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Association between Toll-like receptor 7 Gln11Leu single-nucleotide polymorphism and basal cell carcinoma

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Abstract. Non-melanoma skin cancers (NMSC) are the most common form of human skin cancer. The majority of NMSC are basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) with a BCC:SCC incidence ratio of 4:1 in immunocompetent patients. Toll-like receptors (TLRs) are transmembrane glycoproteins that recognize pathogen-associated molecular patterns and damage-associated molecular patterns, against which they activate the innate immune response and initiate the adaptive immune response. Genetic variations of these receptors can alter the immune system and are involved in evolution and susceptibility of various diseases, including cancer. Imiquimod, an agonist of TLR7, is applied topically in the treatment of premalignant and malignant skin disorders, in particular BCC. The high efficacy of this TLR7 agonist toward BCC supports a possible role of this receptor in the induction of BCC and, consequently, polymorphisms of this receptor could be responsible for a greater or lesser susceptibility to BCC. The aim of the present study was to evaluate whether the presence of the functional *TLR7* rs179008/Gln11Leu promoter polymorphism conferred an increased susceptibility to BCC. A case-control study with 177 BCC cases and 158 controls was performed to highlight the possible association between this polymorphism and the susceptibility to BCC. As the *TLR7* gene is localized on chromosome X, the allelic frequency of this polymorphism was analyzed separately in males and females. The analysis of the distribution of frequencies of wild-type *TLR7* and variant *TLR7* carrying the single-nucleotide polymorphism (SNP) rs179008 in patients with BCC and healthy subjects did not reveal any statistically significant difference between cases and controls. This study does not suggest the involvement of the SNP rs179008 of *TLR7* in the susceptibility to BCC, but cannot exclude a role for TLR7 in BCC carcinogenesis considering the high efficacy of the TLR7 agonist, imiquimod, in the treatment of this neoplastic disorder.

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Introduction

Non-melanoma skin cancers (NMSC) are the most common form of human skin cancer. The majority of NMSC are basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) with a BCC:SCC incidence ratio of 4:1 in immunocompetent patients (1). NMSC are an increasing problem for health care services worldwide. Incidence rates of NMSC continue to increase, possibly due to a combination of factors, notably an increased exposure to sunlight and ultraviolet (UV) radiation, increased longevity, ozone depletion and in certain cases, immunosuppression.

BCC accounts for 80% of NMSC (2) and has become more frequent in younger patients (<40 years) although the average age at first diagnosis is ~60 years (3). The major established environmental risk factors for BCC are skin type and exposure to UV radiation. Other risk factors include immunosuppression, exposure to arsenic, scars and hereditary disorders such as nevoid BCC syndrome (Gorlin-Goltz syndrome) and xeroderma pigmentosum (4). Although there are numerous histological subtypes, a simplified classification divides all BCC into three histological subtypes: Superficial, nodular and infiltrative (5). BCCs have an extremely low metastatic potential and in the majority of cases they may be treated with local therapies, notably surgical excision, photodynamic therapy, cryotherapy or topical imiquimod (6).

Toll-like receptors (TLRs) constitute a family of receptors that directly recognize a wide spectrum of exogenous ligands [pathogen-associated molecular patterns (PAMPs)] and endogenous damage-associated molecular patterns (DAMPs) (7). The human TLR family consists of ≥10 members: TLR1, 2, 4, 5, 6 and 10 are usually expressed on the cell surface, whereas TLR3, 7, 8 and 9 are mainly expressed on the surfaces of endosomes, lysosomes and the endoplasmic reticulum (8). TLRs are implicated in the innate and adaptive immune response (9-11), and also in cellular proliferation, differentiation, mitosis, cell-cycle regulation, apoptosis, angiogenesis and tissue remodelling (12,13). TLRs activate several signaling

