VIA MEDICA

Review article

NOWOTWORY Journal of Oncology
2019, volume 69, number 5–6, 168–178
DOI: 10.5603/NJO.2019.0031

ISSN 0029–540X
www.nowotwory.edu.pl

Repair or perish – the role of p53 protein in a cell's life

Magdalena Kulesza[1], Agnieszka Dansonka-Mieszkowska[2], Barbara Pieńkowska-Grela[1]

[1]*Independent Laboratory of Cytogenetics, Maria Sklodowska-Curie Institute – Oncology Center, Warsaw, Poland*
[2]*Laboratory of Genetic and Molecular Diagnostics, Maria Sklodowska-Curie Institute – Oncology Center, Warsaw, Poland*

The p53 protein is one of the most important suppressors of neoplastic transformation. It regulates transcription of multiple genes and interacts directly with other proteins. It plays a significant role in the most important processes that take place in the cell, including: DNA repair, cell cycle and programmed cell death – apoptosis. Loss of its proper function leads to a disturbance of the mechanisms controlling cell proliferation and survival, which contributes to the development of neoplasms.

The *TP53* gene is called the guardian of the genome. Its mutations occur in a large percentage of tumors. They most often concern sequences that encode the DNA-binding domain (exons 5–8). The *TP53* gene, together with the *TP63* and *TP73* genes, belongs to the oldest evolutionary family of cancer transformation suppressors. Its product, a full length p53 protein, consists of five domains and a flexible consolidator region and functions as a homotetramer. The regulation of p53 activity is caused by MDM2 protein, which contributes to proteasomal degradation of the suppressor.

This review deals with the most important aspects of the regulation of cell activity by p53 protein. It describes the structure of p53 protein and the associated therapeutic possibilities.

NOWOTWORY J Oncol 2019; 69, 5–6: 168–178

Key words: suppressor genes, p53 protein structure, p53, p53 *vs.* cell activity regulation

Introduction

According to the current state of knowledge, the main cause of death in the global population are cardiovascular diseases and cancers. In recent decades, however, cancer has been ranked as a number one in the hierarchy of the most common causes of death in developed countries. Europe accounts for 23.4% of global cancer cases and 20.3% of cancer deaths, while the population of European countries accounts for only 9% of the global population. According to GLOBOCAN, 9.6 million people worldwide died of cancer in 2018 and more than 18 million new cancer cases were diagnosed [1, 2]. This ever-increasing threat has become the driving force behind research into the mechanisms of the cancer process. Genetic research has allowed us to distinguish several classes of genes that are involved in the carcinogenesis process. One of these classes is made up of genes responsible for suppressing abnormal cell proliferation. One of the most important suppressor genes is the *TP53* gene, which is called the guardian of the genome.

The p53 protein – the superior guardian of the genome

The history of p53 research dates back nearly forty years, and the protein itself was discovered in 1979 independently by several groups of scientists. They identified a protein of approximately 53kDa mass present in human and mouse cells, which bound to a large T-antigen of the SV40 virus. Research has shown the existence of a high level of the p53 protein in neoplastic cells with a low level of p53 in normal cells. In turn the overexpression of the newly discovered *TP53* gene resulted in the transformation of a healthy cell into a neoplastic one. Therefore, initially *TP53* was erroneously considered to be an oncogene. It was not until 10 years later that it was classified as a cancer transformation suppressor. It became clear then

that the originally analyzed *TP53* gene was mutated. This gave rise to intensified research on its role in the carcinogenesis process [3, 4].

The role of the wild type p53 protein in suppressing tumor development is well known. Suppressor regulates the transcription of numerous target genes that are involved among other things in cell cycle monitoring, apoptosis and DNA repair. The majority of mutations in this gene are missense type changes, leading to expression of full length but abnormal p53 protein. The altered protein not only loses its suppressive function, but also it can acquire new properties that promote carcinogenesis [5–7].

In normal cells, activation of *TP53* transcription is a response to stress factors, including significant DNA damage and cell hyperproliferation signals. A normal suppressor interacts with proteins and regulates the transcription of many genes affecting the most important cellular processes. Depending on the degree of DNA damage, the p53 protein stops or slows down the cell cycle, giving the cell time to repair the genetic material and preventing duplication of the error. When the damage is too serious, the p53 protein directs the cell to the path of programmed death (apoptosis) [8]. Due to its important role in determining the fate of the cell, p53 is strictly controlled by a number of regulatory proteins: MDM2, MDM4, MDMX, p300/CBP and some kinases. Low p53 protein level is caused by interaction with MDM2, which contributes to proteasomal protein degradation. MDMX works in a similar way and also negatively regulates the p53 level. In stressful conditions the kinases phosphorylating p53 N-terminal domain are activated, which allows to releasing MDM2 and enhances the binding to transcription coactivators, including p300/CBP, leading to the activation of the suppressor [9].

Mutations, i.e. gene pathogenic variants, associated with cancer are usually somatic changes that arise and accumulate in cells during the carcinogenesis process. Somatic mutations are not transferred to reproductive cells, but only to the progeny of cancer clone cells. The pathogenic variant of the gene that occurs in all cells of the organism (inherited or arised *de novo*) is called a germinal mutation. Such a change may increase the risk of cancer development in affected individuals (hereditary cancer).

The presence of a germinal pathogenic variant of the *TP53* gene is associated with Li Fraumeni syndrome (LFS). The ClinVar database, which collects information on gene variants of clinical significance, contains so far over 750 described changes in the *TP53* sequence detected in patients with LFS. Li Fraumeni syndrome is inherited in an autosomal dominant way. It is characterized by patient's predisposition to rare types of cancer, especially in younger age. Half of the persons with LFS develop cancer before the age of 30, while in an unencumbered population the risk of developing the disease is about 1% [10, 11].

The *TP53* gene structure

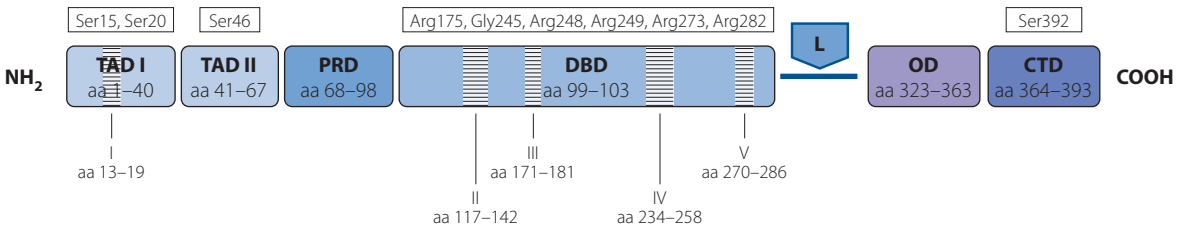
The *TP53* gene is located on the short arm of the chromosome 17 (17p13.1). It consists of 19 198 nucleotides including 11 exons, the first of which is non-coding [12–14]. There are no CAAT or TATA sequences in the *TP53* gene promoter that are recognized by RNA II polymerase. On the other hand (like other areas that regulate transcription without the TATA-box sequence), the *TP53* promoter contains multiple GC repetitions and binding sites for the SP1 transcription factor. It also has motifs specific to many other transcription factors, including: cMyc/Max, ETF, NF-κB complex [15, 16].

The p53 protein structure

The *TP53* gene encodes a 393-amino acid protein that functions as a transcription factor and is biologically active as a homotetramer. Full length protein consists of five main domains and a flexible consolidator region (linker, L), which links the domain binding DNA with the tetramerization domain (Fig. 1) [12–14].

