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Original Article

Assessment of *PTCH1a* promoter methylation in BCC carcinogenesis

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Abstract. Basal Cell Carcinoma (BCC) is the most common cancer in humans. It is known that *PTCH1* mutations in Sonic Hedgehog pathway play significant role in BCC pathogenesis. However *PTCH1 (Patched)* inactivation through promoter methylation is still under investigation. In this study, promoter methylation of the *PTCH1* gene was analysed by Combined Bisulphite Restriction Analysis in 10 formalin-fixed paraffin-embedded (FFPE) BCC tissues. There was no methylation in any of the samples. Results suggest that *PTCH1* promoter methylation is not effective in BCC carcinogenesis and FFPE tissues are not suitable for methylation analysis since 44 specimens were included in the study and only 10 of them gave result.

Key words: Basal Cell Carcinoma, PTCH1, methylation, COBRA

1. Introduction

Basal cell carcinoma (BCC) is the most prevalent malignancy in humans and has distinctive properties from other cancers; as it does not have distant metastasis and is only locally invasive. BCC is known to be induced by UV- irradiation which targets *p53* and *Patched* (*PTCH1*) tumour suppressor genes. PTCH1 protein is a receptor in Sonic Hedgehog (Shh) pathway, a key regulator of embryonic development and also tumorigenesis. PTCH1 is a negative regulatory protein in Shh pathway. Shh protein binding to PTCH1, releases the inhibitory effect of PTCH1 against smoothened protein (SMO) (1). Activation of Shh pathway is important in BCC pathogenesis, and 50-60% of BCC's arise from inactivating *PTCH1* mutations. Methylation of the promoters is one of the inactivation ways for tumour suppressor genes, leading the question of whether methylation of the *PTCH1* plays a mechanistic role in BCC carcinogenesis.

The aim of this study is to assess whether *PTCH1a* promoter methylation is an important factor in BCC tumorigenesis. 44 formalin-fixed parafin-embedded (FFPE) BCC and normal skin tissues were included in the study, from the archives of Kırıkkale University, Faculty of Medicine, Department of Medical Pathology. The study was reviewed and approved by the Ethic Committee of Kırıkkale University.

2. Materials and methods

Genomic DNA was isolated with Standard phenol-chloroform precipitation from FFPE tissue samples. Methylation analysis of the promoter was performed with COBRA (Combined Bisulphite Restriction Analysis) as described previously (2). Briefly, bisulphite modification of genomic DNA was done by "Methylamp DNA Modification Kit" (EPIGENTEK). After PCR amplification, amplicons were cleaned-up with "GF-1 PCR Clean-Up Kit" (VIVANTIS) and were restricted by methylation sensitive TaqI restriction enzyme and electrophoresed in 3% agarose gel.

3. Results

Within 44 specimens, only 10 PCR and COBRA results were able to be obtained, 5 of them were normal skin and 5 of them were BCC specimens from different locations (face, palpebra, temporal zone, nose). Mean age of patients was 70±10,8; three were male and two

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were female and for the normal skin group mean age was 58±4,3; two were male and three were female. None of the samples showed methylation.

4. Discussion

The study revealed no methylation in any of the samples studied, suggesting that *PTCH1* promoter methylation does not play any role in BCC carcinogenesis. However, since COBRA analysis is restricted to specific restriction sites and only PTCH1a promoter was analyzed, it could not be exluded that other promoter sites are methylated. In the literature *PTCH1* methylation studies are quite rare in BCC and related syndromes. Cretnik et al. have investigated the methylation status of the *Patched* promoter in BCC and ovarian tumours. They found methylation changes significantly in ovarian tumours but in BCC, methylation differences were insignificant (3). Pan et al. have investigated genetic and epigenetic changes of the *PTCH1* gene in patients with nevoid basal cell carcinoma syndrome (NBCCS) for which *PTCH1* inactivation is responsible. Furthermore NBCCS patients are predisposed to sporadic keratocystic odontogenic tumors (KCOT). In five selected KCOTS and two normal controls they found insignificant promoter methylation changes between two groups. They proposed that *PTCH1* gene alterations might play significant role in the pathogenesis of NBCC and sporadic KCOTs by different mechanisms, such as standard two-hit model or haploinsufficiency or dominant-negative effect (4). Heitzer et al. analysed *PTCH* promoter methylation in a total of 56 BCCs. They found 5/16 (31%) of the fresh BCC samples and 2/16 (13%) of the short-time stored FFPE DNA from the same tumours were methylated on *PTCH1* promoter, and found no methylation in long term stored DNA in any of the remaining 40 BCC samples (5).

Since 44 FFPE tissues were included in the study and only 10 specimens were able to be analysed by COBRA; this low rate of attainment was attributed to the material used. Although high quality DNA was extracted from FFPE tissues most of them did not reveal successful PCR results. When same procedure was performed for fresh peripheric blood, the technique was fully effective. DNA extracted from FFPE is more prone to degradation depending on the paraffinization steps and the period in paraffin. Probably, FFPE DNA was degraded in the bisulphite modification step which is 90 minutes incubation in 65 °C. In a recent study Heitzer et al. analysed methylation status of *PTCH1* promoter from FFPE and fresh BCC samples by three different methods; direct bisulphite sequencing PCR, MethyLight and high-resolution melting (HRM) and compared their sensitivity. They concluded that HRM analysis of DNA from fresh tissue was the most sensitive method to detect methylation and FFPE tissue was not suitable for methylation analysis (5). Results presented in this paper are consistent with their study.

PTCH1 inactivation is an important step in BCC carcinogenesis, although its mutation is more common in BCC, it is concluded that its inactivation through promoter methylation does not suggest a relation to BCC tumorigenesis and FFPE tissue is not suitable for methylation analysis.

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