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Immunohistochemistry Assessment of P53 Protein in Basal Cell Carcinoma

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ABSTRACT

The most frequently mutated tumor suppressor gene found in human cancer is p53. In a normal situation, p53 is activated upon the induction of DNA damage to either arrest the cell cycle or else induce apoptosis. However, when mutated, p53 is no longer able to properly accomplish these functions. Our aim was to investigate p53 protein alteration in cases of basal cell carcinoma (BCC) and compare it with the control group.

We investigated P53 gene expression in 41 cases of basal cell carcinoma and 20 patients with benign skin disease as control group. The alteration of p53 protein was investigated by immunohistochemistry method. The Data were analyzed using SPSS package, T and Chi-Square tests.

Twenty eight out of 41 basal cell carcinoma and 3 out of 20 control were p53-mutated, and there was a statistically significant difference in cases of BCC in comparison with controls (χ^2 test; p= 0.0001).

Taken together, showing alteration of p53 protein, our findings could add to the knowledge that might contribute to the self-maintenance of cancer cells and development of basal cell carcinoma.

Key words: Basal cell carcinoma; Gene expression; Immunohistochemistry; Tumor Suppressor Protein p53

INTRODUCTION

In a normal situation, *p53* is activated upon the induction of DNA damage to either arrest the cell cycle or to induce apoptosis. However, when mutated, p53 is no longer able to properly accomplish these functions. Apparently, appropriate p53 functioning is crucial for suppression of tumor development. There is considerable evidence that apoptosis plays an important role in the pathogenesis of a wide variety of skin diseases. Apoptosis failure may ensure the survival of transformed cells prone to sustain further genetic damage and it plays an important part in the development of tumors. Genetic alterations of p53, with consequent inactivation of gene protein products, may be involved in transcriptional downregulation of Fas.[1]

DNA-damaging agents constantly challenge the genetic material of living cells. These agents can originate from endogenous cellular processes that produce DNA interactive compounds (like reactive oxygen species, ROS), as well as from exposure to environmental compounds or radiation (e.g. gamma or UV).[2] One particularly well-known result of the induction of gene mutations is cancer, in general caused by loss of efficient cell growth control.[3,4]

Oncogenes are mutated forms of proto-oncogenes, whose function is to stimulate cell growth. This

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stimulation involves a series of steps, beginning at receptors on the cellular membrane.[5] The receptors that are activated by growth factors activate proteins in the cytoplasm (signal transducers), which in their turn activate transcription factors that help to move the cell through its cell cycle. Gene alterations, resulting in structural changes or altered expression levels of the proto-oncogenes, can give rise to activated oncogenes that keep the pathway continuously operational, resulting in uncontrolled cell growth.[6]

In contrast, tumor suppressor genes normally function to inhibit cell growth and division, and maintain a balance with cell cycle progression.[7] Tumor suppressor genes found mutated in human cancer are: RB, WT-1, VHL, NF1, NF2 and APC.[8] In addition, the most frequently mutated tumor suppressor gene found in human cancer is *p53*.[9,10] Appropriate p53 functioning is crucial for suppression of tumor development. This is also demonstrated by the fact that Li-Fraumeni syndrome patients, who carry a germ line mutation in p53, are highly cancer prone.[11]

In this study we investigated p53 protein alteration in cases of skin basal cell carcinoma and compared them with control group.

MATERIALS AND METHODS

Patients and Specimens

This case control study was done to investigat the relationship between basal cell carcinoma and mutation of P53 gene in dermatology and pathology departments of Imam Reza Hospital of Birjand University of Medical Sciences from 2001 to 2002.

Paraffin-embedded, formalin-fixed tissues were obtained from the Pathology Department. Samples were reviewed by two pathologists to ensure adequacy and that they were representative of the actual tumor. For each case, one adjacent slide was cut and stained with hematoxylin and eosin (H&E) for pathological confirmation. 41 paraffin-embedded histologic sections were collected from 41 patients with BCC. Clinical and histologic findings were reviewed in each case. The diagnosis of basal cell carcinoma (BCC) was based on the clinical picture and histologic findings. 20 paraffin-embedded histologic sections were collected from 20 patients with benign skin disease (without any premalignant or malignant skin disease) as control group.

