including Neuroblastomas.

P53 as a subject for drug targeting

P53 is a key mediator of cell response and effective regulator of cell growth. Once P53 dependent mechanisms are broken, conditions for rapid accumulation of genetic changes are established leading to dramatic destabilization of the genome and acceleration of carcinogenesis [23]. Indeed, in the majority of tumors, P53-mediated response is broken either by inactivation of the P53 gene itself or by other members of the pathway: Arf (or) by natural negative P53 regulators of cellular (MDM2)/viral (e6), origin.

Tumor cells are usually very sensitive to wild type P53 and respond to ectopic expression of P53 by apoptosis (or) growth arrest. Frequent inactivation of P53 in cancer and high sensitivity of tumor cells to P53 determine the most straight forward P53 based therapeutic approach to cancer treatment, restoration (or) imitation of P53 function in P53 deficient

tumors, resulting either in a direct (tumor growth inhibition) (or) indirect (sensitization to treatment) therapeutic benefit. This general strategy is being extensively explored through a variety of approaches:

The structural information obtained in this provides us a road map for the rational design of strong inhibitors of MDM2:P53 binding by structural based virtual screening and molecular dynamic simulation studies.

Conclusion

P53 is an important tumor suppressor gene with profound role in causation of multiple human cancers. It can be effectively targeted as a chemotherapeutic agent, to control and eradicate cancers. MDM2 plays a major role with its interaction with P53, effectively inhibiting its transcriptions. Therefore, MDM2 has been identified as a P53 interacting protein towards represses P53 transcriptional activity. Inhibiting the MDM2- P53 interactions has been proven to be one of the most promising approaches for anti-cancer therapy, and further work is need to develop agents that can interfere and modify the P53-MDM2 interaction with therapeutic effectiveness.

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