



Role of p53 as a Tumour Marker

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ABSTRACT

Tumor marker is a biochemical substance indicative of neoplasia and is ideally specific, sensitive, and proportional to tumor load. p53, is a 53 KD nuclear phosphoprotein which functions as tumor suppressor by inhibiting cell proliferation. p53 plays a dominant role in cellular apoptosis. p53 gene mutations are reported in approximately 50% of all types of cancers. The importance of its presence either in wild or in mutated form in pathogenesis of various neoplasms definitely invokes scientific attention towards this molecule, both as gene as well as protein.

INTRODUCTION

Tumor markers are substances that can often be detected in higher-than normal amounts in the blood, urine, or body tissues of some patients with certain types of cancers. Tumor markers are produced either by the tumor itself or by the body in response to the presence of cancer or certain benign conditions.¹ Tumor marker is a biochemical substance indicative of neoplasia and is ideally specific, sensitive, and proportional to tumor load.²

Tumor markers are allocated to 4 groups according to function.²

1. Enhancement of Tumor growth (Tumor promoters): These cause cell cycle acceleration & thus help in proliferation. This category includes PCNA, EGFR, Ki67, AgNOR.
2. Tumor suppressor: These are active in tumor suppressor & anti tumor response. Under this category Retinoblastoma protein, CDK inhibitors, p53, Bax are considered.

3. **Angiogenesis:** Angiogenesis is required for tumour progression and metastasis. It includes Vascular endothelial growth factor/receptor, Nitric oxide synthase type II, Platelet-derived endothelial cell growth factor.
4. **Tumor invasion & Metastatic potential:** These includes adhesion molecules and matrix degrading enzymes that attribute to tumour invasion and metastasis. It includes Matrix-Metallo-Proteases, Cathepsins, Integrins, Cadherins etc.

Among various tumor markers, p53 is the most commonly studied marker. p53, is a 53 KD nuclear phosphoprotein which functions as tumor suppressor by inhibiting cell proliferation. p53 plays a dominant role in cellular apoptosis. p53 gene mutations are reported in approximately 50% of all types of cancers.³

p53 is the name given to tumor suppressor gene, as well as the protein encoded by gene. The name is due to its molecular mass: it is in the 53 kilodalton fraction of cell proteins. The p53 is located on short arm (p) of chromosome 17 & consist of 11 exons, of which first one is non encoding. The p53 consist of 393 aminoacids and comprises of four regions with different functions.⁴

N- Terminus region: Transactivational domain important for specific activation of genes.

Central part: DNA binding domain through which the p53 protein binds to specific binding sites.

C Terminus: Tetramerisation domain responsible for p53 protein tetramers which is the most active form of p53 in transactivation.

Regulatory domain: Negatively regulates central DNA binding domain by binding to it and thereby inhibits specific binding of p53 protein to different promoters.

p53 is of two types, Wild type & Mutant type. The Wild type is essential for normal cell growth and the eventual suppression of the malignant phenotype. Inactivation of p53 induces the development of malignancy. Thus, the normal p53 act as the molecular policeman monitoring the integrity of genome. It is present in a very low concentration in the nucleus of normal cells and tissues and has half-life of about 20 min, so wild type is undetectable by immunohistochemical analysis. The wild type level strongly increases after DNA damage, and this leads to specific arrest of cell cycle at G1 phase. If DNA is damaged, wild type p53 accumulates and switches off replication to allow extra time for DNA repair. If the DNA repair fails, wild type p53 may trigger cell suicide by apoptosis.

