P53 – "THE GUARDIAN OF GENOME"

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Summary. p53 is a transcription-suppressing factor, which participates in control of cell cycle and apoptosis and in the regulation of cell genomic integrity. p53 is a tumor suppressor protein that in humans is encoded by the TP53 gene. p53 is crucial in multicellular organisms, where it regulates the cell cycle and, thus, functions as a tumor suppressor that is involved in preventing cancer. The name p53 is in reference to its apparent molecular mass: It runs as a 53-kilodalton (kDa) protein on SDS-PAGE. But, based on calculations from its amino acid residues, p53's mass is actually only 43.7 kDa. This difference is due to the high number of proline residues in the protein, which slow its migration on SDS-PAGE, thus making it appear heavier than it actually is. This effect is observed with p53 from a variety of species, including humans, rodents, frogs, and fish. Mutations of p53 are observed in 50% of all human carcinomas and in 90% of melanomas being the most frequently genome disturbance in carcinomas. Also, allele loss and mutation of the p53 gene are detected in more than 60% of gastric cancers regardless of histological type. Different mechanisms of activation, physiological functions and the role of p53 in cancerogenesis and in different neoplasms are viewed. Immunohistochemical recognition of p53 in normal cells and neoplastic altered cells gives information about genesis, prognosis and sensitivity to therapy of different types of cancers. Nevertheless, prospective studies need to address this issue definitely and this marker need further investigation and confirmation.

Key words: *p53, activation, biological function, immunohistochemical expression, malignant melanoma, gastric cancer*

Carcinomas form a very heterogeneous group of diseases. Although their occurrence and development are influenced by various factors, two of these factors – namely, the disturbed regulation of cellular proliferation and the inhibition of apoptosis – play a major role in the pathogenesis of all tumors. The disturbed regulation of cellular proliferation in tumors is due to gene mutations affecting normal cellular growth and division at various stages of cellular cycle. Typical examples are the changes in pRb and p53 regulating cellular cycle at different stages. On the other part, the survival of both normal somatic cells and tumor cells is related to the regulation of apoptosis. The suppressed apoptosis in tumor cells is a result of the combination of direct oncogene activity, genetic mutations of regulating proteins, and the interaction of cellular proliferation and apoptosis.

1.1. p53 – Introduction. The p53 protein is a transcription factor taking part in the maintenance of human genomic integrity through regulation of cellular growth within the cellular cycle as well as through regulation of cellular apoptosis. That is why it is frequently named “the guardian of genome”. Mutations in the p53 gene represent the most frequently occurring genetic damage observed in various types of human cancer. These are found in more than 50% of all cancers and in more than 90% of cutaneous malignant diseases. Besides, malignancies or melanomas with p53 mutations respond poorly to treatment and tend to have a poor prognosis [29].

1.2. Structure of p53. p53 is a protein with a short half-life of about 20 minutes that is found at a very low level in normal cells. Human p53 contains 393 amino acids arranged structurally and functionally in 4 domains: amino acidic (transcriptional domain); central nuclear domain (linking DNA); tetramerizing domain; and c-terminal regulatory domain. The amino transcriptional domain reacts with various proteins that positively regulate p53 transcription function in physiological conditions. The central nuclear domain plays an important role in the linkage of DNA sequences (fragments) as well as the various oncogenes and proteins that negatively affect p53 transcription activity. The mutations affecting p53 suppressor activity emerge in this domain. The tetramerizing domain maintains p53 as a tetramer in dissolved state at physiological conditions. The oligomerization of p53 is important for its suppressor activity. The c-terminal domain regulates the linkage of DNA fragments by the central nuclear domain. The structural changes in the c-terminal domain are required for p53 activation in view of linking DNA fragments to the nuclear domain of p53.

