ORIGINAL ARTICLE

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Mutation Analysis of Exon 23 of the PTCH Tumor Suppressor Gene in Multiple Basal Cell Carcinoma Patients with a History of Radiodermatitis

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ABSTRACT

Background: Basal Cell Carcinoma (BCC) with its slow-growing and rarely metastatic nature is the most common human neoplasm. Multiple BCCs mostly result from germline mutations in the tumor suppressor gene, PTCH with a genetic transmission pattern. Multiple BCCs may also originate from radiodermatitis which is a significant side effect of ionizing radiation exposure delivered to the skin in various skin treatments. PTCH is a critical member of the Sonic Hedgehog signalling pathway and mutations in this gene have been reported in as many as 40-80% of skin cancers. Exon number 23 is a critical exon in the function of the PTCH protein. Mutations have been reported in codon 1315 of PTCH in non-melanoma skin cancers.

Methods: We assessed mutations in exon 23 of the PTCH gene by polymerase chain reaction and direct sequencing in the peripheral blood cells of 10 patients with multiple BCCs. All of the subjects were selected from among patients with a history of radiation exposure and subsequent radiodermatitis.

Results: Direct sequencing revealed a Cytosine to Thymine mutation in codon 1315 of the PTCH gene in 60% of patients, 50% of which were heterozygotes, possessing both the C and T allele, and 10% were homozygotes for the T allele in the same position. Four subjects (40%) were normal homozygotes of the C allele, similar to the normal population.

Conclusion: Mutations with ID: rs 3575564 were detected in codon 1315 which transform the proline amino-acid to leucine in the PTCH protein. This transformation may affect the normal function of the PTCH protein, as reported previously. Patients with multiple BCCs who had a history of radiation exposure show a transformation from cytosine to thymine in codon number 1315 of the PTCH gene in their peripheral blood cells. Subsequent assessment of BCC tissues will clarify the somatic mutagenesis effects of radiation.

Keywords: BCC, PTCH, Sonic Hedgehog, Radiodermatitis

INTRODUCTION:

Basal Cell Carcinoma (BCC) is the most common human malignant neoplasm. Although BCC is a slow-growing tumour that rarely metastasizes or causes death, it can result in extensive morbidity through local invasion and tissue destruction[1]. Mutation of the PTCH gene appears to be an important event in skin cancer development[2]. Studies have reported that the mutation has a prevalence of 40- 80% in these patients[3].

Hedgehog (Hh) pathway inhibitors are clinically effective in the treatment of basal cell carcinoma[4]. Vismodegib acts as a cyclopamine-competitive antagonist of the smoothened receptor (SMO) which is part of the hedgehog signalling pathway. SMO inhibition prevents the activation of transcription factors GLI1 and GLI, which prevents the expression of tumor mediating genes within the hedgehog pathway. This pathway is pathogenically relevant in more than 90% of basal-cell carcinomas. Vismodegib targets a mutation in the hedgehog pathway identified in basal cell carcinoma and basal cell nevus syndrome[5]. Targeted therapies provide a novel and potentially effective treatment alternative for patients with eyelid carcinoma not amendable for surgery, including those with metastatic or locally advanced disease, advanced age, and significant co morbidities[6]. Sonic Hedgehog (SHH) pathway genetic studies may be essential for preventing misdiagnosis of metastatic BCCs and helping in the development of therapeutic strategies able to overcome resistance in both types of Hh inhibitors. Genotyping and molecular analysis can help to understand the nature and aggressiveness of the tumors.

Radiodermatitis is a side effect of exposure to ionizing radiation to the skin in cancer treatment. It appears that 95% of such cancer patients will develop some type of radiodermatitis[8]. Treatment of a dermatophyte fungus, tinea capitis using radiotherapy was introduced at the beginning of the twentieth century. Patients with previous radiodermatitis exposure had BCCs with aggressive biological behavior or multiple basal cell carcinomas with up to 40 lesions in a single patient, with no predisposing condition such as Gorlin's or Bazex's syndromes. Radiation exposures to the scalp during childhood for tinea capitis were associated with a four-fold increase in skin cancer, primarily basal cell carcinomas[9].