TAD (N-terminal transactivation domain)

A transactivation domain (N-terminal), due to its function, is also called a transcription activating domain. It consists of



Ser15, Ser20, Ser46, Ser392 – most frequent phosphorylation sites,
Arg175, Gly245, Arg248, Arg249, Arg273, Arg282 – most often mutated codons,
TAD – transactivation domain,
PRD – proline-rich domain,
DBD – DNA-binding domain,
L – consolidator,
OD – oligomerization domain,
CTD – regulatory domain,
I–V – regions of high evolutionary conservation

Figure 1. The p53 protein structure

two independent subdomains: TAD I (1–40 amino acids) and TAD II (41–67 amino acids) that contain ϕ-X-X-ϕ-ϕ evolutionary conserved sequences (where ϕ stands for a hydrophobic amino acid, X for any amino acid). Each of the subdomains functions autonomously and is necessary for the regulation of different cellular processes [13, 17–20]. Mouse model studies have shown that TAD II inactivation impairs the transactivation of some genes, while other p53 functions are preserved. In turn, inactivation of TAD I leads to much more serious cell function defects. It causes the lack of target gene transactivation and disturbs cell cycle inhibition and apoptosis initiation in response to DNA damage. It does not affect the regulation of cell senescence and inhibition of the carcinogenesis process. Simultaneous elimination of both subdomains gives an effect similar to the total removal of p53 protein from the cell. This leads to disturbances in transcription of suppressor-dependent genes, lack of activation of senescence and apoptosis, dysfunction of cell cycle control points and loss of ability to inhibit neoplastic transformation.

The transactivation domain, except for two regions – Phe19 to Leu25 and Pro47 to Trp53, which form a pair of helixes (Pα1 and Pα2) – has an unordered structure. Pα1 and Pα2 play a key role in binding TAD to other proteins and the DNA-binding domain (DBD). Interaction with DBD affects the selectivity of the p53 binding to its specific sequences that are present in the target gene promoters [17]. It can also prevent premature binding of the TAD with transcription coactivators and other cofactors by masking their binding sites [9, 21]. Proteins, with which the transactivation domain creates a complex, are among others: MDM2, p300/CBP acetyltransferase, subunit of transcription factor TFIIH and BCL-XL. The formation of the complex with MDM2 and p300/CBP affects the activation and stability of the p53. The p300/CBP protein plays an important role both in the activation of transcription with p53 and in the regulation of the stability of the p53-DNA complex by acetylation of lysines in the regulatory domain located at the carboxyl-terminus [9]. The transactivation domain, as a result of interaction with BCL-XL (anti-apoptotic mitochondrial protein), is also responsible for activating the apoptosis pathway in a cell, without the need to activate transcription of genes [4].

The transactivation domain contains one of the two nuclear export signal sequences (NES) that are responsible for transporting the p53 protein from the cell nucleus to the cytoplasm. TAD also includes the first of five evolutionary conserved regions, HCD (highly conserved domain) [20, 22].

PRD (proline-rich domain)

The proline-rich region is mostly evolutionary conserved and includes amino acids 68–98. It consists of multiple repetitions of the PXXP motif, where P means proline and X corresponds to any amino acid [8, 23]. These repetitions are a specific ligand for proteins containing SH3 domains and facilitate their direct interaction with p53 [12]. Studies of cell lines have shown that the PRD domain is significant for activation of apoptosis by p53 [24, 25]. Moreover, PRD may influence the kinetics of protein folding and facilitate its splicing [12]. It also plays an important role in the stabilization of the p53 protein through interaction with proline isomerase Pin1. *In vitro* studies have proven that phosphorylation of p53 in response to stress signals results in increased interaction with Pin1. This changes the protein conformation and reduces its availability for MDM2 [25].

DBD (DNA-binding domain)

The DNA binding domain is located centrally and includes amino acids 99–303 [8]. Its core forms a β-sandwich structure, similar to that of immunoglobulins, which consists of two antiparallel β-sheets and a helix [26]. There are four of the five evolutionary preserved sites here (HCD II-V). These are the areas where mutations are most frequently detected [27, 28]. The DBD domain is a region necessary for specific DNA binding.

Direct contact with the double helix involves a complex of three elements. It includes: a β-hairpin structure formed by two antiparallel strands (S2 and S2'), L3 strand including a short α-helix (H1) and a zinc ion tetrahedrally coordinated by His-179 and three side chains of cysteines: Cys-176, Cys-238 and Cys-242. The last component is the evolutionarily preserved α-helix (H2) located at the C-terminus of the domain. It is a functionally important part of the domain and is crucial for biological activity of p53 [12, 29, 30].

A domain that binds DNA connects to the specific RE (response elements) sequence present in the target gene promoter region [28]. RE consists of two decameric palindrome sequences – 5'-RRRCWIGYYYY-3' (where R stands for purine, Y for pyrimidine and W for alanine or threonine). Single sequences can be separated by a short 0–13 base pairs insertion. The p53-dependent gene promoters contain different numbers of RE sequences, which may be associated with differences in the activation strength of their transcription by the suppressor. Crystallographic findings suggest that the p53 homotetramer is capable of forming a complex with a single decameric sequence, with two of the four DNA-binding domains involved in the interaction. It is also possible that one p53 homotetramer binds to two RE sequences [28].

The selectivity of the DBD binding to specific target gene sequences is related to the interaction with the transactivation domain. TAD competes with a DNA molecule to bind to DBD. RE sequences specific for p53 are bind with higher affinity and replace TAD from the interaction with the central domain. Intramolecular interaction between TAD and the surface of DBD in the p53 homotetramer reduces by more than five times the ability of DBD to non-specific binding to DNA, but does not affect the interaction with the suppressor-specific sequence [21].

OD (oligomerization domain)

In the C-terminal part of p53 protein there is an oligomerization domain, which is responsible for the tetramerization of the

molecule and the formation of a functional homotetramer. It includes amino acids 323–363 and contains the second NES signal sequence [31, 32]. Structurally, OD is inscribed in the hairpin pattern. It consists of β-sheet (Glu326-Arg333 amino acids) and α-helix (Arg335-Gly356 amino acids) connected by glycine (Gly-334) [31–33].

CTD (C-terminal domain)

C-terminal region p53 includes amino acids 364–393. CTD is characterized by internal disorder and its important feature is the presence of lysines, which co-participate in the non-specific p53 binding to DNA. This domain also regulates the ability of the DBD domain to bind DNA, making it easier to match each other and favoring the maintenance of the p53-DNA complex. The unmodified form of the CTD domain inhibits DNA binding, while phosphorylation of Ser392 has a positive effect on the interaction with nucleic acid [18, 20, 27].

Similarly to the N-terminal domain, the C-terminal region is responsible for p53 interactions with other proteins. It is a region where transcription factors (TFs) bind [43, 45]. Furthermore, the CTD is essential for interaction with MDM2, thus affecting the stability of p53 and its nuclear location. It contains sites that are the target of post-translational modifications that regulate p53 activity (e.g. acetylation of the domain modulates affinity to DNA) [18, 20, 27, 34].

The CTD may adopt different conformations depending on the interaction with other proteins: α-helix in contact with low-molecular-weight S100 calcium-binding protein B, β-sheet in contact with Sir2 (deacetylase NAD+-dependent), β-sheet in complex with p300/CBP (coactivation protein with histone acetyltransferase activity) and undefined secondary structure in connection with cyclin A/CDK2 complex. Therefore, depending on the conformation adopted by the C-terminal domain, the functionality of p53 protein is regulated both negatively and positively [20, 34].

Activity control of p53

The activity of p53 protein is regulated by a number of mechanisms. These include post-translational modifications, regulation of the degradation and oligomerization process. The inhibition of degradation contributes to the activation of p53 protein through its stabilization and accumulation in the cell. The level of suppressor in cells that are not exposed to a stress factor is low. It is facilitated by the short half-life of the molecule. Responsible for this are the MDM (murine double minute) proteins – MDM2 and MDM4, which bind p53 within the TAD domain.

MDM2 has the ability to degrade p53 through proteasome due to its ubiquitin E3 ligase activity. MDM4 does not has ligase activity, but by heterodimerization it regulates enzymatic activity of MDM2, increasing the efficiency with which MDM2 degrades p53 [35]. MDM2 synthesis is positively regulated by p53, which leads to an increase in MDM2 levels at a higher p53 concentration. In inactive cells, p53 binds to MDM2, which promotes its ubiquitination. However, under stress conditions, such as the occurrence of DNA damage, the balance between proteins is disturbed. As a result of post-translational modifications blocking the interaction with MDM2, p53 is stabilized, its amount in the cell is increased and it is activated [8, 18, 36].