1.3. Function and activation of p53. p53 was identified in 1979 by Lionel Crawford, David P. Lane, Arnold Levine, and Lloyd Old, working at Imperial Cancer Research Fund (UK), Princeton University/UMDNJ (Cancer Institute of New Jersey), and Sloan-Kettering Memorial Hospital, respectively. It had been hypothesized to exist before as the target of the SV40 virus, a strain that induced development of tumors. The TP53 gene from the mouse was first cloned by Peter Chumakov of the Russian Academy of Sciences in 1982, and independently in 1983 by

Moshe Oren in collaboration with David Givol (Weizmann Institute of Science). The human TP53 gene was cloned in 1984, and the full length clone in 1985. The major function of p53 is to respond to DNA damage as well as to protect from accumulation of oncogene mutations, thus preventing the occurrence of a state of genomic instability. Several stress-related conditions lead to the emergence of signals for p53 activation. Other p53-activating factors are vital or cellular oncogenes, hypoxia and hypoglycemia [18]. Transcriptional activation of p53 is a major component of its biological effects [4]. Activated p53 is bound to specific DNA fragments and activates the process of transcription [8, 9]. The exact mechanism through which p53 is being activated in case of cellular stress is not completely clarified. Activation might include increased p53 level as well as appear as a post-translation phenomenon – due to DNA fragment linkage. The main question for p53 activation is how the cell interprets DNA damage and transfers a signal for p53 stabilization. One possibility is that cellular proteins recognize damaged DNA and bind to p53. Another possibility is that specific phosphorylation, dephosphorylation and acetylation lead to p53 activation [1, 29].

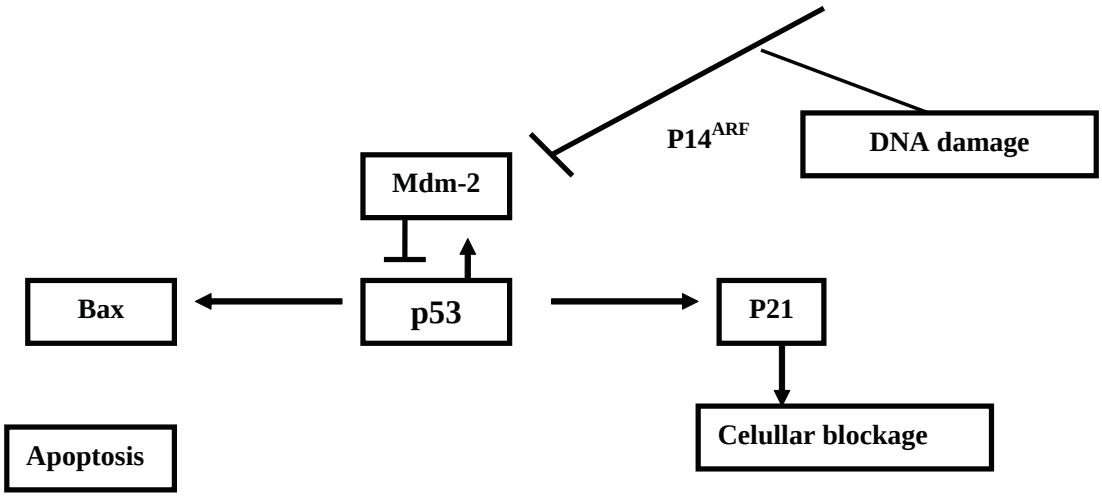


Fig. 1. Mdm-2 and ARF – their role in p53 regulation

The Mdm2 protein plays a major role in the control of p53 protein stability. Mdm2 is a target of p53 transcriptional activation. At the same time, Mdm2 interacts with and inhibits p53 transcriptional activity. This is done through rapid p53 disintegration, and this route is probably responsible for the low p53 level in normal cells. In cancer cells with p53 mutations, it is believed that the low Mdm2 level contributes to p53 stabilization [11]. The phosphorylation of p53 amino terminal occurring after DNA damage reduces Mdm2 binding affinity and leads to p53 stabilization. Various factors provoking alterations in cellular DNA specifically inhibit the transcription of Mdm2 protein and induce an indirect response through p53. The reduced expression of Mdm2 in these cases is not dependent on p53, and the exact mechanism is not known [41] (Fig. 2).