There is limited data regarding ionizing radiation-induced BCCs. Higher rates of p53 and PTCH abnormalities in post-ionizing radiation BCCs have been reported[10]. There is a lack of documented differences in gene expression that would account for the different biological behavior of radiotherapy-related BCCs, coupled with the aggressive and recurrent nature of these lesions. According to the limited data and despite the existence of few appropriate samples, the aim of this study was to evaluate germline mutations in the critical exon 23 of PTCH in patients with multiple BCCs who had a history of radiodermatitis.

METHODS:

Sample collection

Peripheral blood samples were collected from 10 subjects who had been diagnosed with BCC through histological assessment. Patients were selected at the time of diagnosis at Razi Skin Hospital in Tehran, Iran, after obtaining written informed consent. The subjects comprised BCC patients who were enrolled on the basis of specific criteria; three or more simultaneous BCC lesions or five or more within 2 years, with a history of radiodermatitis from x-ray exposure. Two ml of peripheral blood was collected in canonical tubes containing ethylene diamine tetraacetic acid (EDTA) as anticoagulant.

DNA extraction

Genomic DNA was extracted from blood samples using

DNA extraction Blood Mini Kit (Qiagen, Chatsworth, CA) according to the manufacturer's instructions. The quality, purity and quantity of extracted DNAs were determined using NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington,DE) and electrophoresis on 1% agarose gel.

Genotyping

Genotyping was performed by polymerase chain reaction (PCR) using two specific primers (forward: AACCCAAGGAGGGAAGTGTG and reverse: GAACCTTGTCCTCCTCTTTG) to amplify a 648 bp product.

The PCR mixture included 10 pmol of each of the primer pairs, 2.5 µl of 10x buffer including 1.5 mM MgCl2, 0.2 mM of mixed dNTPs, 1U of the enzyme Taq DNA polymerase (Cinnagen, Iran) and 100 ng of genomic DNA from the subjects. The mixture was adjusted with ddH2O to reach a final volume of 25 µl.

Thermal cycling was performed as follows: The reactions were started with an initial denaturation at 95˚C for 3 minutes, followed by 33 cycles of PCR comprising: denaturation at 94˚C for 30 seconds, annealing at 58˚C for 30 seconds, extension at 72˚C for 45 seconds and a final extension at 72˚C for 5 minutes by TC-512 Techne Thermal Cycler instrument (Bibby Scientific Limited, Staffordshire, England). DNA sequencing was performed by Prism_3130 Geneic Analyser (Applied biosystems) ABI and analysed using Chromas software. Finally, the Blast software on the NBCI website was used and results were mapped with genomic DNA in existing databases.

RESULTS:

Five men and five women were enrolled in the study. The age of the subjects ranged from 53 to 79 with a mean of 61.22± 4.33. The number of lesions ranged from 2 to 6 with a mean of 2.66±1.57. Two out of 10 patients stated a history of significant sun exposure, both of whom were determined as carrying the mutation. None of the subjects suffered from cancer, but 5 of them had a positive family history of cancer (3 breast cancers, 1 uterus and 1 foot cancer), all of whom carried the mutation. Most of the subjects had been subjected to radiation before the age of 19, and for 60%, radiation had occurred between the ages of 6-9. Treatment of tinea capitis (infection of the scalp with a dermatophyte fungus) was mentioned as the most common cause of radiation uptake. Mean age at diagnosis of the first lesion was 53.6±4.32 (42-69). The time between receiving radiation therapy and onset of BCC was 46.5±4.6 (40-55) years. A total of 6 (60%) of the subjects were detected as carrying the mutation in the 1315 codon of the PTCH gene, where a transition from C to T had occurred. Five of these subjects (50%) were heterozygotes (CT) and one patient (10%) was homozygote (TT) and the 4 remaining subjects (40%) had a normal homozygote phenotype (CC) in the same position (**Figure.1**). The detected mutation is variation ID: rs3575564 in codon 1315. The detected mutation transforms the proline amino-acid to leucine in the structure of the PTCH protein.

