Abstract

A p73 is a new member of p53 family of transcription factor, having two types. First is TAp73, transcriptionally active and expressed via upstream promoter as a tumor suppressor and vital apoptotic inductor, it also has a key role in cell cycle arrest/differentiation and Second is ΔNp73 that is transcriptionally inactive and expressed via downstream regulator as oncogenes. Both types are expressed in various isoforms, which originate from alternative splicing events at the C-terminus. Upon DNA damage, post-translational modifications cause conformational changes in various amino acid residues via induction or inhibition of various proteins, which are present in the structural domains of p73. These modifications may cause up- or down-regulation of p73 expression levels, as well as alters the transcriptional activity and/or stability of the protein. In this review, we have made an effort to assemble all existing data regarding the role of p73, its modification and after effects in cancer.

Introduction

A monoallelically articulated tumor suppressor gene is found at 1p36 region in numerous tumor cell lines, someway similar to p53, named as p73 gene. It is rarely mutated in cancer, but its level increased in various cancers, including hepatocellular carcinomas, neuroblastomas, lung, prostate, colon, breast and ovarian cancer. It allocates a noteworthy sequence resemblance with the p53 structure of DNA-binding, transactivation and oligomerization domain that is 63%, 29% and 38% respectively and intermingles with p53-binding proteins. Its wild type and mutant form bind with DNA more competently than p53. In this review, we have tried our best to summarize all information regarding p73 as a tumor suppressant as well as its oncogenic potential.

Structure and Isoforms of p73

p73 protein contains an amino-terminal Transactivation Domain (TA) which is having two Isoforms, TAp73 (tumor suppressant form, transcriptionally active because it includes exons 2 and 3, which encode the transactivation domain due to the presence of NH2-terminal) and ΔNp73 (oncogenic form, transcriptionally inactive due to lack of NH2-terminal) (Figure 1), A Proline Rich Domain (PR), a foundation and highest homology region with p53, DNA Binding Domain (DBD) and p73 protein contains an amino-terminal Transactivation Domain (TA) which is having two Isoforms. It is a globular domain, comprises of 4 α-helixes and finds in proteins those participate in the development and arbitrate in protein/protein and protein/RNA interactions. The alternative splicing of p73 at the COOH - terminal generates six diverse TAp73 isoforms which includes α, a longer isoform with SAM domain, β, g, d, e and z (Figure 2) and NH2-terminal generates assorted ΔNp73 isoforms, Δ2p73, Δ3p73, Δ2, 3p73 etc. which are originating from alternative splicing events at the C-terminus.

Promoters of p73

Two promoters, P1 and P2 promote TAp73 upon DNA damage and trigger the transcription of p53-responsive genes, like p21 that control the cell-cycle arrest, NOXA, PUMA and BAX (apoptosis regulators), cause apoptosis and cell cycle arrest and ΔNp73 upon non apoptotic DNA damage correspondingly.

P1 is TAp73 promoter having 3 potential consensuses regions of E2F1 which is a fundamental apoptosis and cell cycle inductor and adjust the p73 expression in G1/S phase. It increases the level of p16 and p14ARF which inhibit the MDM2 and degrade p53 by forming MDM2-p53 complex and elevates its level in cancer cells. E2F1 influences p73 level and cause p73 up-regulation in a p53 dependent manner due to huge structural homology with p53 (Figure 3A). Another fact about MDM2 is that it causes p73 inactivation instead of degradation by E3 ubiquitin ligase.

E2F1 also induces p53-independent cell death because it triggers the TAp73 expression at P1 promoter. E2F1 binding to
TAp73 P1 promoter and triggers its acetylation after DNA damage. It stabilizes E2F1/p73 complex and induces apoptosis and cell cycle arrest12 (Figure 3B). P2 is ΔNp73 promoter with no E2F1 binding region, but having p53/p73 responsive element. The non-apoptotic DNA damage activates P2p73 in p53-dependent manner and cause abundance of ΔNp73 proteins that only causes cell cycle arrest but do not undergo apoptosis.13,14 It exhibits negative auto-regulatory feedback by inhibiting p53 and TAp73 isoforms while both proteins induce ΔNp73 level.15,16 ΔNp73 deregulation promotes malignant transformation of fibroblasts and tumor growth.17