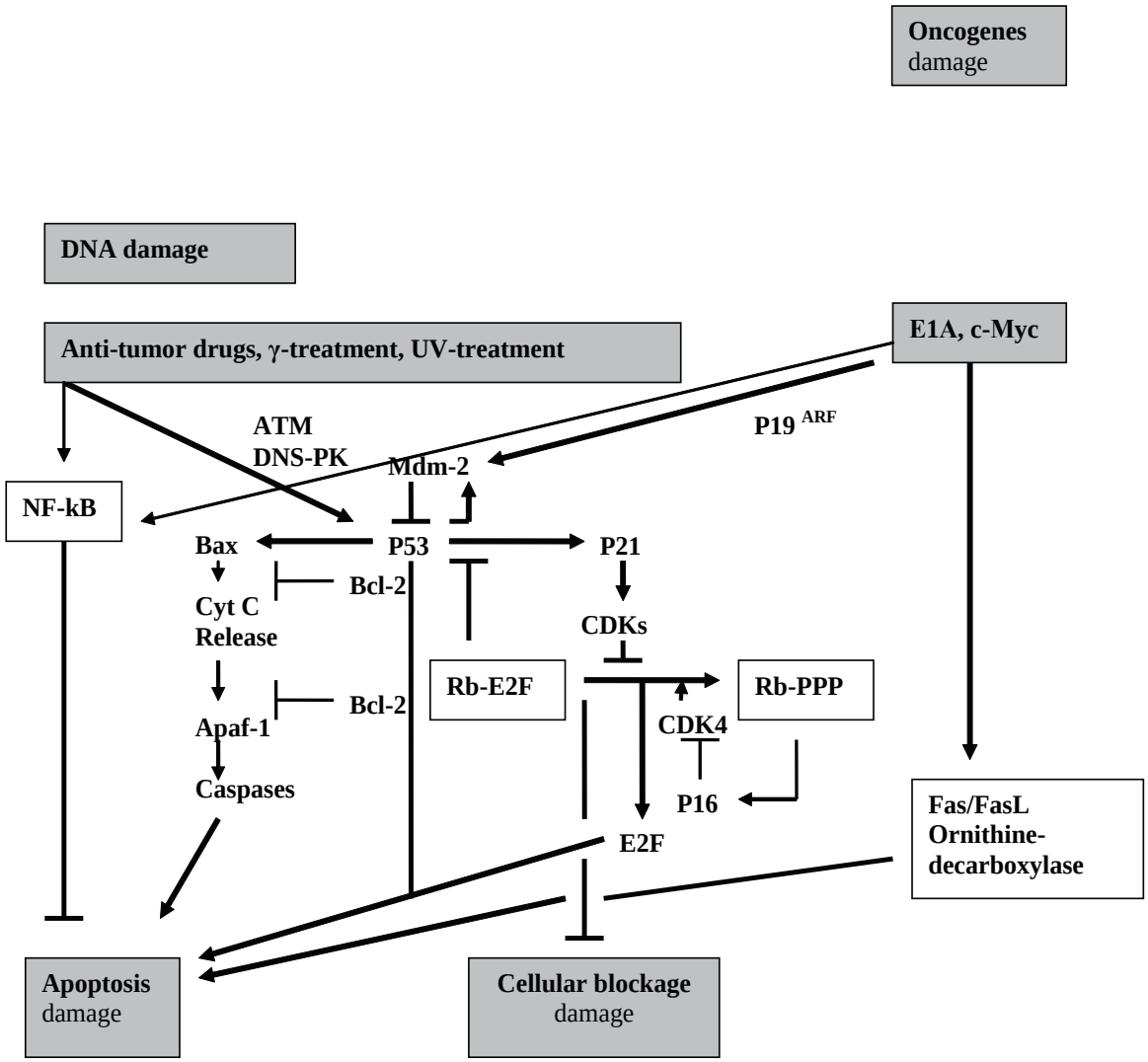


Fig. 2. Routes of regulating carcinogenesis

Meanwhile, it has been shown that several cellular oncogenes like E1A adenovirus, SV4OT antigens and Human papilloma virus 16E7 stabilize p53. Surprisingly, oncogenic viruses inducing DNA replication stabilize p53 that leads to growth arrest or apoptosis. One plausible explanation is that p53 stabilization influenced by viral oncogenes represents a defensive cellular response to viral infection. However, oncogenic viruses develop auxiliary mechanisms aimed at overcoming this defensive cellular response. E1A inhibits p53 transcriptional activation that leads to degradation of the protein and to inhibition of the negative growth signals from p53. The mechanism of p53 stabilization in response to the expression of viral oncogenes was clarified following the discovery of p19ARF / p14ARF in humans. ARF belongs to the INK4 proteins, p19ARF is a tumor suppressor-provoking blockage of the cellular cycle like p53. ARF directly binds to p53 and/or Mdm2, altering p53 level and activity. E2F1 activates 14ARF binding also the E1A oncogene to p14ARF and

p53. Several other cellular oncogenes like c-myc and ras also stabilize p53 through the route of ARF (Fig. 2). The damage (inactivation) of AFR in tumors complicates the link between oncoproteins (mic, ras, E2F) and the subsequent p53 activation which allows for cellular proliferation and survival in spite of the dysregulation of oncogenes. The mutations specifically damaging AFR are not so frequent in human carcinomas. However, the disturbances of a series of regulatory mechanisms of ARF activity frequently become the reason for its poor expression in a series of tumors. Typical examples are adenomas and carcinoma of the colon, where ARF is inactivated due to disturbed methylation of its promotor [7, 32].