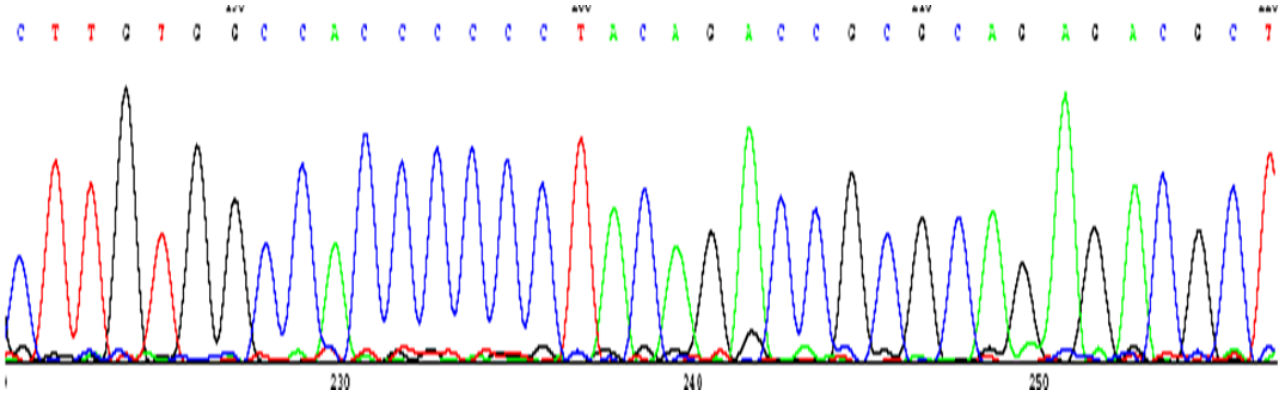
DISCUSSION:

The majority of the participants (90%) had been subject to radiation before the age of 12. Studies have suggested that radiodermatitis may result in specific clinicopathophysiological features of BCCs including multifocal involvement and scalp preference[11,12,13].

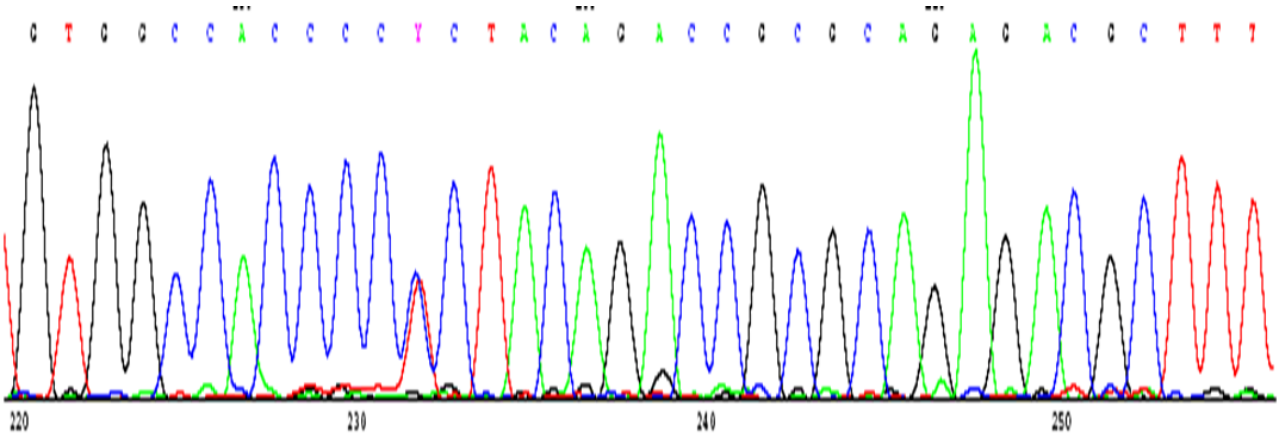
Given the significant association between sun exposure and mutations, life style modifications such as avoidance of exposure to UV need to be emphasized in order to prevent BCC. It has been noted that all subjects carrying mutations with significant sun exposure developed primary tumors at a younger age, 10 years sooner than those without the mutation. It could be suggested the sunlight may promote the development of the phenotype. Mutations found in the peripheral blood cells of patients emphasize the role of PTCH germline mutations

in the pathogenesis of BCC. Based on our results, the prevalence of mutations in exon 23 of the PTCH gene of patients with multiple BCC was 60%. This finding is in agreement with other studies that suggested a prevalence of 40-80% for PTCH gene mutations[14]. The effects of ionizing radiation in a Japanese study on survivors of the Hiroshima and Nakazaki atomic bombs reported that alteration of both PTCH and P53 genes