Post-translational modifications, including phosphorylation, acetylation, methylation, ubiquitination, neddylation, SUMOylation and the addition of N-acetylglucosamine amino acids at the end of the chain also contribute to p53 activation. Phosphorylation occurs mainly within the N- and C-terminal domains, as well as in the linker region. Lysines which are located in those regions, may also be subjected to methylation or neddylation [36]. Neddylation is the attachment of the Nedd8 molecule to the target protein. Nedd8 is a protein similar to ubiquitin, which promotes protein degradation by activating ubiquitin E3 ligases [37].

The number and type of DNA damage determine post-translational modifications of p53 protein, which directly affects the cell response. Induction of apoptosis pathway requires phosphorylation of the remaining amino acid Ser46 by p38, DYRK2 or HIPK2 proteins. Acetylation of the Liz120 residue by PCAF *(p300/CBP-associated factor*) activates transcription of genes responsible for cell cycle arrest. In turn, acetylation by Tip60 *(60-kDa tat-interactive protein*) or hMof (human males absent on the first) introduces the cell on the p53-dependent pathway of apoptosis. Acetylation of Liz164 via CEP and p300/CBP is necessary for both stopping the cell cycle and apoptosis [36].

The p53 protein is phosphorylated by the protein kinases ATM (ataxia telangiectasia mutated) and ATR (ataxia-telangiectasia and Rad3-related). In response to DNA damage, they interact with the Chk2 and Chk1 kinases respectively, which stabilize p53 by attaching the phosphate residue to Ser20. In addition, ATM protein phosphorylates MDM2 that prevents its interaction with p53. This results in inhibition of degradation and accumulation of the suppressor [8, 18, 36]. ATM or ATR kinases also directly phosphorylate of p53 at Ser15. Sequential phosphorylation of amino acid residues facilitates acetylation at the C-terminus of the chain, enabling oligomerization of the protein and its nuclear transport. This has a positive effect on the transcription activity of p53 and at the same time strengthens the bond with DNA [7].

Another process that affects p53 activity is its oligomerization. This dynamic process is regulated by protein-protein interactions and depends on the level of p53 in the cell. Numerous proteins that directly bind to the OD domain can modify the oligomerization of the molecule and change its stability. Interactions between proteins enhance or inhibit the oligomer formation process, thus promoting the monomeric forms of p53 [31]. When creating a tetramer, the NES sequence is masked, suggesting that the regulation of tetramerization and nuclear export is correlated [31, 32].

Homotetramer of p53 protein is structurally a dimer of two dimers and the oligomerization process takes place in stages. Primary dimer is created by interaction between β-sheets of two p53 monomers, which in an antiparallel way bind together with hydrophobic interactions between α-helixes. Subsequently, the two primary dimers merge with each other and form a p53 protein homotetramer [31, 38, 39]. In case of other the p53 family members: p63 and p73, it is observed an additional helix that stabilizes the formed tetrameter [38]. Under normal conditions most of the p53 molecules are in the form of dimers – nearly 60% of the cell pool. It was shown that the structure of the tetramer increases the affinity and ability of p53 to bind to DNA in relation to the dimeric form. Therefore, during cellular stress, homotetramer formation is observed with simultaneous reduction of p53 degradation. Correct oligomerization of the molecule affects the functionality of the suppressor, it is necessary to direct the cell to the apoptosis pathway, inhibit its growth or allow for proliferation. Defects during the oligomerization process decrease the transcription activity of the protein and contribute to the formation of a number of neoplasms [31, 39].

The p53 protein as the main driver of cellular processes

The p53 protein plays a key role in the control of many of the most important cellular processes.

It participates in the regulation of proliferation, programmed death and repair of genetic material. It is also involved in the control of cell metabolism, autophagy and cell senescence processes [8]. The suppressor works by interacting directly with other proteins and regulating gene transcription. For example, it forms complexes with proteins that are involved in DNA repair (including RAD51, RecA, BRCA2). It also interacts with individual members of the BCL-2 family regulating the mitochondrial pathway of apoptosis. In addition, p53 influences the control of transcription of target genes in different ways:

- it directly activates genes expression through an extended mechanism that includes the interaction of p53 with the RE in the promoter,
- directly inhibits target genes expression, which occurs after interaction of p53 with RE or by binding p53 via an adaptor, in particular the NF-Y factor (*nuclear transcription factor Y*),
- indirectly inhibits the expression of target genes through:
  - activation of p21, DREAM (p53-DREAM pathway; DP, RB-like, E2F4 and MuvB) or pocket protein complexing (RB/E2F),
  - interaction with transcription activators, in particular NF-Y, Sp1 (specificity protein 1) and TBP (TATA-box binding protein),
  - activation of transcriptions of non-coding RNAs (ncRNAs), including: *mir34a* (*MicroRNA 34a*), *lincRNA-p21* (*long intergenic non-coding RNA p21*) and *PANDA* (*p21 associated ncRNA DNA damage activated*) [27, 40–44].

The role of p53 in DNA repair

DNA in cells of living organisms is exposed to numerous damage related to both endogenous factors (reactive oxygen species, replication errors) and the effects of exogenous factors, including ionizing radiation and UV radiation, as well as genotoxic compounds. In response to abnormalities in the genetic material, in a cell the pathway of response to DNA damage – DDR (DNA damage response) is activated. Its important element is the p53 protein [36, 45].

The main role in DDR pathway activation play ATR and ATM kinases, which belong to the PIKK (phosphatidylinositol 3-kinase-related kinase) family. The first one is involved in the response to single-strand breaks (SSB) damage caused by e.g. stopping the replication fork. Its activation takes place mainly during the S-phase of the cell cycle. The ATM kinase, on the other hand, is active during all phases of the cell cycle. It intermediates in response to double stranded DNA damage – DSB (double strand breaks). The consequence of the signal cascade activated by the above mentioned kinases is the activation of effector proteins, including p53. Depending on the extent of damage, various post-translational modifications of the suppressor occur. This affects the cell response: an attempt to DNA repair or in the case of irreversible changes, launching the apoptosis pathway [36, 46, 47].

There are many mechanisms for DNA repair. One of them is a direct repair without breaking the continuity of the DNA strands. It is related to the action of O^6-methylguanine methyltransferase (MGMT), which transcription is activated by p53 protein. MGMT reduces alkylation by removing the alkyl group from the guanine O^6 atom, transferring it to its own cysteine residue (Cys145) in the active center [36, 48, 49]. High methyltransferase activity in neoplastic cells affects resistance to treatment with alkylating agents such as nitrosourea or dacarbazine derivatives and thus to therapy failure and chemoresistance in patients [50].

Another mechanism of DNA repair is the repair by cutting out the nucleotides – NER (nucleotide excision repair). Depending on the type of damage, p53 protein stimulates this pathway in a way dependent or independent of the activation of the transcription. It facilitates the availability of repair proteins to chromatin or mediates the activation of gene expression responsible for diagnosing damage and initiating DNA repair [51]. The first mechanism is related to the interaction between p53 and p300/CBP protein, which has the acetyltransferase activity. The p53 protein, when bound to p300/CBP, modifies its conformation, thus activating the enzyme, which relaxes heterochromatin and facilitates access of repair proteins to DNA [52, 53]. A similar mechanism can be observed in the interaction of p53 with p33ING1 (inhibitor of growth 1) and p33ING2 proteins, which by increasing the level of H4 histone acetylation activate the relaxation of chromatin structure and reveal the DNA damage sites for the repair proteins [54].

In turn, the NER repair mechanism dependent on activation of transcription by p53, includes, among others, the following genes: *XPC* (*xeroderma pigmentosum*) and *Gadd45* (*growth arrest and DNA-damage-inducible*) [51, 55]. The DDB2 protein (*damage specific DNA binding protein 2*) encoded by *XPC* is necessary to repair DNA in response to damage caused by UV rays. It is part of a complex that acetylates histones [56]. A product of the *Gadd45* gene by interaction with the PCNA (proliferating cell nuclear antigen) increases the access of repair proteins to damaged DNA. In this way, it plays a key role in preventing neoplastic transformation [54, 57].