1.4. Consequences of p53 activation. p53 activation leads to suppression of cellular growth. p53 realizes these effects through individual or combined effects on the cellular cycle (blockage of the cycle) or apoptosis. p53 also takes part in cellular differentiation, in the inhibition of angiogenesis and in the process of ageing of tumorous cells [27, 29]. Though it is not quite clear how p53 exerts its effects on the cells, now it is confirmed that p53 transcriptional activation is a major component of its biological effects [7].

1.4.1. Regulation of the cellular cycle. p53 activation provokes cellular blockage in G1 and G2/M which are the check-points of the cellular cycle (Fig. 3).

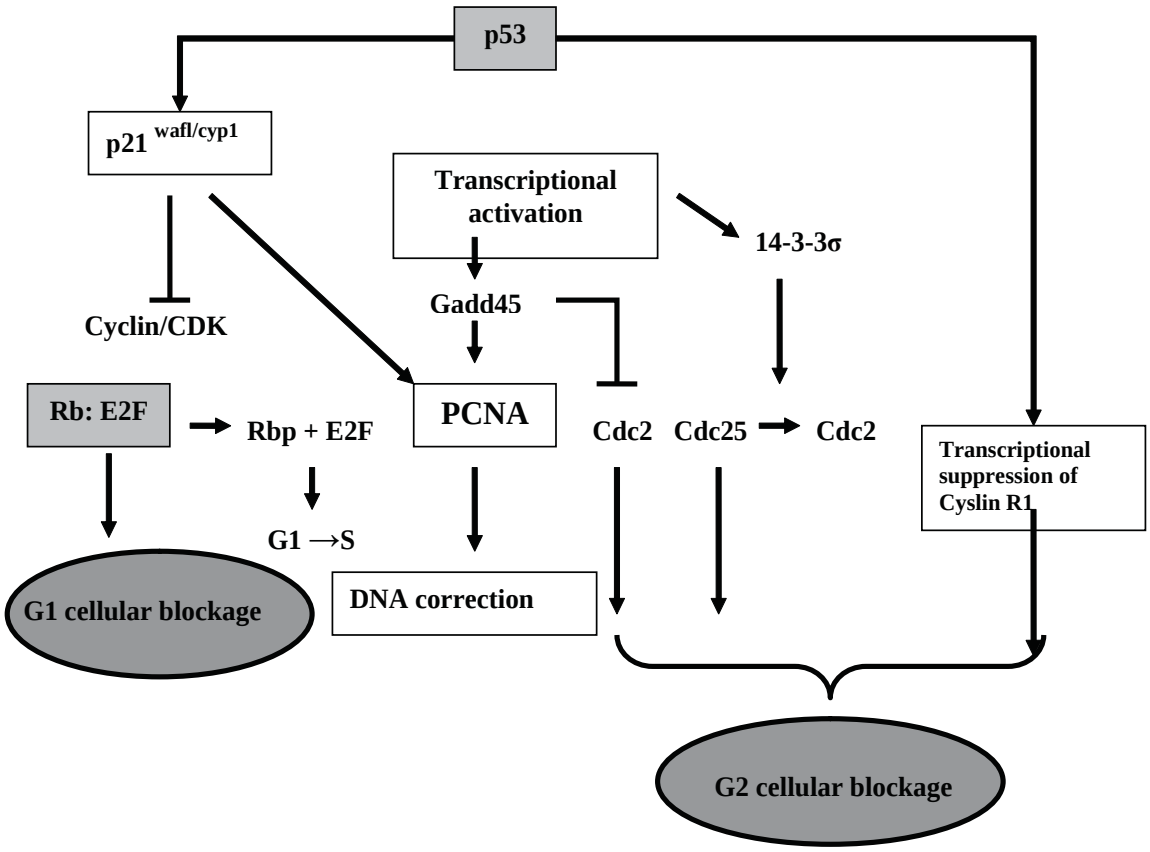


Fig. 3. Cellular regulation through p53

A crucial role for the induction of the G1 cellular blockage plays p21 transcriptional activation. p21 binds and inhibits the activity of the cyclin-CDK complexes that leads to increased expression of the phosphorylated (active) form of pRb and of a subsequent G1 cellular blockage. p53 takes part in the induction of cellular blockage during the G2 phase. p53 causes decreased transcriptional regulation of cyclin B as well as cellular blockage at the margin of the G2/M phase. Cyclin B is a major regulator required during the mitosis. GADD-45 and 14-3-3σ are other transcriptionally activated proteins taking part in the induction of cellular blockage in the G2 point of the cellular cycle. p21 and GADD-45 affect the activity of PCNA that takes part in the process of DNA repair [7] (Fig. 2).

1.4.2. Induction of apoptosis by p53. p53 plays a major role in the regulation of apoptosis under physiological conditions. As in cellular arrest, here it is also not quite clear how activated p53 affects apoptosis. It is admitted that p53 induces apoptosis in a transcriptionally dependent and independent manner [4]. The apoptosis is activated most frequently after DNA damage or tumor mutations. The phosphorylation and activation of p53 are realized by DNA protein kinases (DNA-PK) or ATM (ataxia teleangiectasia gene). Other activators of p53 are various antitumor drugs, gamma and ultraviolet radiation, and a series of oncogenes (Fig. 3) [18]. Activated p53 relays the apoptotic signal to Bax that is translocated from the cytoplasm to the mitochondria [25]. Bax