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Original Article

Mutations of p53 Gene in Skin Cancers: a Case Control Study

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Abstract

Background: 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 to induce apoptosis. However, when mutated, p53 is no longer able to properly accomplish these functions. The aim of this study was to investigate the expression of p53 gene in cases of skin cancers and compare those with control subjects.

Methods: The expression of P53 gene was investigated in samples form 62 patients who diagnosed as having various skin cancers including 41 basal cell carcinoma, 15 squamous cell carcinomas, 5 malignant melanoma, and one basosquamous carcinoma using immunohistochemistry method. The control group comprised 20 samples from patients with benign skin diseases. Data were analyzed using *t* and Chi-Square tests.

Results: The ratios of p53 mutations in different skin malignancies comprised of 28/41 basal cell carcinoma, 9/15 squamous cell carcinoma, 1/1 of basosquamous carcinoma, and 1/5 of malignant melanoma as well as 3/20 of control group. Statistically significant differences from the control group were found in the ratios of p53 mutations in cases of basal and squamous cell carcinomas. However, the ratio of p53 mutations in malignant melanoma was not significantly different from that of the control group.

Conclusion*:* Our findings suggest that mutations of p53 gene may contribute to the persistence of cancer cells and development of basal and squamous cell carcinomas.
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Keywords ● Mutation ● p53 gene ● skin cancers

Introduction

DNA-damaging agents constantly challenge the genetic material of living cells. Such agents can originate from endogenous cellular processes that produce DNA interactive compounds, namely reactive oxygen species. They are also derived from exposure to environmental compounds or radiations including gamma or UV.[1,2] Fortunately, the cell has several ways to defend itself against damage to DNA. For instance, a cell cycle arrest will be induced, presumably to give the cell time to repair the lesion by specific DNA repair proteins. Alternatively, the cell can undergo apoptosis, resulting in the elimination of cells containing severe DNA damage. However, when the recognition of DNA damage or its repair

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process fails, gene mutations may occur upon cell proliferation. These mutations can, depending on the affected genes, influence the proper function of the cell. One particularly well-known result of the induction of gene mutation is cancer, which is generally caused by loss of efficient cell growth control.[3,4] Mutations in two classes of genes can lead to this uncontrolled cell growth. These classes of genes are known as oncogenes and tumor suppressor genes. At present, more than 100 different oncogenes and tumor suppressor genes have been identified. It is likely, however, that a considerable number of genes have not been discovered yet.

Oncogenes are mutated forms of proto-oncogenes, whose function is to stimulate cell growth. This stimulation involves a series of steps, beginning at receptors on the cellular membrane.[5] The receptors that are activated by growth factors activate signal transducers proteins in the cytoplasm, which in 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] Gene alterations can occur by mutations in the gene itself, gene amplifications through promoter or enhancer mutation, chromosomal rearrangements or by viral integration.

In contrast, tumor suppressor genes normally function to inhibit cell growth and division, and maintain a balance with cell cycle progression.[7] Mutated tumor suppressor genes in human cancer include 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] 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 function is crucial for suppression of tumor development. This is also demonstrated by the fact that patients with Li-Fraumeni syndrome, who carry a germ line mutation in p53, are highly prone to cancer.[11]

The objective of the present study was to investigate the expression of p53 gene in cases of skin cancers and compare those with control subjects.

Patients and Methods

The study was conducted in the Deparments of Dermatology and Pathology, at Birjand University of Medical Sciences, from 2001 to 2002. It included tissue samples from tumors of 62 patients with various skin cancers such as basal cell carcinoma (BCC, 41 patients), squamous cell carcinoma (SCC, 15 patients), basosquamous carcinoma (1 patient), and malignant melanoma (5 patients). Tissue samples from 20 patients with benign skin disease without any premalignant or malignant skin diseases were selected as controls. The diagnoses of the tumors were based on the clinical picture and histologic findings.

Paraffin-embedded, formalin-fixed tissues were reviewed by a pathologist to ensure the adequacy of sample tissues for the study and representativeness of the actual tumor. For each case, one adjacent slide was cut and stained with hematoxylin and eosin (H&E) for pathological confirmation.