The role of p53 in the regulation of the cell cycle

The cell cycle is a cascade of consecutive events that lead to the division of a cell into two offspring cells. Its correct course is monitored at checkpoints, whose correct realization determines the start of the next phase of the cycle. The key protein involved in this process is p53.

The role of p53 at G1/S checkpoint

The first stage of the cell cycle is the G1 phase, in which the cell makes the final decision about the division. However, if damage of genetic material is detected, a sequence of processes, which leads to the arresting of the cell cycle at the turn of the G1/S phases is started. DNA damage is detected by ATM/ATR kinases, which directly and indirectly – through Chk1/Chk2 proteins – phosphorylate p53 and stimulate its activation [58]. The active suppressor is transported to the cell nucleus. There it acts as a transcription factor and influences the expression of target genes, in particular cyclin-dependent kinase inhibitors from the Kip/Cip family: *CDKN1A* (p21) and *CDKN1B* (p27) [58–60]. The p21 and p27 proteins bind to the complex of CDK2 kinase and cyclin E, inhibiting its activity. The p21 protein is also an inhibitor of the cyclin D/CDK4/6 complex [61]. Diminished activity of CDK2 and CDK4 blocks RB phosphorylation. It favors the binding of RB protein to transcription factor E2F1 and prevents its action. This leads to the silencing of transcription of the genes necessary to carry out the cell to the subsequent stages of division. This provides time to repair the DNA. Removal of damage allows the division to continue. In the case of significant, irreparable changes, p53 starts a programmable cell death [62].

The role of p53 at the checkpoint in S-phase

The condition for proper cell division is complete and error-free replication of genetic material, which takes place during the S-phase. The occurrence of errors causes phosphorylation of p53 by ATR and CHK1 kinases. The activated p53 suppressor induces expression of p21 protein, which by interaction with PCNA and CDK2 inhibits DNA replication [58, 61]. The association of p21 with PCNA interferes its DNA dependent polimerase activity, but does not affect the repair functions of PCNA [63].

The role of p53 at G2/M checkpoint

The G2 checkpoint protects the cell from passing into the last stage of division with unreplicated DNA. As a result, it prevents the inappropriate chromosome segregation into offspring cells. The condition for a cell's entering into M-phase is the activation of Cdk1/cyclin B complex. The p53 protein stimulates the expression of *Gadd45*, *SFN* (14-3-3σ) and *CDKN1A (p21)* genes in that way contributes to the inhibition of cell cycle in the G2 phase [5]. The p21 molecule binds to the cyclin-dependent kinase Cdk1, blocking its active center. Its results in inactivation of the Cdk1/cyclin B complex [62]. In turn, *Gadd45*, the negative regulator of the cell cycle, contributes to the decomposition of the cyclin B and Cdk1 complex. Cyclin B dissociates and changes its location from nuclear to cytoplasmic, which reduces its availability for cyclin-dependent kinase. This hinders the further stages of the division [64]. The next protein controlling the G2/M checkpoint is 14-3-3σ. This protein binds to Cdk1 to prevent the formation of a complex with cyclin B. In addition, it contributes to the activation of Chk1 kinase, which by phosphorylation inactivates Cdc25 and inhibits the interaction between Cdk1 and cyclin B [65, 66].

Another mechanism of control of cell division by p53 is repression of topoisomerase II promoter. Topoisomerase II is an enzyme that regulates the correct chromatin condensation in the process of forming mitotic chromosomes. Decreasing its concentration during the G2 phase results in the cell cycle arrest [67].

Cell self-destruction process – the role of p53

Cell cycle arrest and programmed cell death are among the most noticeable effects of p53 activity in response to DNA damage, occurrence of stress factors or activation of oncogene. A neoplastic cell's entering into the apoptosis pathway is a desirable event [62].

There are two main pathways of apoptosis initiation: intracellular – mitochondrial and extracellular – associated with death receptors [35]. The p53 protein is actively involved in both. It is closely related to the activation of transcription of pro-apoptotic genes and direct interaction with anti-apoptotic proteins. Another mechanism targeted at initiation apoptosis by the suppressor is stimulation of microRNA expression, which is aimed at silencing genes associated with the cell cycle and DNA repair [36, 68].

Participation of p53 in intracellular pathway of apoptosis

Intracellular cell death pathway is associated with the interaction of p53 with one of the two protein groups that belong to the BCL-2 family (B-cell leukemia/lymphoma 2). The common feature of these proteins is the presence of one or more BH domains (Bcl-2 homology): BH1, BH2, BH3 and BH4. Proteins from the first group, including BAX and BAK, contain BH domains

from 1 to 3 and form channels in the mitochondrial membrane. This allows pro-apoptotic factors to enter the cytoplasm. In an inactivated cell, these proteins are associated with anti-apoptotic proteins such as: BCL-2, BCL-XL, MCL-1. Proteins from the second group have only one domain type – BH3. These are:

- PUMA (p53 upregulated modulator of apoptosis),
- NOXA (phorbol-12-myristate-13-acetate-induced protein 1),
- BID (BH3 interacting domain death agonist),
- BIM (Bcl-2 interacting mediator of cell death).

Under the influence of stress factors, they cause the release and activation of pro-apoptotic proteins. This results in mitochondrial outer membrane permeabilization (MOMP) [36, 68, 69].

Numerous studies indicate that p53 protein positively regulates the transcription of genes encoding proteins from the second group (which have only the BH3 domain). Moreover, it may also interact directly with the anti-apoptotic BCL-2, BCL-XL or MCL-1 proteins, inhibiting their action, which leads to the activation of pro-apoptotic BAX and BAK proteins [36, 68, 70]. As a result of permeabilization of the mitochondrial membrane, cytochrome c is released into cytoplasm and together with APAF1 protein (apoptotic protease activating factor 1) and caspase-9 (initiator caspase) forms apoptosome. This complex initiates activation of the remaining effector caspases and degradation of individual elements of the cell. There are also released proteins: SMAC/DIABLO (second mitochondria-derived activator of caspases/direct IAP binding protein with low pI) and OMI/HTRA2, that block inhibitors of effector caspases -3, -7 and -9 – IAPs (inhibitors of apoptosis) [36, 69, 71]. Studies on the role of p53 in cell death have shown that this protein activates apoptosis by regulating the expression of APAF1 and also inhibits transcription of caspase inhibitors (IAPs), which are responsible for blocking apoptosis [71, 72].

Marchenko and Moll proved that p53 can also perform a direct pro-apoptotic function independent of transcription [68]. This is closely related to the specific post-translational modifications of this protein. In stressful conditions, p53 molecules are translocated and placed in mitochondria, where the suppressor behaves as a pro-apoptotic factor with the BH3 domain and initiates MOMP.

The role of p53 in extracellular pathway of apoptosis

The extracellular apoptosis pathway is activated by signals from outside the cell. The stimuli are received by death receptors (DR), located on the cell surface, which belong to the TNFR (tumor necrosis factor receptor) family. They consist of the extracellular domain, the transmembrane part and death domain (DD) extending into cytoplasm, which directly interacts with the complex of adaptor proteins. The stimulation of the death receptor occurs by binding a specific ligand, which leads to conformational changes in the internal domain and formation of the DISC complex (death-inducing-signaling-complex). This complex is initiated by combining the death domain with adaptor protein FADD (Fas associated death domain protein) and initiator procaspase 8 or 10. Autoproteolysis of caspase 8 leads to direct activation of effector caspase 3. This, in turn, entails irreversible changes – the beginning of cutting the cytoskeleton of the cell and, as a result, its death [36].

In response to gamma radiation and DNA damage, the p53 protein regulates the transcription of genes that encode death receptors and their ligands, CD95/FAS/Apo-1 and DR5/KILLER respectively [70, 71, 73]. Numerous studies also indicate the important role of p53 in the induction of apoptosis via TNFR1 (tumor necrosis factor receptor 1) [71, 74]. In addition, p53 sensitizes the cell to signals from the external environment and may be directly responsible for activating caspase 8, which initiates the cascade of effector caspases [72].