Immunohistochemistry

Immunostaining for p53 was performed on formalin-fixed, paraffin-embedded tissues based on an avidin-biotin-peroxidase complex technique. Sectioned tissue (3 μm thick) was deparaffinized with xylene and rehydrated through descending strengths of alcohol. Endogenous peroxidase activity was blocked by incubating specimens in 2% hydrogen peroxide in methanol for 5 minutes. Before blocking for non-specific protein binding with normal goat serum (Jackson Immunoresearch) and bovine serum albumin (Sigma) an antigen retrieval step was performed by boiling the sections for 15 minutes in 10 mmol/L citrate buffer at pH 6.0. The slides were then incubated overnight at 4°C in a humidified chamber with a primary antibody. The primary antibody used for detection of p53 protein was the DAKO antihuman p53 protein clone DO-7, which recognizes an epitope in the N-terminus of the human p53 protein residing between amino acids 19 to 26.

After rinsing with a phosphate-buffered saline solution for 15 minutes, the biotinylated secondary antibody was applied for 30 minutes at room temperature. After an incubation step with avidin-biotin-horseradish peroxidase conjugate (Vectostain Elite ABC kit, Vector) for 30 minutes, sections were stained with 0.04% 3,3[1]-diamino-benzidine tetrahydro-chloride (Sigma) and counterstained with hematoxylin (Gill No. 3). Secondary antibodies included biotiny-lated goat antimouse and goat antirabbit (Jackson Immunoresearch).

Scoring of Immunostaining

Tumors were scored by a pathologist who was blinded to the clinical outcomes of the patients. Scoring was based on the estimated proportion of tumor: nuclear cells staining positively for p53. Cases were considered negative for a specific marker if 10% or less of the malignant cells stained for the antibody were negative. Cases were scored positive if more than 10% of the malignant cells were stained with the antibody. Assessment of p53 protein in benign types of skin diseases was done.

Statistical Analysis

Data were analyzed using SPSS package, T and Chi-Square tests. Statistical significance was defined as $P<0.05$.

RESULTS

Representative material was identified for 41 patients with basal cell carcinoma and for 20 patients with benign skin disease (without any premalignant or malignant skin disease) as control group.

The mean (± SD) age of the patients with basal cell carcinoma and the patients with benign skin disease was 58.7 (±11.5) and 57.6 (±10.8) years respectively (T test, P>0.05). Twenty eight out of 41 basal cell carcinomas (68.3%) and three of the 20 controls (15%) were p53-mutated, and there was a significant statistically difference in cases of basal cell carcinoma in comparison with the control group ($p< 0.0001$) (Figure 1).

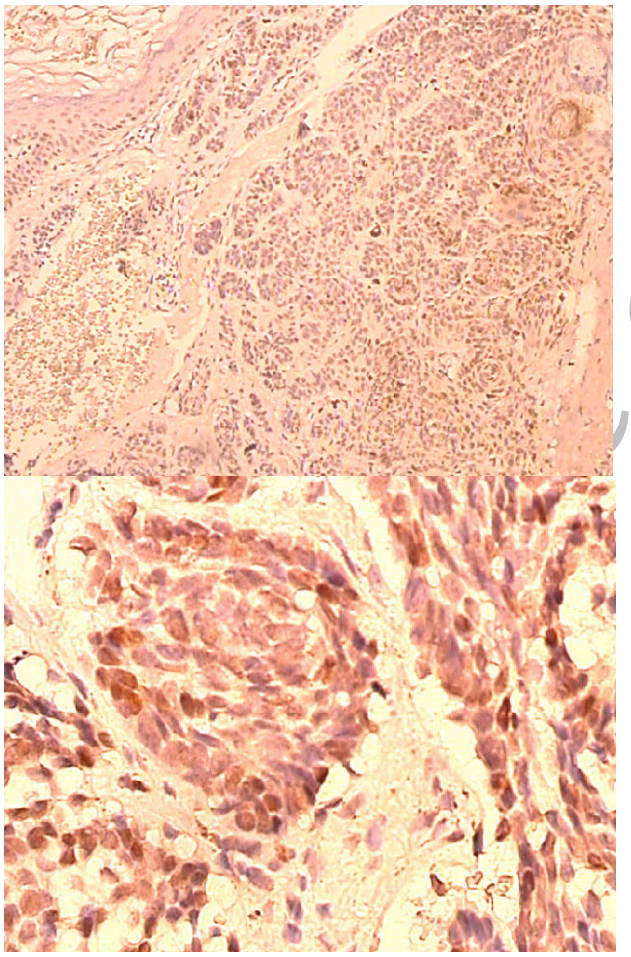

Figure 1. Positive immunostaining for P53 Protein in Basal Cell Carcinoma. Tumor is represented by compact areas, well delineated and invading the dermis. Tumor cells resemble normal basal cells (small, monomorphous) are disposed in palisade at the periphery of the tumor nests. Tumor cells were stained with antiP53 Protein.