pathways that are responsible for the induction of nuclear transcription factors (14). Genes induced by the activation of TLRs encode for several inflammatory cytokines, notably tumor necrosis factor-α (TNF-α), including interferon (IFN) 1, interleukin-6 (IL-6), IL-1, granulocyte-colony stimulating factor, and different chemokines, including CCL2 and CXCL10 (7,14). Certain TLRs have been associated with the pathogenesis of several inflammatory and autoimmune skin diseases (15). Furthermore, their role in tumor development and progression has attracted increasing attention recently (11). Imiquimod, an agonist of TLR7 (16) has been shown to induce the expression of several cytokines such as IFN-α, IL-12 and TNF-α, which mediate its anticancer effects (pro-apoptotic, anti-invasive and anti-angiogenic) (17). It may be applied topically in the treatment of premalignant and malignant skin disorders, in particular BCC (17,18). The high efficacy of this TLR7 agonist toward BCC supports a possible role of this receptor in the induction of BCC, and consequently, polymorphisms of this receptor could be responsible for a greater or lesser susceptibility to BCC.

In the present study, a functional single-nucleotide polymorphism (SNP) within the promoter of *TLR7* gene (SNP rs179008/Gln11Leu) was investigated in order to determine whether the carriers of this variant were more or less susceptible to BCC.

Materials and methods

Patients. A prospective case-control study with 177 cases of histologically confirmed BCC and 158 controls from healthy blood donors was performed. The study comprised 177 Caucasian patients who underwent surgical excision for BCC, 104 males and 73 females, with an average age of 76.8 years, were recruited at the Unit of Dermatology and the Unit of Plastic Surgery of the University of Padova (Padova, Italy) (Table I). Participants provided written informed consent and the study was approved by the local research ethics committee. Blood samples from 158 normal healthy Caucasian individuals, 69 males and 89 females, were used as the controls.

Polymorphism analysis. The *TLR7* gene is located on chromosome Xp22.2. The *TLR7* exon polymorphism SNP rs179008 (A>T), base pair 17,961 relative to start codon ATG on exon 3, was analyzed. DNA was extracted from a peripheral blood sample and the rs179008 *TLR7*-SNP genotyping was performed by TaqMan allelic discrimination using the assays C_2259574_10 (Applied Biosystems, Foster City, CA, USA). The two alleles were scored using primers and TaqMan minor groove binder probes labeled with VIC and FAM dye (forward, 5'-CTT TCA GGT GTT TCC AAT GTG GAC-3' and reverse primers, 5'-CCC CAA GGA GTT TGG AAA TTA GGA T-3'; probes, 5'-TGA AGA GAC AAA TTC-3' and 5'-ACT GAA GAG ACT AAT TC-3'; the underlined character indicates the polymorphism).

Polymerase chain reaction (PCR) was performed according to the manufacturer's protocols on the StepOnePlus™ Real-Time PCR system (Applied Biosystems).

Statistical analysis. The genotypes obtained were statistically analyzed by applying the χ^2 test. The allele frequency of rs179008 *TLR7*, which is an X-linked gene, was analyzed separately for males and females, as well as overall frequency in the BCC patient and control groups.

Table I. Distribution of patients and genotype frequencies of the *TLR7* Gln11Leu single-nucleotide polymorphism.

Polymorphism	Basal cell carcinoma, n (%)	Controls n (%)
TLR7 rs179008 (A>T)		
Females		
AA	47 (64.38)	50 (56.18)
AT	19 (26.03)	37 (41.57)
TT	7 (9.59)	2 (2.25)
Males		
A	82 (78.85)	57 (82.61)
T	22 (21.15)	12 (17.39)

TLR7, Toll-like receptor 7.

Table II. Allelic distribution of the *TLR7* Gln11Leu single-nucleotide polymorphism.

Polymorphism	Basal cell carcinoma, n (%)	Controls n (%)
TLR7 rs179008 (A>T)		
Females		
A	113 (77.40)	137 (76.97)
T	33 (22.60)	41 (23.03)
Males		
A	82 (78.85)	57 (82.61)
T	22 (21.15)	12 (17.39)
Total A	195	194
Total T	55	53

TLR7, Toll-like receptor 7.