The p53 protein as a superior regulator of apoptotic proteins

In addition to the the basic pathways of cell death, the p53 protein directly regulates the expression of other genes associated with apoptosis. These include PERP (p53 apoptosis effector related to PMP-22), whose product is a response to DNA damage as well as the hyperproliferation of cells induced by E2F1 and its overexpression initiates apoptosis [75, 76]. Another target for p53 is AEN (apoptosis-enhancing nuclease) – a gene activated by genotoxic stress, encoding nuclease, which during apoptosis digests double-stranded DNA [77].

The p53 protein also influences the transcription of the following genes:

- *PIDD* (p53-induced protein with a death domain) [78],
- *p53DINP1* (p53-dependent damage-inducible nuclear protein 1),
- *p53AIP1* (tumor protein p53 regulated apoptosis inducing protein 1) [79].

PIDD expression occurs in response to genetic damage. The *PIDD* gene product forms, together with RAIDD (RIP-associated protein with a death domain), a p53-dependent complex called PIDDosome, which cooperates with caspase 2. Caspase 2 activates the BID protein, which initiates the mitochondrial pathway of apoptosis [36, 75, 80].

Expression of the *p53DINP1* gene is initiated when serious damage is detected in DNA. Its product regulates the phosphorylation of Ser46 in p53. This is necessary for the transaction of the pro-apoptotic *p53AIP1* gene, which is involved in the mitochondrial pathway of apoptosis dependent on p53 [4, 79]. The *p53AIP1* product interacts directly with BCL-2, inhibiting its activity and increasing the permeability of the mitochondrial membrane. This results in releasing cytochrome c and initiates the cell death [81].

The *TP53* gene mutations

The loss of proper function of p53 protein causes disturbance of proliferation control mechanisms and plays a key role in the development of a large group of neoplasms. *TP53* is the gene

whose mutations in human cancer cells are described most frequently and concern more than half of all cases of malignant tumors [23]. They are detected, among other things, in:

- 43% of cases of colorectal cancer,
- 41% of cases of HNC (head and neck cancers),
- 41% of all esophageal cancers,
- nearly 39% of epithelial ovarian carcinomas.

(Database of the International Agency for Research on Cancer (IARC), R19 edition) [82].

Loss (deletion) of one of the *TP53* gene copy in cells is a common disorder in various types of leukemia. For example, in chronic CLL (chronic lymphocytic leukemia), both *TP53* deletion and its somatic point mutations cause genetic instability of cancer cells, which results in worse prognosis. *TP53* deletion occurs in about 10% of patients with CLL at the time of diagnosis, but in patients during relapse after ineffective treatment with fludarabine it is as much as 40%. Deletion is associated with the loss of one gene allele and it is an important factor that determines the classification of hematological tumors. It is associated with a worse response to treatment, shorter disease free survival and shorter overall survival [83]. Mutations of *TP53* cause various types of disturbances in the activity of p53 protein itself, as well as a number of dysfunctions in interactions of p53 with other proteins, which significantly affects the probability or progression of cancer [84].

The prognostic significance of mutations in the *TP53* gene in solid tumors is not fully clear. Most publications on cancers such as ovarian, prostate, breast, bladder, lung and gastrointestinal cancers suggest that the presence of p53 disorders is associated with worse prognosis. On the other hand, however, other analyses of the same cancers do not indicate such a connection [85].

Somatic mutations of *TP53* are mostly missense type changes, resulting in the formation of full length protein with a single amino acid change. In most cases, the physiological effect of this mutation is a significantly prolonged half-life of p53, manifested by protein accumulation in the cell. Mutations can contribute to the acquisition of new functions by incorrect protein, which was not present in the wild form of the p53 – GOF (gain-of-function) [8, 86–88]. The second type of changes are mutations leading to the formation of a premature termination codon. These include mutations of the nonsense type, i.e. substitutions leading to the formation of STOP codon and frameshift mutations (deletions and insertions), which cause the shift of the reading frame. Nonsense type mutations represent about 10% of all changes [82].

Mutations in the *TP53* gene are detected in the whole length of the coding sequence, but their frequency in particular regions varies. The most disturbances are observed in the sequences that encode the DNA binding domain – exons 5–8. A lower percentage of mutations is recorded in exons 2–4 and 9–11, which encode transactivation, proline-rich and oligomerization domains [89, 90]. The majority of mutations within the DNA binding domain are missense changes, while outside of this region the proportion changes in favor of alterations causing the shortening of the reading frame [91]. The codons in which the highest percentage of substitution is detected are: Arg175, Gly245, Arg248, Arg249, Arg273 and Arg282 [30, 86, 92, 93].

Mutations in the *TP53* gene can be divided into two groups depending on the type of changes they cause in the protein. The first one involves modifications in the region involved in the direct interaction with DNA (class I) and the second one refers to the conformational changes of the molecule (class II). The first class includes the substitutions transforming Arg248 and Arg273 codons (L3 loop and loop-sheet-helix motif) which are involved in the interaction with DNA [12, 30, 93]. Such mutations do not lead to defects within the protein spatial structure. Mutated molecules are able to adopt correct conformation, but the contact with the DNA sequence recognized by p53 protein is impaired.

The second class includes mutations within the Arg175, Gly245, Arg249 and Arg282 codons. The changes concern amino acids responsible for stabilization of native DNA binding domain structure (L2 loop in the region of zinc atom) and directly affect the tertiary structure of p53. Adoption of incorrect conformation by the protein causes partial or total unwind of DBD. This affects the stability of the molecule and interferes with the interaction with the target proteins [12, 30, 82, 91, 93–95]. Mutations that lead to a change in conformation seem to be of greater importance in the process of carcinogenesis than modifications within the part in the direct contact with DNA [96].

The analysis of the mutation spectrum of *TP53* gene showed that the most frequent substitution is G:C transition in A:T, with the highest percentage of such changes occurring in CpG dinucleotides. This is related to the susceptibility of cytosine to methylation by DNA methyltransferase and its spontaneous deamination leading to the formation of thymine [82, 91]. It is also important that many of the functionally important residues of the DNA-binding domain are arginines. They are coded by three nucleotides particularly susceptible to damage due to the presence of GC dinucleotides, which are repaired with lower fidelity [12].

Family of cancer transformation suppressors

For a long time, the p53 was believed to be a one-of-a-kind suppressor. However, in the late 1990s, two similar proteins were discovered. This allowed for the separation of a new family of proteins, which is one of the oldest evolutionary families of proteins, to which p53, p63 and p73 belong. Studies to identify differences and similarities between the different members of the group, particularly concerning regulation and the possibility of interaction, are still ongoing [97].

It was shown that p53 family members were characterized by a significant similarity in the structure of the protein chain.

Within the DNA-binding domain, the homology between p53 and p73 is about 63%, and between p53 and p63 – about 60% [97]. This allows the p63 and p73 to interact directly with gene promoters regulated by p53, such as: *CDKN1A (p21*), *PUMA*, *NOXA*, *BAX* and *MDM2* [98]. In terms of the structure, also the other domains of p53 family proteins – the proline-rich domain, C-terminal oligomerization domain and the transactivation domain – are characterized by significant similarity between the group members. However, in some of p63 and p73 isoforms is described an additional, not observed in p53, domain that inhibits transactivation - TID (transactivation inhibitory domain) [99, 100]. It is believed that it reacts with the TAD domain of the second molecule, thus generating a closed and inactive dimeric conformation that inhibits the transactivation properties of isoforms [101]. The second additional domain, located at the C-terminus, is the sterile alpha motif (SAM) domain, which is responsible for the protein-protein interaction. It is essential for the tetramerization process – the formation and stabilization of an active molecule. At the level of amino acid sequences, p63 and p73 proteins show higher homology to each other than to p53 [99, 100]. Unlike the *TP53*, genes encoding p63 and p73 are rarely mutated in neoplastic cells [97].