A: Normal sequence



B: Heterozygote mutation C to T



C: Homozygote mutation T-T

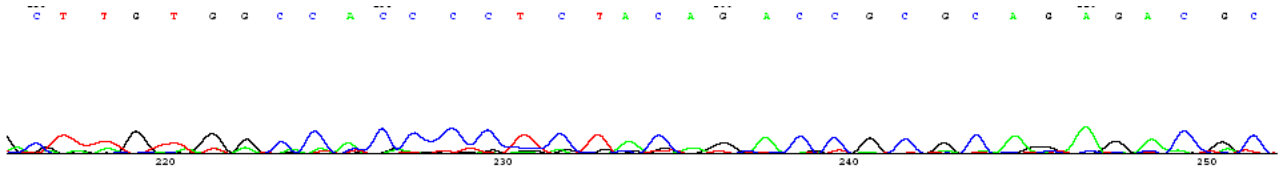


Figure 1: The sequences graphs of; **A**: Normal sequence, **B**: Heterozygote sequence, **C**: Mutated homozygote sequence

are likely to play a role in radiation-induced basal cell carcinogenesis[12].

In the current study, a mutation in codon1315 was detected in the peripheral blood. This resulted in a change in the proline codon (CCC) to leucine (CTC) at the C terminal domain of the protein. Studies have shown that this region is an essential regulatory region of the PTCH protein with a vital role in the SHH/PTCH pathway. This mutation may change the structure and function of the protein leading to changes in the signaling pathway and potentially increasing the risk of BCC formation. An American study suggested that codon1315 of the PTCH gene may increase the risk of non-melanoma skin cancers in populations as well as individuals[15]. Failure to lose proline during the shift to phaeomelanin may be associated with an increased population risk for BCC and increased individual risk for multiple BCCs. The effect of proline may be amplified by loss of the leucine allele during development of a tumor[15]. Hassanpour et al[16], suggested vigilant follow-up for patients with a history of radiodermatitis given their higher risk of recurrence or multiple new tumors. The high frequency of mutations in the PTCH gene suggests that this may be used as a diagnostic marker, but further studies are required to confirm this.

Based on our results, all patients with a positive family history of cancer carried the mutation. This emphasizes the need for further studies to indicate the importance of genetic counselling and screening among family members. In addition, the most common cancer reported in patients with a positive family history of cancer was breast cancer (3 out of 5: 60%). Assessing PTCH status, combined with assessment of clinical risk factors could be useful in identifying high-risk patients to be targeted for prevention or more rigorous surveillance.

CONCLUSION:

Mutations in the PTCH gene have been reported in association with basal cell carcinoma. Multiple BCCs can result from both germline mutations and mutations induced by environmental factors such as radiation. The results of the current study revealed the high prevalence of a mutation in codon 1315 of the PTCH gene in patients with multiple BCCs who had been exposed to radiation therapies, however, there was no evidence to establish that the detected mutation had been induced by radiation exposure, therefore assessment of somatic mutations in BCC tissues in comparison with the germline mutations which have been reported in the present study is recommended to clarify the issue.

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CONFLICTS OF INTEREST:

The authors have no conflicts of interest to declare.

REFERENCES:

1. Ghafouri-Fard S, Abbasi A, Moslehi H, Faramarzi N, Taba Taba Vakili S, Mobasheri MB, Modarressi MH. Elevated expression levels of testis-specific genes TEX101 and SPATA19 in basal cell carcinoma and their correlation with clinical and pathological features. British Journal of Dermatology. 2010 Apr;162(4):772-9.
2. Abdollahzadeh M, Mobasheri MB, Moslehi H, Modarressi MH. Association study of three single nucleutid polymorphisems in PTCH1 gene with basal cell carcinoma. Basic & Clinical Cancer Research. 2016 Aug 22;8(1&2):36-41.
3. Soufir N, Gerard B, Portela M, Brice A, Liboutet M, Saiag P, Descamps V, Kerob D, Wolkenstein P, Gorin I, Lebbe C. PTCH mutations and deletions in patients with typical nevoid basal cell carcinoma syndrome and in patients with a suspected genetic predisposition to basal cell carcinoma: a French study. British journal of cancer. 2006 Aug;95(4):548-53.
4. Shin K, Lim A, Zhao C, Sahoo D, Pan Y, Spiekerkoetter E, Liao JC, Beachy PA. Hedgehog signaling restrains bladder cancer progression by eliciting stromal production of urothelial differentiation factors. Cancer cell. 2014 Oct 13;26(4):521-33.
5. Basset-Séguin N, Hauschild A, Kunstfeld R, Grob J, Dréno B, Mortier L, Ascierto PA, Licitra L, Dutriaux C, Thomas L, Meyer N. Vismodegib in patients with advanced basal cell carcinoma: primary analysis of STEVIE, an international, open-label trial. European Journal of Cancer. 2017 Nov 1;86:334-48.

6. Yin VT, Merritt H, Esmaeli B. Targeting EGFR and sonic hedgehog pathways for locally advanced eyelid and periocular carcinomas. World Journal of Clinical Cases: WJCC. 2014 Sep 16;2(9):432-8.
7. Sharpe HJ, Pau G, Dijkgraaf GJ, Basset-Seguin N, Modrusan Z, Januario T, Tsui V, Durham AB, Dlugosz AA, Haverty PM, Bourgon R. Genomic analysis of smoothened inhibitor resistance in basal cell carcinoma. Cancer cell. 2015 Mar 9;27(3):327-41.
8. Singh M, Alavi A, Wong R, Akita S. Radiodermatitis: a review of our current understanding. American journal of clinical dermatology. 2016 Jun 1;17(3):277-92.
9. Meibodi NT, Maleki M, Javidi Z, Nahidi Y. Clinicopathological evaluation of radiation induced basal cell carcinoma. Indian journal of dermatology. 2008;53(3):137-9.
10. Tessone A, Amariglio N, Weissman O, Jacob-Hirsch J, Liran A, Stavrou D, Haik J, Orenstein A, Winkler E. Radiotherapy-induced basal cell carcinomas of the scalp: are they genetically different?. Aesthetic plastic surgery. 2012 Dec 1;36(6):1387-92.
11. Mseddi M, Dammak A, Jellouli M, Ghorbel S, Bouassida S, Marrekchi S, Zahaf A, Turki H. Profile of basal cell carcinomas of the scalp after radiotherapy for tinea capitis (about 63 cases). Revue medicale de Liege. 2006 Oct;61(10):724-7.
12. Mizuno T, Tokuoka S, Kishikawa M, Nakashima E, Mabuchi K, Iwamoto KS. Molecular basis of basal cell carcinogenesis in the atomic-bomb survivor population: p53 and PTCH gene alterations. Carcinogenesis. 2006 Jun 15;27(11):2286-94.
13. Maalej M, Frikha H, Kochbati L, Bouaouina N, Sellami D, Benna F, Gargouri W, Dhraief S, Nasr C, Daoud J, Hajji M. Radio-induced malignancies of the scalp about 98 patients with 150 lesions and literature review. Cancer/Radiotherapie. 2004 Apr 1;8(2):81-7.
14. Soufir, B G, et al. (2006) PTCH mutations and deletions in patients with typical Nevoid basal cell carcinoma syndrome and in patients with a suspected genetic predisposition to basal cell carcinoma: a French Study; British Journal of Cancer 95, 548-553
15. Asplund A, Gustafsson AC, Wikonkal NM, Sela A, Leffell DJ, Kidd K, Lundeberg J, Brash DE, Pontén F. PTCH codon 1315 polymorphism and risk for nonmelanoma skin cancer. British Journal of Dermatology. 2005 May;152(5):868-73.
16. Hassanpour SE, Kalantar-Hormozi A, Motamed S, Moosavizadeh SM, Shahverdiani R. Basal cell carcinoma of scalp in patients with history of childhood therapeutic radiation: a retrospective study and comparison to nonirradiated patients. Annals of plastic surgery. 2006 Nov 1;57(5):509-12.