The association between 331A/T polymorphism in the SHH gene and 385G/A polymorphism in the SMO gene and the development of basal cell carcinomas

Związek polimorfizmów 331A/T w genie SHH oraz 385G/A w genie SMO z rozwojem raków podstawnokomórkowych skóry

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SŁOWA KLUCZOWE:
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ABSTRACT

Introduction. Basal cell carcinomas (BCCs) are the most common of all cancers in the Caucasians, and their incidence has been rising in younger populations. BCC usually occurs in sun-exposed body areas, most commonly on the head, neck and upper extremities. Experimental data have shown that the sonic hedgehog pathway may be involved in BCC development, but the mechanism by which activation of the hedgehog pathway leads to carcinogenesis is still not clear.

Objective. The aim of the study was to assess the association between 331A/T polymorphism in the SHH gene and 385G/A polymorphism in the SMO gene and the development of basal cell carcinomas.

Material and methods. The study group consisted of 142 Caucasians with histopathologically confirmed BCC and 142 healthy volunteers as a control group. All patients were diagnosed and treated in the Department of Dermatology and Venereology in Lodz. Polymorphisms in SHH and SMO genes were assessed by RFLP-PCR.

Results. Associations between 331A/T polymorphism in the SHH gene and 385G/A polymorphism in the SMO gene and the development of basal cell carcinomas were found. Additionally, significant differences in genotype distribution of all examined polymorphisms between BCC patients and controls were detected.

Conclusions. The sonic hedgehog pathway is implicated in the etiopathogenesis of basal cell carcinoma, the most common human cancer. A better understanding of the molecular background of this pathway might be useful for introduction of new therapeutic methods.

STRESZCZENIE

Wprowadzenie. Raki podstawnokomórkowe (ang. *basal cell carcinoma* – BCC) to najczęstsze nowotwory u ludzi rasy kaukaskiej. W ostatnich latach obserwuje się wzrost częstości ich występowania w coraz młodszych grupach wiekowych. Większość BCC zlokalizowana jest w okolicy eksponowanej na promieniowanie ultrafioletowe, tj. na głowie, szyi i kończynach górnych. Badania eksperymentalne wskazują na udział

szlaku transdukcji *sonic hedgehog* w rozwoju tych nowotworów, jednak dotychczas nie przeprowadzono dogłębnej analizy wpływu polimorfizmów w genach tego szlaku na rozwój BCC.

Cel pracy. Określenie związku polimorfizmu 331A/T w genie SHH oraz 385G/A w genie SMO z rozwojem BCC.

Materiał i metodyka. Grupę badaną stanowiły 142 osoby z rozpoznanym histopatologicznie BCC oraz 142 zdrowych wolontariuszy – grupa kontrolna. Wszyscy pacjenci byli diagnozowani w Poradni Kliniki Dermatologii i Wenerologii Uniwersytetu Medycznego w Łodzi. Polimorfizmy w genie SHH i SMO zostały zbadane przy użyciu metody RFLP-PCR.

Wyniki. Wykazano związek polimorfizmu 331A/T w genie SHH oraz 385G/A w genie SMO z rozwojem BCC. Dodatkowo zaobserwowano różnice w rozkładzie genotypów analizowanych polimorfizmów w grupie osób z rakiem w porównaniu z grupą kontrolną.

Wnioski. Szlak *sonic hedgehog* jest niewątpliwie związany z rozwojem najczęstszych nowotworów – BCC. Lepsze zrozumienie molekularnego podłoża tego szlaku może w przyszłości zostać wykorzystane do wprowadzenia nowych metod terapeutycznych.

INTRODUCTION

Basal cell carcinoma (BCC) is the most common of all cancers in Caucasians, and the most common human malignancy in general. BCC is typically a disorder of the elderly, but its incidence has been rising in younger populations in recent years [1].

BCC is slow growing, with local malignancy and a destructive characteristic, although it is rarely metastatic [2]. The most common form is the nodular variety, which accounts for 40–60% of BCCs [3]. It is known that this clinical subtype usually occurs in sun-exposed body areas, most commonly on the head, neck, and upper extremities. This may be the confirmation that ultraviolet radiation (UVR) is the main environmental risk factor for this tumor [4]. UVB radiation damages DNA and its repair system and alters the immune system, resulting in progressive genetic alterations and the formation of neoplasms [5]. Experimental data indicate that a range of mutations are caused by UVR, and in one of them, the hedgehog intercellular signaling pathway genes are activated. The hedgehog signaling pathway was first described in genetic studies of embryonic mutants of the fruit fly *Drosophila melanogaster*, although it is more complex in vertebrates than in drosophila. In mammals, three hedgehog homologue proteins have been identified: Sonic hedgehog (SHH), Indian hedgehog (IHH), and Desert hedgehog (DHH). SHH is the most commonly expressed and well characterized. SHH activates the SHH signaling pathway by binding to the membrane receptor Patched (Ptch1)/Smoothened (SMO). In the next stage, SMO protein is released and acts as a transcriptional activator through the Gli nuclear factor, which moves from the cytoplasm to the nucleus. This results in controlled cell proliferation and differentiation through the activation of target genes [5].