is a major representative of the Bcl-2 protein family and possesses proapoptotic activity. It is due to the opening of a polyprotein transmission pore (PTP) in the mitochondrial wall caused by Bax activity. This is related to cytochrome C departure from the mitochondria. Cytochrome C binds to Apaf 1 and procaspase 9. The subsequent activation of caspase 3 leads to induction of the apoptosis. Bcl-2 inhibits apoptosis by blocking Bax function. Thus described signal path is the target of many oncogenic mutations in malignant melanoma [25].

Apoptosis might be activated by the oncogenes independently of p53. One of these routes is NFKkB (the nuclear factor – kB). The myc oncogene possesses a potent effect on the mitochondria. Myc strongly increases the sensitivity for signals of the Fas/FasL, CD95, TNF and TRAIL death receptors. E2F directly affects apoptotic signals from death receptors [13]. Another common route through which many of the various signals for proliferation affect the apoptosis programme is the induction of ARF the function of which is to activate p53 using its inhibitory effect on Mdm2 [28]. Oncogenes also lead to apoptosis by activation of Rb tumor suppressor gene. The potential of Rb to take active part in the processes of proliferation and apoptosis depends on its linkage to E2F (Fig. 3) [7].

1.4.3. Apoptosis in tumor cells. In tumor cells, apoptosis is suppressed below the critical threshold which gives these cells the chance to survive. In the majority of carcinomas, suppression of apoptosis and the chance of cellular survival is due to inhibition of the paths through which p53 affects apoptosis [23, 35]. The restoration of p53 function is enough to induce apoptosis in many tumor cells. Sev-

eral mechanisms exist for p53 reactivation in the treatment of tumors. In principle, these mechanisms include the introduction of the wild type of p53 into the tumors expressing a mutant protein or inhibiting the negative regulators of p53 (like Mdm2) in the tumors including the wild type of p53 [7, 28].

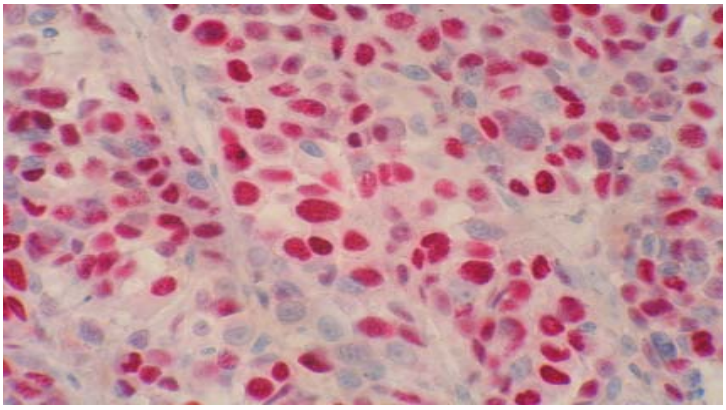


Fig. 4. Immunohistochemical determination of p53 in melanoma cells of initial-stage melanoma (Anti-human p53 monoclonal antibody, own studies). Nuclear accumulation of strong intensity of staining. 80% of tumor cells show staining for the p53 marker