Regulation of p73

The comparative intensity of both forms TAp73 and ΔNp73 decide cell fortune either growth/development arrest or unrestrained propagation. After DNA damage in the steady state level of TAp73 isoforms is up-regulated but ΔNp73 degraded quickly. E2F1 causes direct up-regulation of p73 via recognition and transactivation of TAp73 gene on P1 promoter site as discussed above while E1A and c-myc (oncogenes) promote p73-dependent apoptosis in tumor cells by binding with E1A and p300/CBP and RB (E2F1 regulators).18,19 So, E1A and c-myc put forth a synergistic effect on p73 activity which is sometimes not related to E2F1 activation.16

Post translational modification

These modifications establish p73 specificity/sensitivity to specific proteins and regulate its transcriptional activities like p53 by methylation, phosphorylation, acetylation, ubiquitination and sumoylation under normal circumstances and DNA damage.

Methylation: DNA methylation, which is a part of epigenetic alterations and histone modification, is a covalent chemical adaptation at CpG nucleotides in which CH3 group is shifted from donor S-adenosylmethionine (SAM) to C-5 cytosine via DNA cytosine-5-methyltransferases. It regulates the genomic expression and silencing of tumor suppressant genes which look after cells from malignant alteration called as TSG like p73, identified in 1997.20 Four isoenzymes of methyltransferase are DNMT1, DNMT2, DNMT3A and DNMT3B21 accountable for maintenance and de novo DNA methylation during embryogenesis and cause alterations in genomic methylation which pursue epigenetics and cancer in cells. For example, malignant tumor shows overall hypomethylation and silencing of TSG by directly blocking the specific binding sites of transcription factor by the methyl-CpG-binding proteins (MBDPs)22 (Figure 4).

These methylated TSGs play important role in regulation of cell cycle, DNA repairs, apoptosis, transcriptional regulation, angiogenesis and metastasis.23,24 TSGs become nonfunctional via mutation, deletion and in expression of promoter due to its hypermethylation which leads to epigenetic silencing and induces oncogenic potential.

Phosphorylation

Phosphorylation by the non-receptor tyrosine-kinase c-Abl was first revealed p73 post-translational modification. The c-Abl tyrosine kinase is dispersed in the nucleus and cytoplasm of proliferating cells. Nuclear c-Abl activity is pessimistic regulated by the retinoblastoma protein (RB) and optimistically regulated by DNA damaging agents like Cisplatin and ionizing radiation (gIR).25 C-Abl tyrosine kinase interacts with the p73 proline-rich region in the
SH3 domain via p73 PxxP motif at COOH-terminal OD and cause phosphorylation of p73 primarily at Tyr-99 then Tyr-121 and Tyr-240. It leads to p73 stabilization, accumulation in the nucleus, half-life (T1/2), transcription, apoptosis and cell cycle arrest in growth phase.26

Other important kinases are mitogen-activated protein kinase (MAPK), Checkpoint 1 and 2 kinases, protein kinase C and cyclin dependent kinase. 1: Activated c-Abl tyrosine kinase also promotes MAPK after DNA damaging signals and causes p73 phosphorylation at thr-167 residues that are located adjacent to proline via p38 protein. p38 has ability to phosphorylate p73 threonine residue without c-Abl tyrosine kinase. So, it enhances the p73 transcriptional activity and apoptosis by phosphorylation pathways instead of cell cycle arrest.27

2: In response to DNA damage, Checkpoint kinase 1 (Chk1) causes phosphorylation of p73α at Ser-47 residues and causes TAp73α accumulation and apoptosis induction. Chk1 activates p73 expression, but Chk2 has a role in p73 up-regulation.

They contribute to E2F1-mediated p73 up-regulate transcription by phosphorylation and activation of E2F1 transcription factor28,29 (Figure 5). In fact, p73 does not require DNA damaging signals for phosphorylation. It becomes phosphorylated during cell cycle as well. So, it requires Protein kinase Cd and cyclin dependent kinase. During the cell cycle, PKCδ causes phosphorylation of p73 at Ser-388 residues at second TAD. It causes activation of second TAD and regulation of cell cycle progression but remains unable to induce apoptosis. But in response to DNA damage, PKCδ is sliced by caspase-3 to active catalytic fragment (PKCδ CF) that causes phosphorylation of p73β at Ser-289 residues of

Figure 4. DNA methylation, a part of epigenetic modification leads to silencing of TSGs by direct obstructing the specific binding sites of transcription factor by MBDPs via aberrant methylation and leads to p73 down regulation resulting oncogenes and apoptosis inhibition.