Immunostaining for p53 was performed on formalin-fixed, paraffin-embedded tissues using avidin-biotin-peroxidase complex technique. Tissue sections, 3 µm thick, were 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 Laboratories, West Grove, PA) and bovine serum albumin (P5147, Sigma, St Louis), an antigen retrieval step was performed by boiling 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 the detection of p53 protein was the DAKO (DO-7, code M7001; DAKO, Glostrup, Denmark) 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. The slides were then rinsed with a phosphate-buffered saline solution for 15 minutes. The biotinylated secondary antibody was then applied for 30 minutes at room temperature. The sections were treated with avidin-biotin-horseradish peroxidase conjugate (Vectostain Elite ABC kit, Vector) and following incubation for 30 minutes, the sections were stained with 0.04% 3,3^1-diamino-benzidine tetrahydrochloride (P5147, Sigma, St Louis) and counterstained with hematoxylin (Gill No. 3). Secondary antibodies included biotinylated goat antimouse and goat antirabbit (Jackson Immunoresearch Laboratories, West Grove, PA).

Tumors were scored by a pathologist who was blind to the clinical outcomes of the illnesses. Scoring was based on the estimated proportion of tumor nucleated cells positive for p53. Cases were considered negative for a specific marker if 10% or less of the malignant

cells stained positive with the antibody, or if staining intensity was 1+ or negative. Cases were scored positive if more than 10% of the malignant cells were stained positive with the antibody, or the staining intensity was moderate (2+) or more. Assessment of p53 gene expression in benign types of skin diseases was also performed using the same methods.

Statistical Analysis

Data were analyzed using Chi-Square and *t* tests using SPSS software (version12). Statistical significance was considered at a P value of ≤0.05.

Results

There was no significant difference between the age of the patients with skin cancers (56.9±10.5 years) and those with benign skin diseases (57.6±10.8 years).

The rate of p53 mutations was significantly higher in patients with skin cancers (62.9%, n=39) than that in control subjects (15%, n=3). Moreover, the rate of p53 mutations was significantly higher in patients with basal cell carcinomas (68.3%, n=41) than that in control subjects (15%, n=3). Also, the rate of p53 mutations was significantly higher in patients with squamous cell carcinomas (60.0 %, n=9) than that in control subjects (15%, n=3). There was only one patient with basosquamous cell carcinoma, which showed p53 mutation. The rate of p53 mutations in patients with malignant melanoma (20.0 %, n=1) was not siginificantly different from that in control subjects (15%, n=3).

Discussion

This study showed that 28 (68.3%) of patients with BCC and 3 (15%) of 20 patients in the control group had p53 mutations. Despite the small number of cases, our study suggested that mutation of p53 gene might be involved in BCC tumorigenesis. In Caucasians, mutations in p53 were detected in sporadic BCC at a frequency of 40–56%.[12-15] The racial and regional difference in prevalence of BCC may reflect the observed differences in the incidence of mutations of tumor suppressor genes.

The study also found that 9 (60%) SCC tumors did express the p53 gene. Despite the small sample size, the finding suggests that mutation of p53 gene might be involved in SCC tumorigenesis in the patients. The p53 mutation frequency in SCC was reported to be between 60 and 90%, and the mutations often display a typical UV signature.[16] Mutations in the p53 gene are common in SCC and precursor lesions and are suggested to be early events in the development of SCC.[16]

The findings of the present study are in agreement with those reported for SCCs in human,[17-20] and mice.[21] However, they are not consistent with a previous report,[22] indicating no mutations in the p53 gene. One possible explanation for such a differential finding might be that large number of genetic alterations did slow down the repair process. The other possible explanation may result from a smaller sample size and differences in sensitivity of the procedures in different studies.

Although our study might be overshadowed by small sample size, it also demonstrated that one (20%) of malignant melanoma tumors showed p53 gene mutations. Our study suggested that mutation of p53 gene might not be involved in the development of malignant melanoma. However, a major role played by p53 gene in melanoma genesis remains controversial. Our results agree with the findings of malignant melanomas from human in other studies.[23-27] In contrast to findings of the presnet study, it was shown that MM96L melanoma did express p53 gene mutation.[28]

Further studies to analyze the invlovement of p53 gene mutation in Iranian skin malignancies is required. New modalities designed to target specific p53 gene protein may represent promising approach for treatment of human skin cancers.[29]