2. Significance of the immunohistochemical determination of p53 in malignant melanoma. The immunohistochemical determination of the markers characterizing the processes of proliferation and apoptosis, especially of p53, in the case of various carcinomas is performed to allow subsequent pathogenetic conclusions, to predict the prognosis of the disease, and to determine the susceptibility of the tumor to chemo- and/or radiotherapy. Immunohistochemically, p53 expression is confirmed in the cell nucleus [34].

The evidence for increased expression of p53 in melanoma probably shows better prognosis and therapeutic response. The data on the changes in the expression of p53 in malignant melanoma are controversial and should be subjected to critical consideration [9, 11, 12, 23, 37, 39]. The decreased expression of p53 in melanoma cells is indicative of the impossibility for direct induction of apoptosis through the route of Bax as well as probably decreased induction of cellular blockage indirectly through the route of p21 (Fig. 5). Melanomas are often positive when stained for mutant p53. As a result of these processes, tumor cells proliferate uncontrollably and cannot be neutralized by the mechanisms of apoptosis. In these cases, melanoma advances very fast. Our recent studies show an increase in the number of cases with loss of expression as well as a decrease in the number of cases with strong p53 expression when melanoma progresses. This is in support of the thesis that tumor progression is accompanied by decreased control of the cellular cycle and impossibility of inducing apoptosis [23, 30, 31, 32, 33, 38, 41].

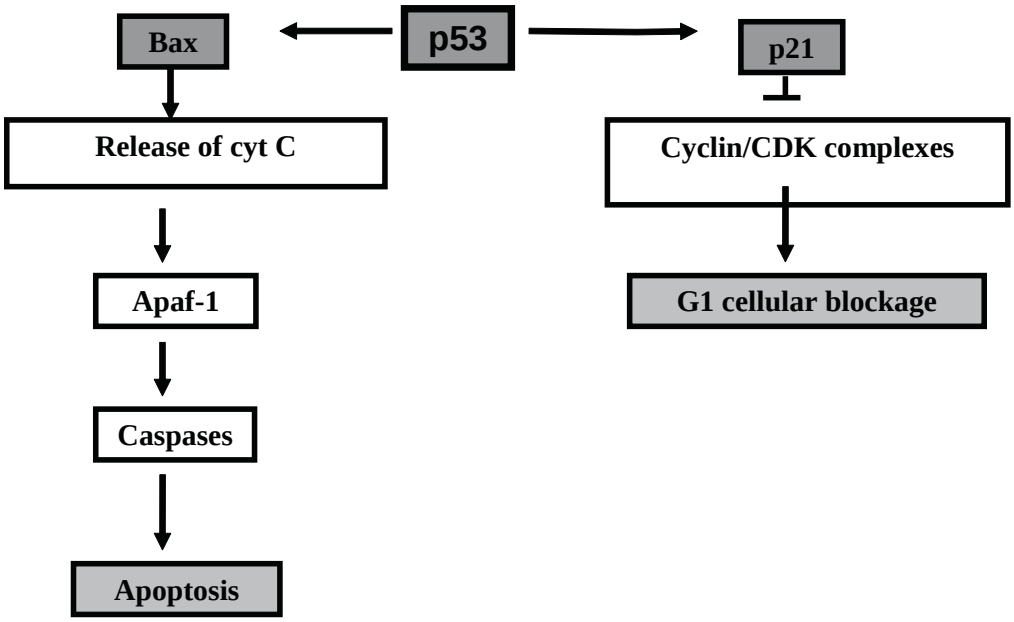


Fig. 5. Major p53 functions in the induction of apoptosis and cellular blockage

It is important to stress that various factors playing a role in the occurrence of a tumor are involved at various time points of its evolution, i.e. in the stage of carcinogenesis. This means that they activate p53 at various time points of the progression of carcinogenesis. For example, the disturbed regulation of tumor markers by oncogenes appears in the early stage of carcinogenesis while hypoxia plays a role only after the tumor has become macroscopically apparent. Respectively, p53 exerts its tumor-suppressor role in various stages of the progression of carcinogenesis. This explains why p53 loss has such a profound effect on the development of melanoma and of tumors in general [7, 21, 36].