Despite sharing some activities with p53, homologous proteins are characterized by a high level of functional specificity. Studies have shown that p73 protein plays an important role in the development and differentiation of nervous system cells and regulates non-specific (congenital) immune response. The p63 protein, on the other hand, is significant for embryonic development and functioning of the epidermis and other squamous epithelia. It is responsible for regulating the development of the cells of the nipple, prostate, cervix and internal reproductive system both during human embryonic development and in adult life. As a protein, which belongs to the family of anti-oncogenic transcription factors, it also plays an important role in the neoplastic process. It was shown that p63 is involved in inhibition of neoplastic metastases [97–101].

Defective p53 protein as a therapeutic target

The prevalence of mutations in the *TP53* gene in human tumors makes the disturbances in p53 an important therapeutic goal. The main directions of research are focused on the restoration of the defective protein to normal function, the degradation of abnormal p53 or the affect on the pathways associated with the suppressor [102].

Research has shown that the restoration of p53 function in tumors with mutation inhibits the development of cancer. However, the development of a drug that would restore the proper function of the suppressor seems to be a more difficult challenge than the development of therapies blocking overactive protein, as is the case with the commonly used tyrosine kinase inhibitors. So far, a number of small molecule reactivators have been developed, which when combined with the mutated p53 protein, are able to restore its function completely or partially [102]. Most of them are still in the pre-clinical testing phase, but some of them are already in the patient testing phase. One of them is the APR-246 molecule, which causes the defective p53 protein to assume normal conformation, activate transcription of target genes and inhibit tumor growth [102, 103]. The drug is currently being studied in clinical trials including leukemia, lymphoma, melanoma, ovarian and esophageal cancer. Another molecule tested in the first phase of clinical trials – in gynecological cancer, head and neck cancer – is COTI-2, which functions as a chelator for zinc ions [104]. This drug, similarly to APR-246, combines with incorrectly coiled p53 and changes its conformation as well as restores its proper function. Thanks to this, apoptosis is activated in cancer cells [102–104].

Gene therapies are also used to reactivate normal p53 by providing a wild type copy of the *TP53* gene to tumor cells. Oncolytic viruses, which have a selective ability to replicate in cancer cells, are used for this purpose [105]. The first commercial drug for gene therapy based on *TP53* is Gendicine, which has so far been approved for use only in China. This drug is a genetically modified human adenovirus, which served as a vector delivering the wild type sequence of the *TP53* gene to neoplastic cells [106].

Another therapeutic strategy is to eliminate the abnormal p53 protein, which accumulates in the cell and by aqusiting new functions contributes to the growth of neoplasm. This can be achieved by activating its proteasomal degradation. For this purpose, among other things, chaperone inhibitors are used. Chaperons, when combined with mutated p53, stabilize it and protect it against proteolysis [102, 103]. One example is Ganetespib, small molecule Hsp90 (heat shock protein 90) inhibitor, which as shown in mouse model studies, contributes to increased degradation of the defective p53 [103].

Some therapies are not targeted directly at the damaged p53, but at the associated pathways active in cancer cells. This strategy is used, among others, in the case of atorvastatin and AZD1775, the studies of which in oncological patients have already entered the clinical phase [102, 107]. Atorvastatin is a commonly used statin drug that blocks the production of cholesterol and is used in cardiovascular diseases. Its use in tumor cells with *TP53* mutation results in a decrease in the amount of abnormal protein and a change in the morphology of tumor cells, which results in the inhibition of tumor growth. In turn, AZD1775 is an inhibitor of WEE1 kinase, which initiates cycle stopping at G2/M checkpoint. In cells with *TP53* mutation, inactivation of the G2/M checkpoint leads to cell elimination due to a mitotic catastrophe and increases cancer sensitivity to drugs damaging DNA and radiotherapy [102, 107].

Summary

The presented literature review summarizes data which indicates that p53 protein is one of the most important tumor transformation suppressors. As a result of a wide spectrum of stress factors, such as DNA damage, hypoxia or stimulation

of oncogenes, p53 is activated and through the regulation of the most important processes in the cell decides its fate. Therefore, its dysfunction is a key element in the process of transforming a normal cell into a cancer cell. The prevalence of *TP53* gene mutations in many types of cancer makes it an attractive target for potential therapies that focus mainly on eliminating defective protein or restoring its normal function.

Conflict of interest: none declared

Magdalena Kulesza
Maria Sklodowska-Curie Institute – Oncology Center
Independent Laboratory of Cytogenetics
ul. Wawelska 15B
02-034 Warszawa, Poland
e-mail: mydzia@gmail.com