DISCUSSION

Tumor development is a complex process that occurs either spontaneously or after exposure to genotoxic stress. In skin carcinogenesis, molecular epidemiology studies have clearly shown that UVB radiation can induce mutations, leading to the activation of oncogenes, such as the *ras* gene, or the loss of function of the tumor suppressor genes *p53*. [12]

In this study, we screened 41 BCCs for mutations in the *p53* gene. We have found that twenty eight out of 41 BCCs (68.3%) and three of the 20 controls (15%) showed *p53* mutations. Despite the small number of cases, our study suggests that mutation of *p53* gene might be an early event in BCC tumorigenesis, and the progression of BCC in our Iranian population occurs through the inactivation of *p53* gene. In Caucasians, mutations in *p53* were detected in sporadic BCCs at a frequency of 40–56% [13-16] and some investigators also noted that only one *p53* allele was inactivated.[17] The observed difference in the incidence of mutations of tumor suppressor genes in BCCs may account for the racial and regional difference in prevalence of BCCs.

Most mutations, detected in *p53* gene, occurred within conserved regions II–V, and at conserved amino acid nearby, leading to a predicted amino acid change.[14,18] Interestingly, *p53* alteration affecting codon 178–179 and codon 281–282, found in three of the BCCs analyzed, was frequently observed in skin tumors including BCCs.[14,18,19] These mutations may correspond to mutation hotspots in skin tumors and would result in the synthesis of nonfunctional protein. In fact, codon 282 site has a crucial role in stabilizing the protein–DNA interaction. The fact that these tumors do not commonly contain an inactivated second *p53* allele remains an interesting phenomenon. Mutation of *p53* may produce a possible dominant protooncogene able to add malignant potential to tumors. This so-called 'gain of function' *p53* mutation could be the predominant mechanism of progression for BCC. Alternatively, the well-established tumor-suppressing function of *p53* could be diminished by inactivation of a single allele. Unlike other epithelial tumors, which can progress and invade, these tumors appear to behave in a more benign fashion, further suggesting a different genotype from that of invasive squamous carcinomas of the skin. Perhaps BCCs that do invade and act aggressively may inactivate both *p53* alleles.[12]

In Caucasian populations, mutations of most tumor suppressor genes are frequently deletions or insertions leading to truncated or absent protein product. The frequency of deletions or insertions leading to truncated or absent protein appears to be lower in the BCCs from Korean patients than from Caucasian patients and these observations may be related to the different BCC tumorigenesis between different racial populations.[12]

The spectrum of *p53* mutation in BCCs clearly shows that unrepaired photolesions resulting in UV-induced mutations play a decisive role in tumorigenesis. In the *p53* gene, the CC to CT(TC) substitutions are strongly reminiscent of typical CC→TT transitions, specific to UVB-induced DNA damage.[20] This is in line with previously reported UVB signature mutations in *p53* gene in some sporadic BCCs,[21,22] but the greater UVB signature mutation rate in *p53* when compared with *PTCH* in BCCs implies that UVB plays a greater role in *p53* inactivation than *PTCH* inactivation and that other mutagenic factors may play on additive role in *PTCH* inactivation and tumorigenesis. However, in one study, three out of four UV-specific *PTCH* mutated BCCs also had UV-specific *p53* mutations, which not only indicates the causal role of UV-specific lesions in skin cancer development but also lends additional evidence to the multiplicity of genetic alterations leading to tumor function.[12]

Our results agree with the findings that BCCs from xeroderma pigmentosum patients belong to complementation group C and BCCs from normal individuals.[1,19,23] Contrary to our findings, previous results were obtained in sporadic BCCs from normal individuals in Caucasians.[24] One possible explanation is that the large number of genetic alterations may result in slower repair levels compared with replication, leaving mutations on both strands.

Some authors showed that mutations in HH pathway genes have been identified in sporadic BCC, implying that the principal trigger for tumorigenesis is not simply mutation of the *PTCH* gene, but more likely HH pathway dysregulation. Additional studies in a large patient population and an examination of the BCC samples according to progression are needed to verify these initial observations and delineate the effect of HH pathway genes, including *Gli*, *W*nt, and TGF-β, which have been identified in sporadic BCC.

Taken together, showing alteration of p53 protein, our findings could add to the knowledge that might contribute to the self-maintenance of cancer cells and development of basal cell carcinoma. Also further studies to analyze repair parameters in Iranian BCCs should be performed to reveal the BCC tumorigenesis.