Under physiological conditions, the sonic hedgehog pathway is responsible for the regeneration of damaged tissues, regulation of cell proliferation and embryogenesis [6]. Reports indicate that these pathways particularly determine the development of the neural tube, extremities, intestines, lungs, hair follicles, teeth, and eyes [7–13]. Recent studies suggest that the disruption of the sonic hedgehog pathway is not only associated with various human congenital anomalies, but may also result in cancer [14]. Experimental data have shown that the sonic hedgehog pathway may also be involved in BCC development, but the mechanism by which the activation of the hedgehog pathway leads to carcinogenesis is still not clear.

OBJECTIVE

The aim of the study was to evaluate the association between the 331A/T polymorphism in the SHH gene and the 385G/A polymorphism in the SMO gene and the development of basal cell carcinomas.

MATERIAL AND METHODS

The study group consisted of 142 Caucasians (71 males, 71 females) with histopathologically con-

firmed BCC and 142 healthy volunteers (70 males, 72 females) as a control group. All patients were diagnosed and treated in the Department of Dermatology and Venereology at Lodz Medical University and gave their written consent for participation in the study. The volunteers were generally healthy with a negative personal and familial history of skin cancer. DNA from the peripheral blood of BCC patients and volunteers was isolated by Genomic Maxi AX (A&A Biotechnology, Gdansk, Poland). Genomic DNA supposed to contain the polymorphisms to be analyzed was amplified by polymerase chain reaction. The polymorphisms in the SHH and SMO promoter regions were assessed by RFLP-PCR.

Statistical analysis

Statistical analysis was performed using Statistica software. The results were considered statistically significant for $p < 0.05$. Assessment of the individual genotypes coexisting with the disease and other characteristics was performed using the odds ratio (OR). Logistic regression was used to assess the relationships between the dependent and independent variables. The frequencies of genotypes and alleles in the studied population were analyzed for deviation from Hardy-Weinberg equilibrium and tests for association were performed.

RESULTS

The distribution of genotypes in both groups was consistent with the Hardy-Weinberg equilibrium.

Analysis of the distribution of genotypes for polymorphism 331A/T showed that the AA genotype occurs with a frequency of 50% in the group of patients with BCC and of 78.9% in the control group. Genotype TT was observed in 33% of patients with BCC and in 4.9% of controls; the numbers for AT were respectively 16.4% and 16.2%. Statistical analysis showed the significantly more frequent presence of AA genotype and of the rarer TT genotype in the control group (Table 1).

The distribution of the 385G/A genotype polymorphism was statistically significantly different between the control group and the BCC group. Genotype GG occurred in 47.9%, genotype AA in 30%, and GA in 22.1% of the BCC group, while in the control group genotype GG occurred with a frequency of 24.6%, AA with 53.5%, and GA with 21.8%. In patients with BCC, the presence of the GG genotype was statistically significantly more frequent than in control group, in which the AA genotype was observed more frequently (Table 1).

Multiple logistic regression analysis showed that the 331A/T genotype of SHH increases the risk of BCC development by a factor of more than 20 (OR = 20.3; $p < 0.0001$) and the AT genotype by more than 3 times (OR = 3.16; $p = 0.0254$).

Furthermore, it was shown that the GG genotype of polymorphism 385G/A increases the risk of BCC development more than fivefold (OR = 5.49; $p = 0.0003$). The presence of the GA genotype also increases the risk of BCC, though this association was not statistically significant (Table 2).

DISCUSSION

In recent years, an increasing incidence of BCC has been seen. This has resulted in many studies on the pathogenesis of BCC and attempts to implement new therapeutic methods. The genetic background of BCC has been suggested, but no specific information has been available on the specific genes responsible for its development.

More recently, experimental data have shown that the sonic hedgehog pathway may be involved in BCC formation. Ling *et al.* [15] and Xie *et al.* [16] have demonstrated that genetic mutations in the sonic hedgehog pathway are present in 70% of sporadic BCCs.