3. The immunohistochemical determination of p53 in gastric cancer

p53 has been the subject of interests and research activity over the past 20 years because of its critical role in guarding against cancer development. The biological role and clinical importance of p53 alterations in gastric cancer are still unclear. This may be related to the very complicated and extensive p53 network and to technical problems associated with surrogate markers to identify TP53 gene defects, as most detection tests lack sensitivity and specificity.

Although mutations have been extensively discussed as a means of wild-type p53 inactivation, there are also mutation-independent mechanisms to inactivate p53 function. p53 lies within a pathway and perturbations occurring either upstream or downstream negatively impact p53-dependent tumor suppression. Allelic loss occurs in more than 60% of cases and mutations are identified in approximately 30%-50% of cases depending on the mutational screening method used and variable sample sizes [14]. Some mutations of p53 have even been identified in early dysplastic and apparent intestinal metaplasia gastric lesions; however, most altera-

tions occurred in the advanced stages of neoplasia. The spectrum of mutations in this gene within gastric tumors is not unusual with a predominance of base transitions. Many studies have used immunohistochemical analysis of tumors in an effort to detect excessive expression of p53 as an indirect means to identify mutations of this gene, but this assay does not seem to have consistent prognostic value in patients with gastric cancers [10, 15].

Overexpression of p53 protein as the result of an altered gene has been demonstrated in 40–60% of primary gastric carcinomas [17, 19]. Also, few reports have evaluated the relationship between p53 expression and progression in primary gastric carcinoma [19]. For example, in a study from 2011, Ye et al. showed that the prognosis of familial gastric cancer is closely related with p53 expression. A total of 162 patients had worse 5-year survival rates when they showed positive expression for p53 [42]. Many authors in early studies show the importance of this marker in gastric carcinoma and describe it as a negative predictor in terms of survival and prognosis [3, 20, 22].

Our observations also show that expression of this protein is associated with expression of other protein molecules involved in the development of gastric cancer (such as: TGF-beta-1, Ki-67, HER2/neu), as well as survival and prognosis. In our study from 2010, we examined immunohistochemically expression of p53 and found expression in all of gastric cancer specimens (Fig. 6a, b). The patients with p53 expression had worse prognosis after surgical therapy compared to those without, but 88.9% from patients with p53-positive status were in T1-T2 stage vs. 37.8% in T3-T4.

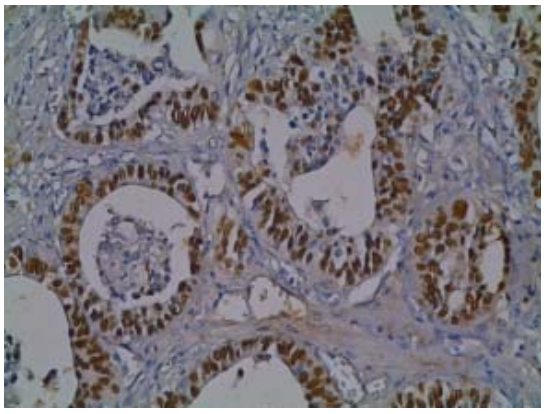
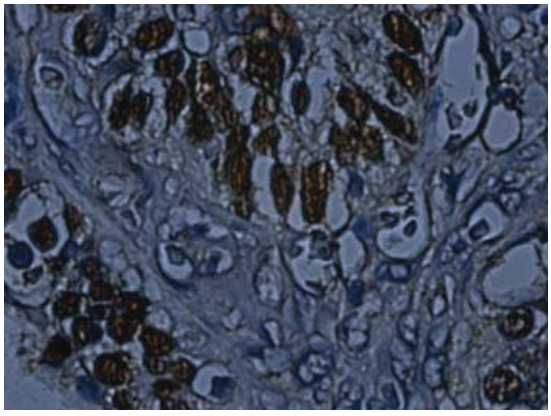
A  B 

Fig. 6a, b. Positive immunohistochemical staining in gastric cancer specimens with monoclonal antibody p53 (Magnification: a x 200; b x 800)

Despite own observations, there are many conflicting results related to the importance of this protein, but the expansion of the groups, and targeted search for meaning and mutations in various tumor types, is likely to lead to a clear conception of the importance of p53.

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