However in tumor cells, mutated p53 is unable to induce cell cycle arrest. It either increases pool of proliferative cells or increases probability of neoplastic transformation by inhibiting apoptosis. It shows increased half-life (4 to 8 hours) of the molecule, which leads to accumulation of mutant p53 in cells, which can be detected as over expression in the lesional cell. Thus p53 act as a tumor suppressor gene in normal form, but when mutated the function is lost leading to tumor progression.⁵

p53 activation and function

Three genetically distinct pathways are known for the activation of the p53 gene:

1. Oncogenic Stimuli
2. UV radiation and the ionizing radiation
3. Hypoxia, Cytokines and growth factors

These stimuli cause the p53 protein to go through certain modifications; the type of modification is specific to, the type of stress, species and cell type. Different stimuli also activate the p53 protein through distinct pathways, e.g. p53 resulting from DNA damage is activated through phosphorylation and acetylation.

Mechanism of Action

p53 protein is a DNA binding protein localized to nucleus, which when activated by some stimuli, functions primarily by controlling the transcription of several other genes. The major functional activities of p53 protein are cell cycle arrest and inhibition to apoptosis by cell damage. p53 is called in to apply emergency brakes, when DNA is damaged by irradiation, UV light or mutagenic chemicals and also in response to other stress conditions that indirectly damage DNA like hypoxia and senescence. Following DNA damage

there is rapid increase in p53 levels. At the same time kinases such as DNA dependant protein Kinases & ATM (ataxia-telangectasis mutated) are activated in response to DNA damage. These enzymes phosphorylate p53 and protein unfolds, which is able to bind to DNA and become an active transcription factor. p53 induced cell cycle arrest occurs late in the G1 phase & is caused by p53 dependant transcription of CDK inhibitors p21. This allows the cells enough time to repair the DNA damage caused by mutagenic agent.¹

p53 also helps in repair process directly by inducing the transcription of GADD45 (growth arrest & DNA damage) which encodes a protein involved in DNA repair. If the DNA damage is repaired successfully, quite ingeniously, p53 activates Mdm2 whose products bind to and degrades p53, thus relieving cell block. If during the pause in cell division, the DNA damage cannot be successfully repaired, normal p53, perhaps as a last ditch effort, sends the cell to the graveyard by inducing the activation of apoptosis inducing genes such as Bax which binds to & antagonizes the apoptosis inhibiting protein Bcl-2, thus promoting cell death (Figure 2).¹