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References

1 Ames BN, Gold LS, Willett WC. The causes and prevention of cancer. *Proc Natl Acad Sci USA* 1995; 92: 5258-65.
2 Bertram JS. The molecular biology of cancer. *Mol Aspects Med* 2000; 21: 167-223.
3 Evan GI, Vousden KH. Proliferation, cell cycle and apoptosis in cancer. *Nature* 2001; 411: 342-8.
4 McDonald ER 3rd, El-Deiry WS. Checkpoint genes in cancer. *Ann Med* 2001; 33: 113-22.
5 Chen Z, Gibson TB, Robinson F, et al. MAP kinases. *Chem Rev* 2001; 101: 2449-76.
6 Krontiris TG. Oncogenes. *N Engl J Med* 1995; 333: 303-6.

7 Sherr CJ. Principles of tumor suppression. *Cell* 2004; 116: 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: 4023-37.
9 Soussi T, Beroud C. Assessing TP53 status in human tumours to evaluate clinical outcome. *Nat Rev Cancer* 2001; 1: 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: 105-13.
11 Malkin D. p53 and the Li-Fraumeni syndrome. *Biochim Biophys Acta* 1994; 1198: 197-213.
12 Rady P, Scinicariello F, Wagner RF Jr, Tyring SK. p53 mutations in basal cell carcinomas. *Cancer Res* 1992; 52: 3804-6.
13 Moles JP, Moyret C, Guillot B, et al. p53 gene mutations in human epithelial skin cancer. *Oncogene* 1993; 8: 583-8.
14 Van der Riet P, Karp D, Farmer E, et al. Progression of basal cell carcinoma through loss of chromosome 9q and inactivation of a single p53 allele. *Cancer Res* 1994; 54: 25-7.
15 Hollstein M, Sidransky D, Vogelstein B, Harris C. p53 mutations in human cancers. *Science* 1991; 253: 49-53.
16 Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res* 1994; 54: 4855-78.
17 Stratigos AJ, Kapranos N, Petrakou 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: 180-6.
18 Boldrini L, Loggini B, Gisfredi S, et al. Mutations of Fas (APO-1/CD95) and p53 genes in nonmelanoma skin cancer. *J Cutan Med Surg* 2003; 7: 112-8.
19 Backvall H, Asplund A, Gustafsson A, et al. Genetic tumor archeology: microdissection and genetic heterogeneity in squamous and basal cell carcinoma. *Mutat Res* 2005; 571: 65-79.
20 Yamazaki Y, Chiba I, Hirai A, et al. Specific p53 mutations predict poor prognosis in oral squamous cell carcinoma. *Oral Oncol* 2003; 39: 163-9.
21 Van Kranen HJ, Westerman A, Berg RJ, et al. Dose-dependent effects of UVB-induced skin carcinogenesis in hairless p53 knockout mice. *Mutat Res* 2005; 571: 81-90.
22 Kanekura T, Kanzaki T, Kanekura S, et al. p53 gene mutations in skin cancers with underlying disorders. *J Dermatol Sci* 1995; 9: 209-14.
23 Dai DL, Martinka M, Bush JA, Li G. Reduced Apaf-1 expression in human cutaneous melanomas. *Br J Cancer* 2004; 91: 1089-95.
24 Kanoko M, Ueda M, Nagano T, Ichihashi M. Expression of p53 protein in melanoma progression. *J Dermatol Sci* 1996; 12: 97-103.
25 Urano Y, Oura H, Sakaki A, et al. Immunohistological analysis of p53 expression in human skin tumors. *J Dermatol Sci* 1992; 4: 69-75.
26 Daya-Grosjean L, Sarasin A. The role of UV induced lesions in skin carcinogenesis: an overview of oncogene and tumor suppressor gene modifications in xeroderma pigmentosum skin tumors. *Mutat Res* 2005; 571: 43-56.
27 Li G, Ho VC, Trotter MJ, et al. p53 mutation in metastatic melanomas and primary melanomas from sun-exposed and sun-protected sites. *J Eur Acad Dermatol Venereol* 1995; 4: 48-53.
28 Ablett E, Pedley J, Dannoy PA, et al. UVB-specific regulation of gene expression in human melanocytic cells: cell cycle effects and implication in the generation of melanoma. *Mutat Res* 1998; 422: 31-41.
29 Green C L, Khavari PA. Targets for molecular therapy of skin cancer. *Semin Cancer Biol* 2004; 14: 63-9.