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REFERENCES

1. Boldrini L, Loggini B, Gisfredi S, Zucconi Y, Baldinotti F, Fogli A, et al. Mutations of Fas (APO-1/CD95) and p53 genes in nonmelanoma skin cancer. J Cutan Med Surg 2003; 7(2):112-8.
2. Bertram JS. The molecular biology of cancer. Mol Aspects Med 2000; 21(6):167-223.
3. Evan GI , Vousden KH. Proliferation, cell cycle and apoptosis in cancer. Nature 2001; 411(6835):342-348.
4. McDonald ER 3rd, El-Deiry WS. Checkpoint genes in cancer. Ann Med 2001; 33(2):113-22.
5. Chen Z, Gibson TB, Robinson F, Silvestro L, Pearson G, Xu B, et al. MAP kinases. Chem Rev 2001; 101(8):2449-76.
6. Krontiris TG. Oncogenes. N Engl J Med 1995; 333(5):303-6.
7. Sherr CJ. Principles of tumor suppression. Cell 2004; 116(2):235-46.
8. Hussain SP, Harris CC. Molecular epidemiology of human cancer: contribution of mutation spectra studies of tumor suppressor genes.Cancer Res 1998; 58(18):4023-37.
9. Soussi T, Beroud C. Assessing TP53 status in human tumours to evaluate clinical outcome. Nat Rev Cancer 2001; 1(3):233-40.
10. Soussi T, Dehouche K, Beroud C. P53 website and analysis of p53 gene mutations in human cancer: forging a link between epidemiology and carcinogenesis. Hum Mutat 2000; 15(1):105-13.
11. Malkin D. P53 and the Li-Fraumeni syndrome. Biochim Biophys Acta 1994; 1198(2-3):197-213.
12. Kim MY, Park HJ, Baek SC, Byun DG, Houh D. Mutations of the p53 and PTCH gene in basal cell

carcinomas: UV mutation signature and strand bias. J Dermatol Sci 2002; 29(1):1-9.

13. Rady P, Scinicariello F, Wagner RF Jr, Tyring SK. p53 mutations in basal cell carcinomas. Cancer Res 1992; 52(13):3804-6.
14. Moles JP, Moyret C, Guillot B, Jeantar P, Bassett-Sequin N. p53 gene mutations in human epithelial skin cancer. Oncogene 1993; 8(3):583-588.
15. van der Riet P, Karp D, Farmer E, Sidransky D. Progression of basal cell carcinoma through loss of chromosome 9q and inactivation of a single p53 allele. Cancer Res 1994; 54(1):25-27.
16. Hollstein M, Sidransky D, Vogelstein B, Harris C. p53 mutations in human cancers. Science 1991; 253 (5015):49-53.
17. Hoogervorst EM, Steeg, HV. Nucleotide excision repair and p53 deficient mouse models in cancer research. Mutat Res 2005; 574(1-2):3-21.
18. Ziegler A, Leffell DJ, Kunala S, Sharma HW, Gailani M, Simon JA, et al. Mutation hotspots due to sunlight in the p53 gene of nonmelanoma skin cancers. Proc Natl Acad Sci U S A 1993; 90(9):4216-20.
19. Dumaz N, Drougard C, Sarasin A, Daya-Grosjean L. Specific UV-induced mutation spectrum in the p53 gene of skin tumors from DNA-repair-deficient xeroderma pigmentosum patients. Proc Natl Acad Sci U S A 1993; 90(22):10529-33.
20. McGregor WG, Chen RH, Lukash L, Maher VM, McCormick JJ. Cell cycle-dependent strand bias for UV-induced mutations in the transcribed strand of excision repair-proficient human fibroblasts but not in repair-deficient cells. Mol Cell Biol 1991; 11(4):1927-34.
21. Campbell C, Quinn AG, Angus B, Rees JL. The relation between p53 mutation and p53 immunostaining in non-melanoma skin cancer. Br J Dermatol 1993; 129(3):235-41.
22. Kubo Y, Urano Y, Yoshimoto K, Iwahana H, Fukuhara K, Arase S, et al. p53 gene mutations in human skin cancers and precancerous lesions: comparison with immunohistochemical analysis. J Invest Dermatol 1994; 102(4):440-4.
23. Stratigos AJ, Kapranos N, Petrakou E, Anastasiadou A, Pagouni A, Christofidou E, et al. Immunophenotypic analysis of the p53 gene in non-melanoma skin cancer and correlation with apoptosis and cell proliferation. J Eur Acad Dermatol Venereol 2005; 19(2):180-6.
24. Bodak N, Queille S, Avril MF, Bouadjar B, Drougard C, Sarasin A, et al. High levels of patched gene mutations in basal-cell carcinomas from patients with xeroderma pigmentosum. Proc Natl Acad Sci U S A 1999; 96(9):5117-22.