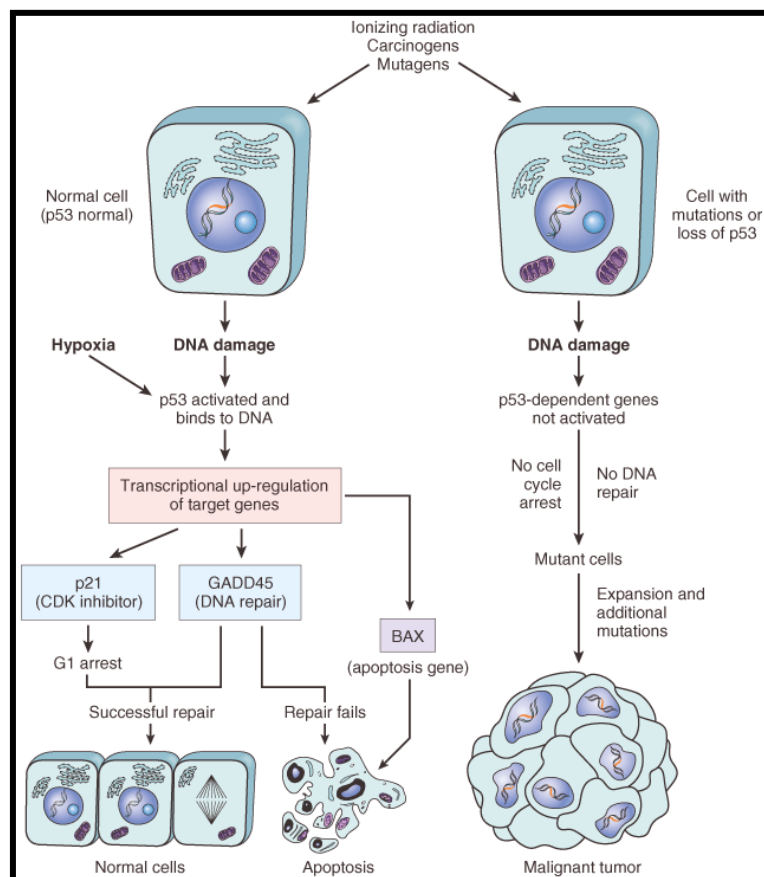


Figure 2: Role of p53 in maintaining integrity of genome.¹

p53 degradation

Physiologically the p53 protein is degraded through the so called ubiquitin pathway.⁴ In normal cells, p53 is kept inactive through a number of mechanisms, including the activity of the oncoprotein Mdm2, a key negative regulator of p53. The interaction between p53 and Mdm2 is necessary for the ubiquitination and degradation of p53 by Mdm2. A highly conserved region in the N terminus of p53 interacts with a hydrophobic binding pocket in the N-terminal domain of Mdm2 and small molecule inhibitors of this interaction stabilize p53 efficiently. The oligomerization domain located in the C-terminal region of p53 also contributes to efficient Mdm2 binding and degradation. Recently, the DBD of p53 has been reported to provide a secondary

binding site for Mdm2. Several studies show that the central acidic domain of Mdm2 is involved in the interaction with p53. The current model suggests that the N-terminal interaction between p53 and Mdm2 induces a conformational change in Mdm2 that promotes the binding of the acidic domain of Mdm2 to the DBD of p53. This second interaction between Mdm2 and p53 has also been shown to contribute to efficient ubiquitination.⁶

p53 and the nature of Mutation

Approximately 80% of p53 mutations present in human cancers are located in the DNA binding domain of the protein. Mutated p53 that does not bind to DNA produces defective protein (missense mutation) that blocks the activity of the normal protein. p53 has 11 exons and out of these, exons

5-8 are the most highly conserved and contain the majority of the mutations in the p53 gene. Mutations or deletions of p53 gene or inactivated protein can alter p53 activity and lead to disturbed cell cycle. p53 can also be inactivated by interaction with other proteins such as Mdm2, by methylation or sequestration.⁵

p53 mutation have been demonstrated in 12% to 100% of HNSCC, mainly in the basal epithelia and at the advancing front.^{4,5}

In oral dysplastic lesions p53 mutations occur in 27-60% of the cases. They are proportional to the degree of dysplasia and they precede dysplastic changes suggesting that p53 changes are early events in carcinogenesis.⁴

Two-thirds of the p53 mutations in head and neck cancer are at guanine (G) nucleotides, since G residues are the preferential targets for chemical carcinogen induced damage. In the large series of HNSCC, most p53 mutations were G:C-A:T (31%) or G:C-T:A(18%) or deletions / inversions(19%).⁴

The most common mutations in oral dysplastic lesions and carcinomas are G-A & G-T transversions. Most p53 mutations have been in exons 2-11, at exons 4-9, in codons 234-248(exon7) and 278-281(exon 8). Hot spot mutation predominantly in codons 220, 245-248 and 278-281 though some have found hot spots at 149 and 274. In oral carcinoma patients, most of whom were women with no obvious risk factors for cancer, a specific exon 8-14 bp deletions in codon 287-292 which results in truncated p53 protein lacking the C terminus has been implicated.^{4,5}

p53 expression may be altered both by gene mutation as a consequence of carcinogens, radiation or by other mechanisms, such as the degradation of p53 protein by viral proteins such as Simian virus (SV40) large T antigen, HPV type 16, E6 protein and cellular proteins such as Mdm2, p21,GADD45, Cyclins, CDKs and PCNA.^{4,5}

Germ line abnormalities in p53 appear to underlie some cancer prone families including cases of Li-Fraumeni syndrome and with less penetrance in some families with HNSCC.⁵

The most common type of p53 mutation is missense mutation in the mid region constituting up to 79% of all mutations. Mutation is found within exon 5-8, but the mutational spectra varies between countries and races.⁵

p53 mutation and expression of p53 protein

Approximately 60% HNSCC tumors have immunohistochemically detected p53, thus suggesting the presence of p53 mutations. Antibody positivity has thus frequently been taken to indicate p53 mutation and conversely negativity has sometimes been regarded as excluding mutations. The frequency of detection of p53 mutations based on IHC studies is not necessarily accurately representative of the state of mutated p53.