Table 1. Distribution of investigated genotypes

Tabela 1. *Rozkład analizowanych genotypów*

Variable	Category	BCC		Control group	
		N	%	*N*	%
SHH	AA	71	50	112	78.9
331A/T	TT	47	33.1	7	4.9
	AT	24	16.9	23	16.2
SHH	CC	116	81.7	13	9.2
349T/C	TC	10	7	28	19.9
	TT	16	11.3	100	70.9
SMO	GG	67	47.9	35	24.6
385G/A	AA	42	30	76	53.5
	GA	31	22.1	31	21.8

Table 2. Associations between BCC development and gene polymorphisms
Tabela 2. *Związek polimorfizmu genów z rozwojem BCC*

Variable	Category	OR	–95% CI	+95% CI	Value of *p*
SHH	AA	Ref.			< 0.0001
331A/T	AT	3.16	1.15	8.67	0.0254
	TT	20.3	5.43	75.5	< 0.0001
SHH	TT	Ref.			< 0.0001
349T/C	TC	2.99	1.03	8.67	0.0434
	CC	87.9	32.6	237	< 0.0001
SMO	AA	Ref.			0.001
385G/A	GA	1.62	0.58	4.57	0.3577
	GG	5.49	2.19	13.7	0.0003

OR – odds ratio, CI – confidence interval

In this study, we confirmed the association between the occurrence of some genotypes and the analyzed polymorphisms in SHH and SMO genes and the development of BCC. We found that the presence of the TT genotype in the 331A/T polymorphism of the SHH gene is associated with an increased risk of cancer development by a factor of more than 20. Published reports on the association between SHH polymorphisms and BCC are scarce. Jorgensen *et al*. [17] investigated the rs7799059, rs7777470, rs2363923, and rs7776456 polymorphisms of the SHH gene but did not confirm a relationship between their presence and an increased risk of BCC. A similar observation concerned rs12540568, rs10954231, and rs6953598 polymorphisms in the SMO gene. In our study, the presence of GG genotype in the 385G/A polymorphism of the SMO gene led to more than a fivefold increase in risk of BCC development. To the best of our knowledge, there are no published reports that confirm our results.

The polymorphisms of the sonic hedgehog pathway that have been most commonly investigated are those pertaining to the Ptch1 gene. Asplund *et al*. [18] and Liboutet *et al*. [19] confirmed the occurrence of some genotypes and polymorphisms of the Ptch1 gene and their association with the development of BCC.

Changes in gene sequences due to mutations have also been recently investigated in a great number of studies. Xie *et al*. [16] reported the identification of activating somatic missense mutations in the SMO gene in sporadic BCC in three patients. In addition, skin abnormalities similar to BCC developed in transgenic mice overexpressing mutant SMO, which may be evidence that mutated SMO can function as an oncogene in BCC.

In another study, Tojo *et al*. [20] analyzed SMO expression in a Japanese population. They found that the expression of SMO mRNA was enhanced in BCC in comparison to normal skin. Furthermore, they observed that the SMO mRNA expression might be associated with BCC progression, and they divided histopathologic types of BCC into two subtypes: superficial and nodular.

Lesiak *et al*. [5] confirmed the increased immunoexpression of the SHH and SMO proteins in a group of 41 patients with BCC, as compared to normal controls.

A very interesting observation has been made by American researchers [21], who suggested that the deregulation of the sonic hedgehog signaling pathway in epidermal keratinocytes is the primary event leading to the formation of BCC. Their study demonstrated that the overexpression of SHH in HaCaT keratinocytes grown in organotypic cultures induced a basal cell phenotype [21].

Further confirmation of the role of the sonic hedgehog pathway in BCC development is the efficacy of vismodegib, which is a first-in-class small-molecule inhibitor of SMO. Sekulic *et al*. analyzed a group of 33 patients with advanced basal cell carcinoma treated with vismodegib, showing a 58% confirmed response rate and a median duration of response of 12.8 months after treatment [22]. The study of Suruchi *et al*. [23] gave a similar observation on the efficacy of vismodegib.

CONCLUSIONS

Our own data show that sonic hedgehog pathway deregulation may play a significant role in skin carcinogenesis, leading to BCC development. Most certainly, further complex multicenter studies are required to confirm these results. A better understanding of the role of impairment of the SHH signaling pathway in this population may lead to an effective strategy for the prevention of BCC development in the general population.

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