Figure 5. Phosphorylation of p73 via different kinases at Ser-47 and 289, Thr-8/167/442 and 482, Tyr-99 and 240 amino acid residues upon exposure with DNA damaging agents and up regulate p73 level else CDK2 which down regulate p73 via phosphorylating Thr-86 at TAD domain in p73 structure

Figure 6. Acetylation of p73α via p300 acetyltransferase at NH2 terminal TAD at Lys-321/327 and 331 with c-Abl kinase in promotion with YAP and Pin 1 by DNA damaging agent doxorubicin resulting in p73 up-regulation

Figure 7. Ubiquitination of TAp73α/β at PY motif domain via E3 Ub ligase upon doxorubicin or cisplatin exposure and leads to p73 up-regulation but ubiquitination of ΔNp73 via E3 Ub ligase down regulate it.


**Importance of p73 expression in human cancer**

The p73 genomic expression in all normal human tissues is at very low intensity. So, recognition is not so easy. All the findings show the adequate balance between two antagonistic classes of proteins TAp73 and ΔNp73 those are encoded by the same gene. Their balanced ratio determines the cell reaction upon growth stimuli, DNA damage or pathogenic attack. Due to depiction of a whole variety of p73 isoforms, it is well recognized by various agents and differentiates between TA and AN forms properly.

The Ip36.3 chromosomes for human gene Tp73 deficient in various types of cancers is enough for functional loss and persuades the cell to cancer like neuroblastoma, colorectal and breast cancer. A diversity of studies depicts the monoallelic expression in various tissues and cancers means when one allele is imprinted. This one allele deficient cell is sufficient to make a cell p73 void. It also demonstrates the loss of imprinting (LOI), biallelic expression or the allele switching of p73. The loss of function transmutations in p73 is very much rarer i.e. 0.6% when taking evidence from almost >1100 primary tumors.

But in spite of loss of expression the most common noticeable alteration is over expression of different isoforms of the wild type p73, A high occurrence of loss of heterozygosity for p73 has observed in neuroblastoma, lung, gastric and ovarian tumors.24 Mutations in TAp73 gene are seldom found in human malignant cells like P405R and P425L, a somatic and a germ line in primary neuroblastoma. P425L replacement decreases the p73a-dependent developmental suppression, but not p73β transcriptional activity, but exchange in P405R has no effect on p73α/β transcription.40

**Chemo sensitivity with regards to p73**

p73 level enhances by small spectrum chemotherapeutic anti-cancer drugs like anthracyclines, topoisomerase I and II inhibitors, microtubule inhibitors and alkylating agents such as cisplatin, adriamycin, taxol, etoposide and doxorubicin treatment in different tumor derived cell lines. The reason of p73 up-regulation is its enhanced transcriptional activity and increased protein stability, which leads to induction of apoptosis via apoptosis commencement.

Chemo resistance increases by blocking TAp73 via inhibiting p73DD fragment or p73 RNA. Up-regulated ΔNp73 also has ability to stop the apoptosis in wild-type p53 cancer cells induced via various chemotherapeutic agents.14-20 But up-regulation of p53 mutant due to Arg-72 polymorphism blocks the p73 transcriptional activity and ultimately apoptosis.38 p73 has ability to induce apoptosis in the absence of p53 but p53 cannot do so when the fibroblast cells lacking p73. All these studies show the importance of p73 in regards to chemotherapeutic agents.

**Concluding annotations**

This review principally stresses the functions of p73, interacting proteins in human cancer and chemosenstivity with regards to p73 gene. p73 functions are mostly trans mutated in various cancers. One of p73 isofom, TAp73 potentially triggers upon DNA damage for cell cycle arrest and apoptosis and vice versa for other isomor ΔNp73. So, the balance between both types is vital to settle down the p73 apoptotic fate in cancerous cells. In spite of high structural and functional homology with p53, both have substantial dissimilarities as well. Post-translational modifications pin point...
their discrepancy behavior throughout growth and malignant makeover. Like E3 Ub ligase mdm2 causes degradation of p53 but stabilizes p73. So all the signals those mutate p53 are not able to infect p73. Eventually, improved perception of all these pathways may direct to the expansion of innovative p73 oriented therapies.

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