Antigen retrieval significantly increases the sensitivity of p53 immunoreactivity but there is no association between the p53 protein over expression and gene mutations. Absence of reactivity with p53 antibodies does not exclude alterations in the gene. Both the frame shift as

well as non- sense mutations may be found in antibody negative tumors since the antibodies fail to detect truncated p53 proteins. Thus, there may be over expression of p53 protein without apparent gene mutation.^{4,5}

DISCUSSION

The nature of mutations at the p53 locus in head & neck squamous cell carcinoma

Mutations of p53 are most common genetic abnormalities found in the development of HNSCC. Mutations in p53 have been shown to result from allelic loss, point mutation, deletion or rearrangement. The p53 mutation causes both a loss of its tumor suppressor function & a gain of its oncogene function by alteration of the repertory of genes controlled by p53. The loss of function of tumor suppressor genes corresponds to an increased risk of cancer. The protein expressed from the mutant p53 gene has been implicated in growth, degradation & malignant progression to cancers.

Largely et al⁷ revealed that loss of heterozygosity in a p53 site was found in more than 70% of oral SCC. About 90% of missense mutations occur in p53 locus leading to faulty or altered protein in cell. Greenblatt et al⁸ in a review of p 53 mutations in HNSCC, 31% mutations were G: C A: T transition & 18% were G: C A: T transversions & 11% were G: C C:G transversions. Yin et al proposed the following sequence of p53 alteration as follows:

- a) p 53 mutation
- b) Deletion of wild type

c) Increased dosage of mutated gene by aneuploid increase in chromosome copies

d) p53 gene amplifications

Alteration in p53 gene is a gradual process that spans many levels of tumor progression , & possibly involve different mechanisms.

Immunoreactivity of p53 in premalignant oral lesions & in squamous cell carcinoma

The prevalence of p53 positivity ranged from 11-69% in oral SCC & 34-79% in HNSCC. The pattern of p53 reactivity in HNSCC is exclusively nuclear & correlates with presence of mutant p53 but cytoplasmic reactivity is observed in few cases. Most authors have found no positive staining in normal mucosa or in mucosa adjacent to neoplasms but other authors have shown p53 positivity in normal mucosa. Similar expression was observed in keratinocytes adjacent to p53 positive carcinomas but not in tumor negative cases. Shin et al⁹ observed p53 overexpression in basal layer in normal epithelium adjacent to tumor. p53 expression is also seen in premalignant conditions such as leukoplakia both with & without dysplasia. p53 expression was seen in the basal region of dysplastic cells¹⁰. In hyperplasia & dysplasia p53 overexpression expanded into parabasal & superficial expression.

p53 overexpression has been correlated with degree of severity with highest expression in severe dysplasia, but Regezi et al found no clear cut relationship between percentage of p53 positive cells & severity of atypia. In oral premalignant lesions that show progression to oral cancer, there is low prevalence of p 53 gene

mutations in the precancerous stage. In cases showing progression to squamous cell carcinoma, p53 expression is a relatively late event in progression to carcinoma¹¹. Ogden et al¹⁰ could identify p53 positive cells from oral smears from positive tumors, thus suggesting that p53 is a useful marker for oral cancer screening.

p53 expression in primary tumor, recurrences & metastases in lymph nodes

Dolcetti et al showed p53 negative metastases invariably derived from primary tumors with no p53 immunostaining. The p53 overexpression status of tumor metastases was identical in all cases, with that of primary tumor. Burns et al⁹⁶ showed that the primary & lymph node metastasis had the same mutations, thus suggesting mutant p53 genes continued to be important for p53 progression. It has been suggested that p53 mutation precedes metastatic spread, however others have found discordant staining between lymph node metastasis & primary tumors. Prevalence of p53 mutation was higher in patients with lymph node involvement than those without lymph node metastases thus correlating p53 expression with invasiveness⁵. Girod et al¹² noted that the number of p53 positive tumors was highest in the group of recurrent SCC, where as neither the recurrence rate nor the time to recurrence were dependant on p53 positivity or negativity.