Received: 27 Mar 2019
Accepted: 22 Oct 2019

References

1. Latest global cancer data, International Agency for Research on Cancer, World Health Organization. 2018.
2. Ferlay J, Soerjomataram I, Dikshit R et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International Journal of Cancer.* 2014; 136: E359–E386.
3. Soussi T. The history of p53. *EMBO Rep.* 2010; 11: 822–826.
4. Sznarkowska A, Olszewski R, Zawacka-Pankau J. Farmakologiczna aktywacja supresora nowotworu, natywnego białka p53 jako obiecująca strategia zwalczania nowotworów. *Postepy Hig Med Dosw.* 2010; 64: 396–407.
5. Brosh R, Rotter V. When mutants gain new powers: news from the mutant p53 field. *Nat Rev Cancer.* 2009; 9: 701–713.
6. Meek DW. Regulation of the p53 response and its relationship to cancer. *Biochem J.* 2015; 469: 325–346.
7. Szybińska A, Leśniak W. Dysfunction in neurodegenerative diseases – the cause or effect of pathological changes? *Aging Dis.* 2017; 8: 506–518.
8. Bieging KT, Mello SS, Attardi LD. Unravelling mechanisms of p53-mediated tumour suppression. *Nat Rev Cancer.* 2014; 14: 359–370.
9. Lee CW, Martinez-Yamout MA, Dyson HJ et al. Structure of the p53 transactivation domain in complex with the nuclear coactivator binding domain of CBP. *Biochemistry.* 2010; 49: 9964–9971.
10. Kamihara J, RanaHQ, Garber JE. Germline TP53 mutations and the changing landscape of Li-Fraumeni syndrome. *Hum Mutat.* 2014; 35: 654–662.
11. Sorrell AD, Espenschied CR, Culver JO et al. TP53 testing and li-fraumeni syndrome: current status of clinical applications and future directions. *Mol Diagn Ther.* 2013; 17: 31–47.
12. Stavridi ES, Huyen Y, Sheston EA et al. The three-dimensional structure of p53. zambetti gp. the p53 tumor suppressor pathway and cancer. Springer: Boston; 2005; 25–52.
13. Kim S, An SS. Role of p53 isoforms and aggregations in cancer. *Medicine.* 2016; 95: e3993.
14. Paskulin Dd, Paixão-Côrtes VR, Hainaut P et al. The TP53 fertility network. *Genet Mol Biol.* 2012; 35: 939–946.
15. Reisman D, Polson-Zeigler A. The bi-directional nature of the promoter of the p53 tumor suppressor gene. *J Leuk.* 2015; 3: 187.
16. Reisman D, Takahashi P, Polson A et al. Transcriptional regulation of the p53 tumor suppressor gene in s-phase of the cell-cycle and the cellular response to DNA damage. *Biochem Res Int.* 2012; 2012: ID 808934.
17. Brady CA, Jiang D, Mello SS et al. Distinct p53 transcriptional programs dictate acute DNA-damage responses and tumor suppression. *Cell.* 2011; 145: 571–583.
18. Jenkins LM, Durell SR, Mazur SJ et al. p53 N-terminal phosphorylation: a defining layer of complex regulation. *Carcinogenesis.* 2012; 33 : 1441–1449.
19. Natan E, Baloglu C, Pagel K et al. Interaction of the p53 DNA-binding domain with its N-terminal extension modulates the stability of the p53 tetramer. *J Mol Biol.* 2011; 409: 358–368.
20. Sullivan KD, Galbraith MD, Andrysik Z et al. Mechanisms of transcriptional regulation by p53. *Cell Death Differ.* 2018; 25: 133–143.
21. Krois AS, Dyson HJ, Wright PE. Long-range regulation of p53 DNA binding by its intrinsically disordered N-terminal transactivation domain. *Proc Natl Acad Sci USA.* 2018; 115: E11302–E11310.
22. Varna M, Bousquet G, Plassa LF et al. TP53 status and response to treatment in breast cancers. *J Biomed Biotechnol.* 2011; 2011: ID 284584.
23. Kumari R, Sen N, Das S. Tumour suppressor p53: understanding the molecular mechanisms inherent to cancer. *Current Science.* 2014; 107: 786–794.
24. Campbell HG, Mehta R, Neumann AA et al. Activation of p53 following ionizing radiation, but not other stressors, is dependent on the proline-rich domain (PRD). *Oncogene.* 2013; 32: 827–836.
25. Garcia PB, Attardi LD. Illuminating p53 function in cancer with genetically engineered mouse models. *Semin Cell Dev Biol.* 2014; 27: 74–85.
26. Lukman S, Lane DP, Verma CS. Mapping the structural and dynamical features of multiple p53 DNA binding domains: insights into loop 1 intrinsic dynamics. *PLoS One.* 2013; 8: e80221.
27. Laptenko O, Shiff I, Freed-Pastor W et al. The p53 C terminus controls site-specific DNA binding and promotes structural changes within the central DNA binding domain. *Mol Cell.* 2015; 57: 1034–1046.
28. Kearns S, Lurz R, Orlova EV et al. Two p53 tetramers bind one consensus DNA response element. *Nucleic Acids Res.* 2016; 44: 6185–6199.
29. Joerger AC, Fersht AR. Structural biology of the tumor suppressor p53. *Annu Rev Biochem.* 2008; 77: 557–782.
30. Cho Y, Gorina S, Jeffrey PD et al. Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. *Science.* 1994; 265: 346–355.
31. Fischer NW, Prodeus A, Malkin D et al. p53 oligomerization status modulates cell fate decisions between growth, arrest and apoptosis. *Cell Cycle.* 2016; 15:3210–3219.
32. Itahana Y, Ke H, Zhang Y. p53 Oligomerization is essential for its C-terminal lysine acetylation. *J Biol Chem.* 2009; 284: 5158–5164.
33. Chillemi G, Kehrloesser S, Bernassola F. et al. Structural evolution and dynamics of the p53 proteins. *Cold Spring Harb Perspect Med.* 2017; 7: a028308.
34. Iida S, Mashimo T, Kurosawa T et al. Variation of free-energy landscape of the p53 C-terminal domain induced by acetylation: Enhanced conformational sampling. *J Comput Chem.* 2016; 37: 2687–2700.
35. Haupt S, Vijayakumaran R, Miranda PJ et al. The role of MDM2 and MDM4 in breast cancer development and prevention. *J Mol Cell Biol.* 2017; 9: 53–61.
36. Korwek Z, Alster O. Rola szlaku indukowanego uszkodzeniami DNA w apoptozie i starzeniu komórkowym. *Postępy Biochemii.* 2014; 60: 248–262.
37. Enchev RI, Schulman BA, Peter M. Protein neddylation: beyond cullin–RING ligases. *Nat Rev Mol Cell Biol.* 2015; 16: 30–44.
38. Heering J, Jonker HR, Löhr F. Structural investigations of the p53/p73 homologs from the tunicate species Ciona intestinalis reveal the sequence requirements for the formation of a tetramerization domain. *Protein Sci.* 2016; 25: 410–422.
39. Lui K, Sheikh MS, Huang Y. Regulation of p53 oligomerization by Ras superfamily protein RBEL1A. *Genes Cancer.* 2015; 6: 307–316.
40. Marmorstein LY, Ouchi T, Aaronson SA. The BRCA2 gene product functionally interacts with p53 and RAD51. *Proc Natl Acad Sci USA.* 1998; 95: 13869–13874.
41. Fischer M, Steiner L, Engeland K. The transcription factor p53: not a repressor, solely an activator. *Cell Cycle.* 2014; 13: 3037–3058.
42. Beckerman R, Prives C. Transcriptional regulation by p53. *Cold Spring Harb Perspect Biol.* 2010; 2: a000935.
43. Engeland K. Cell cycle arrest through indirect transcriptional repression by p53: I have a DREAM. *Cell Death Differ.* 2018; 25: 114–132.
44. Baldassarre A, Masotti A. Long Non-Coding RNAs and p53 Regulation. *Int J Mol Sci.* 2012; 13: 16708–16717.
45. Franklin DA, He Y, Leslie PL et al. p53 coordinates DNA repair with nucleotide synthesis by suppressing PFKFB3 expression and promoting the pentose phosphate pathway. *Sci Rep.* 2016; 6: 38067.
46. Sun B, Ross SM, Rowley S et al. Contribution of ATM and ATR kinase pathways to p53-mediated response in etoposide and methyl methanesulfonate induced DNA damage. *Environ Mol Mutagen.* 2017; 58: 72–83.
47. Awasthi P, Foiani M, Kumar A. ATM and ATR signaling at a glance. *J Cell Sci.* 2015; 128: 4255–4262.
48. Baran K, Yang M, Dillon CP et al. The proline rich domain of p53 is dispensable for MGMT-dependent DNA repair and cell survival following alkylation damage. *Cell Death Differ.* 2017; 24: 1925–1936.