Relationship between the expression of p53 and the development of multiple tumors

OSCC originates in a multicentric fashion by a process of field cancerisation in which area of the epithelium has ben preconditioned by spontaneous alteration or by carcinogenic agents⁵.The prognosis for patients suffering from HNSCC is adversely affected by the very high occurrence of multiple primary & secondary tumors. Gallo & Bianchi¹³ showed p53 staining in 71% of multiple SCC of which 50% showed positive staining both first & second primary tumors thus showing discordance between p53 gene mutations in primary cancers & the corresponding secondary primary cancers. Moreover 10 out of 12 head & neck cancer patients with multiple cancers showed p53 positive staining in normal epithelium from sites at significant distance from site of first & second primary malignancies¹³.

The expression of p53 in normal epithelium from HNSCC patients would indicate an increased risk for second primary cancers. The presence of multiple foci of p53 overexpression in the mucosa at distance of upto 1 cm from the main lesion indicate multifocality of these events.p53 positivity was limited to basal & suprabasal cells with loss of positivity with differentiation. The expression of p53 adjacent to normal epithelium adjacent to tumor might support the concept of field cancerization, whereby the whole epithelium accumulates genetic damage overtime & is at increased risk of developing multiple independent lesions that may become malignant¹⁴. The procees of multiple tumors development in the HNSCC might be initiated by lateral movement of

pre-malignant basal keratinocytes, thus favouring a monoclonal nature for the development of multiple primary, secondary, & recurrent tumors. However Nees et al¹³ detected different genetic changes in p53 in different tumor distant mucosal biopsies from the same patients & dramatic changes occurred in different regions within single biopsy. But others have showed distinct p53 mutations in two separate tumors in the same patient thus suggesting a multifocal polyclonal process. This concept is strongly substantiated by data showing that the genetic lesions were discordant between the initial primary cancer & the second or third tumors, which suggested that these cancers arose as independent events.

SUMMARY

As a tumor suppressor gene, the inactivation of p53 can induce the development of malignancy. Alterations of p53 may be excellent biomarkers as indicators of the predisposition of a particular oral pre-malignant lesion towards malignancy as well as for other neoplasms.

The proportion of p53 positive cases increase from hyperplasia to dysplasia to Oral Squamous Cell Carcinoma. Thus, indicating an involvement of p53 in neoplastic transformation as well as in proliferating events. In other neoplasms, such as benign and malignant salivary gland tumours, p53 immunopositivity is detected. TP53 gene alterations suggestive of mutations are frequently observed in salivary gland neoplasms, mostly in those with a more aggressive behavior.

The ideal marker in tumour prognosis is the one that when present indicate tumor development and

when excludes this possibility. However, it is unlikely that such a marker exists, and it is therefore also important to look at the status of other factors closely related to p53, as it is more likely that p53 could be one factor in a panel of factors with importance for outcome, etc. rather than the single prognostic factor.

Indeed, it is unlikely that a single parameter will ever be identified as the ideal prognostic or predictive marker. Nevertheless, it is clear that the p53 pathway is very important in various neoplasm biology and also potentially in its treatment.

Mutations in the p53 gene is the most common genetic change found in human tumours. p53 is over-expressed in many cases of cancer & this overexpression is detectable by immunostaining of the tissues. There is a progressive increase in p53 expression in oral squamous cells as they acquire a more malignant phenotype. Its evaluation in the pre-malignant stages may assist in identifying lesions that are more likely to progress to malignancy. p53 mutation is postulated as a strong and independent variable for prognosticating survival.

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