49. Shamsara J, Sharif S, Afsharnezhad S et al. Association Between MGMT Promoter Hypermethylation and p53 Mutation in Glioblastoma. *Cancer Invest.* 2009; 27: 825–829.
50. Blough MD, Zlatescu MC, Cairncross JG. O6-methylguanine-DNA methyltransferase regulation by p53 in astrocytic cells. *Cancer Res.* 2007; 67: 580–584.
51. Adimoolam S, Ford JM. p53 and regulation of DNA damage recognition during nucleotide excision repair. *DNA Repair (Amst).* 2003; 2: 947–954.
52. Wang QE, Han C, Zhao R et al. p38 MAPK- and Akt-mediated p300 phosphorylation regulates its degradation to facilitate nucleotide excision repair. *Nucleic Acids Res.* 2013; 41: 1722–1733.
53. Rubbi CP, Milner J. p53 is a chromatin accessibility factor for nucleotide excision repair of DNA damage. *EMBO J.* 2003; 22: 975-986.
54. Vélez-Cruz R, Johnson DG. E2F1 and p53 Transcription Factors as Accessory Factors for Nucleotide Excision Repair. *Int J Mol Sci.* 2012; 13: 13554–13568.
55. Adimoolam S, Ford JM. p53 and DNA damage-inducible expression of the xeroderma pigmentosum group C gene. *Proc Natl Acad Sci. USA* 2002; 99: 12985–12990.
56. Luijsterburg MS, Lindh M, Acs K et al. DDB2 promotes chromatin decondensation at UV-induced DNA damage. *J Cell Biol.* 2012; 197: 267–281.
57. Tamura RE, de Vasconcellos JF, Sarkar D et al. GADD45 proteins: central players in tumorigenesis. *Curr Mol Med.* 2012; 12: 634–651.
58. Hyun SY, Jang YJ. p53 activates G1 checkpoint following DNA damage by doxorubicin during transient mitotic arrest. *Oncotarget.* 2015; 6: 4804–4815.
59. Waldman T, Kinzler KW. Vogelstein B. p21 is necessary for the p53-mediated G1 arrest in human cancer cells. *Cancer Res.* 1995; 55: 5187–5190.
60. Coqueret O. New roles for p21 and p27 cell-cycle inhibitors: a function for each cell compartment? *Trends Cell Biol.* 2003; 13: 65–70.
61. Abukhdeir AM, Park BH. p21 and p27: roles in carcinogenesis and drug resistance. E*xpert Rev Mol Med.* 2008; 10: e19.
62. Chen J. The Cell-Cycle Arrest and Apoptotic Functions of p53 in Tumor Initiation and Progression. *Cold Spring Harb Perspect Med.* 2016; 6: a026104.
63. Abbas T, Dutta A. p21 in cancer: intricate networks and multiple activities. *Nat Rev Cancer.* 2009; 9: 400–414.
64. Gao H, Jin S, Song Y et al. B23 regulates GADD45a nuclear translocation and contributes to GADD45a-induced cell cycle G2-M arrest. *J Biol Chem.* 2005; 280: 10988–10996.
65. Dobrzańska-Kaczanowska J, Piwońska D, Kaczanowski A. Rola polo-kinaz(y) w regulacji cyklu komórkowego – mechanizm translokacji i tworzenia kompleksów białkowych przez polokinazy. *Postępy Biochemii.* 2006; 52: 24-34.
66. Li Z, Liu JY, Zhang JT. 14-3-3σ, the double-edged sword of human cancers. *Am J Transl Res.* 2009; 1: 326–340.
67. Taylor WR, Stark GR. Regulation of the G2/M transition by p53. *Oncogene.* 2001; 20: 1803–1815.
68. Marchenko ND, Moll UM. Mitochondrial death functions of p53. *Mol Cell Oncol.* 2014; 1: e955995.
69. Lopez J, Tait SW. Mitochondrial apoptosis: killing cancer using the enemy within. *Br J Cancer.* 2015; 112: 957–962.
70. Amaral JD, Xavier JM, Steer CJ et al. The role of p53 in apoptosis. *Discov Med.* 2010; 9: 145–152.
71. Sharp AN, Heazell AE, Crocker IP et al. Placental apoptosis in health and disease. *Am J Reprod Immunol.* 2010; 64: 159–169.
72. Maximov GK, Maximov KG. The role of p53 tumor-suppressor protein in apoptosis and cancerogenesis. *Biotechnol & Biotechnol Eq.* 2008; 22: 664–668.
73. Pfeffer CM, Singh ATK. Apoptosis: a target for anticancer therapy. *Int J Mol Sci.* 2018; 19: E448.
74. Goretsky T, Dirisina R, Sinh P et al. p53 Mediates TNF-induced epithelial cell apoptosis in IBD. *Am J Pathol.* 2012; 181: 1306–1315.
75. Attardi LD, Reczek EE, Cosmas C et al. PERP, an apoptosis-associated target of p53, is a novel member of the PMP-22/gas3 family. *Genes Dev.* 2000; 14: 704–718.
76. Ihrie RA, Reczek E, Horner JS et al. Perp is a mediator of p53-dependent apoptosis in diverse cell types. *Curr Biol.* 2003; 13: 1985–1990.
77. Fischer M. Census and evaluation of p53 target genes. *Oncogene.* 2017; 36: 3943–3956.
78. Lin Y, Ma W, Benchimol S. Pidd, a new death-domain-containing protein, is induced by p53 and promotes apoptosis. *Nat Genet.* 2000; 26:122–127.
79. Okamura S, Arakawa H, Tanaka T et al. p53DINP1, a p53-inducible gene, regulates p53-dependent apoptosis. *Mol Cell.* 2001; 8: 85–94.
80. Baptiste-Okoh N, Barsotti AM, Prives C. A role for caspase 2 and PIDD in the process of p53-mediated apoptosis. *Proc Natl Acad Sci USA.* 2008; 105: 1937–1942.
81. Ozaki T, Nakagawara A. p53: the attractive tumor suppressor in the cancer research field. *J Biomed Biotechnol.* 2011; 2011: 603925.
82. http://p53.iarc.fr/TP53SomaticMutations.aspx
83. Yu L, Kim HT, Kasar S et al. Survival of Del17p CLL depends on genomic complexity and somatic mutation. *Clin Cancer Res.* 2017; 23: 735–745.
84. Stracquadanio G, Wang X, Wallace MD et al. The importance of p53 pathway genetics in inherited and somatic cancer genomes. *Nat Rev Cancer.* 2016; 16: 251–265.
85. Robles AI, Harris CC. Clinical outcomes and correlates of TP53 mutations and cancer. *Cold Spring Harb Perspect Biol.* 2010; 2: a001016.
86. Giorgi C, Bonora M, Missiroli S. Alterations in Mitochondrial and endoplasmic reticulum signaling by p53 mutants. *Front Oncol.* 2016; 6: 42.
87. Muller PA, Vousden KH. p53 mutations in cancer. *Nat Cell Biol.* 2013; 15: 2–8.
88. Kamada R, Toguchi Y, Nomura T. Tetramer formation of tumor suppressor protein p53: structure, function, and applications. *Biopolymers.* 2016; 106: 598–612.
89. http://p53.free.fr/p53_info/p53_Protein.html
90. Leroy B, Anderson M, Soussi T. TP53 mutations in human cancer: database reassessment and prospects for the next decade. *Hum Mutat.* 2014; 35: 672–688.
91. Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: origins, consequences, and clinical use. *Cold Spring Harb Perspect Biol.* 2010; 2: a001008.
92. Kremenetskaya OS, Logacheva NP, Baryshnikov AY et al. Distinct effects of various p53 mutants on differentiation and viability of human K562 leukemia cells. *Oncol Res.* 1997; 9: 155–166.
93. Eldar A, Rozenberg H, Diskin-Posner Y et al. Structural studies of p53 inactivation by DNA-contact mutations and its rescue by suppressor mutations via alternative protein-DNA interactions. *Nucleic Acids Res.* 2013; 41: 8748–8759.
94. Makarov EM, Shtam TA, Kovalev RA. The rare nonsense mutation in p53 triggers alternative splicing to produce a protein capable of inducing apoptosis. *PLoS One.* 2017; 12: e0185126.
95. Shajani-Yi Z, de Abreu FB, Peterson JD et al. Frequency of somatic TP53 mutations in combination with known pathogenic mutations in colon adenocarcinoma, non-small cell lung carcinoma, and gliomas as identified by next-generation sequencing. *Neoplasia.* 2018; 20: 256–262.
96. Sigal A, Rotter V. Oncogenic mutations of the p53 tumor suppressor: the demons of the guardian of the genome. *Cancer Res.* 2000; 60: 6788–6793.
97. Wysocka-Dubielecka KM, Majewski S, Łoza K. Rola białek p63 w kancerogenezie oraz znaczenie ich ekspresji w diagnostyce nowotworów skóry i żeńskiego układu rozrodczego. *Przegl Dermatol.* 2015; 102: 550–557.
98. Costanzo A, Pediconi N, Narcisi A et al. TP63 and TP73 in cancer, an unresolved "family" puzzle of complexity, redundancy and hierarchy. *FEBS Lett.* 2014; 588: 2590–2599.
99. Kunst C, Haderer M, Heckel S et al. The p53 family in hepatocellular carcinoma. *Transl Cancer Res.* 2016; 5: 632–638.
100. Manzella L, Stella S, Pennisi MS et al. New insights in thyroid cancer and p53 family proteins. *Int J Mol Sci.* 2017; 18: E1325.
101. Candi E, Agostini M, Melino G et al. How the TP53 family proteins TP63 and TP73 contribute to tumorigenesis: regulators and effectors. *Hum Mutat.* 2014; 6: 702–714.
102. Muller PA, Vousden KH. Mutant p53 in cancer: new functions and therapeutic opportunities. *Cancer Cell.* 2014; 25: 304–317.
103. Parrales A, Iwakuma T. Targeting Oncogenic mutant p53 for cancer therapy. *Front Oncol.* 2015; 5: 288.
104. Bykov VJN, Eriksson SE, Bianchi J et al. Targeting mutant p53 for efficient cancer therapy. *Nat Rev Cancer.* 2018;18: 89–102.
105. Bressy C, Hastie E, Grdzelishvili VZ. Combining oncolytic virotherapy with p53 tumor suppressor gene therapy. *Mol Ther Oncolytics.* 2017; 5: 20–40.
106. Zhang WW, Li L, Li D et al. The first approved gene therapy product for cancer Ad-p53 (Gendicine): 12 years in the clinic. *Hum Gene Ther.* 2018; 29: 160–179.
107. Blandino G, Di Agostino S. New therapeutic strategies to treat human cancers expressing mutant p53 proteins. *J Exp Clin Cancer Res.* 2018; 37: 30.