Results

Distribution of allele frequencies between BCC cases and controls. No statistically significant differences were observed in the distribution of allele frequencies between the BCC cases and controls (P=0.97) (Table II). Similar results were identified in males (P=0.54) and females (P=0.93) (Table II). Therefore, the present results do not suggest the involvement of the SNP rs179008 of *TLR7* in the susceptibility to BCC, but cannot exclude a role for *TLR7* in BCC initiation and progression.

Discussion

The role of TLRs in cancer development and progression has been investigated previously. TLRs have been reported

as expressed in different cancer cell lines and TLR polymorphisms have been associated with different types of neoplasms (12,19,20). In healthy tissues, activation of TLRs may cause chronic inflammation, which has been demonstrated to represent a favorable environment for tumor initiation and progression (21-24). The role of TLRs in cancer is therefore ambiguous. TLRs have been found to be overexpressed in certain pre-malignant conditions and neoplasia (25-28), responsible for increasing angiogenic and metastatic potential of tumors activating nitric oxide synthase 2 and cyclooxygenase 2, and upregulating vascular endothelial growth factor and transforming growth factor-β within the tumor microenvironment (29-31). By contrast, TLRs can cause tumor inhibition (32-35) and TLR ligands can have an anticancer effect, as demonstrated by studies on TLR7/8 and TLR9 agonists (36-38). In particular, TLR7 and TLR8 have emerged as key targets in immunotherapy. They are expressed on the endosomal surfaces of monocytes, macrophages, dendritic cells, B lymphocytes and mast cells (39). They induce the activation of nuclear factor-κB, which leads to the transcription of cytokines and chemokines through a myeloid differentiation protein 88-dependent pathway. Topical imiquimod was the first Food and Drug Administration approved drug acting as an agonist of TLR7 (40,41). Several studies reported high efficacy of imiqimod for the treatment of superficial BCC with a cure rate ranging from 43-94% (42-45), whereas it is less efficacious for nodular BCC with a cure rates of 50-65% (43,46). Imiquimod has also been used in the treatment of warts, actinic keratosis, Bowen's disease and lentigo maligna melanoma (17,18). In addition to imiquimod, other topical agents, such as nicotinamide, all-trans retinoic acid, adapalene, zinc and sodium tosylchloramide have also been reported to act through TLRs (47).

Genetic variations in TLRs may alter host immune responses and lead to a different susceptibility to inflammatory disorders, autoimmune diseases and cancer (12). Certain TLR polymorphisms have been already analyzed in order to assess their impact on severity and prognosis of several autoimmune-inflammatory diseases and cancer (12). In the present study, the SNP rs179008 of the *TLR7* gene, localized on chromosome X 22.2 at the ATG start codon of exon 3, was investigated. It is a functional non-synonymous polymorphism that consists in the replacement of an adenine with a thymine (A/T) in the DNA, resulting in the substitution of the glycine with a leucine in position 11 of the polypeptide chain of the *TLR7* (Gln11Leu) (48). To evaluate whether the presence of this polymorphism may confer an increased susceptibility to BCC, a case-control study was performed in which the distribution of frequencies of the wild-type and variant *TLR7* carrying the SNP rs179008 in healthy subjects and in patients with BCC were analyzed. If the frequency of this polymorphism in patients with BCC had been greater compared to the healthy subjects, the SNP rs179008 of *TLR7* could be considered as a cofactor in the carcinogenesis of BCC. The present results, however, do not suggest the involvement of the SNP rs179008 of *TLR7* in the susceptibility to BCC, but cannot exclude a role for *TLR7* in BCC initiation and progression. As a TLR7 agonist (imiquimod) shows a high therapeutic efficacy in the treatment of BCC, this indicates that alterations of this receptor can be implicated in the carcinogenesis of BCC. Exogenous factors may alter the function of this TLR leading to a defect of the innate immunity response. In this regard, it has been demonstrated that heat-shock proteins (49) and microRNAs (50) are ligands for TLR7 and that exogenous factors, in particular UV radiations, may deregulate their expression (51,52). As a consequence, under certain circumstances the *TLR7* expression can be downregulated, diminishing its activity toward innate and acquired immunity, and consequently, also toward the control of UV-induced skin cancers.

In conclusion, although the genetic alteration of the *TLR7* promoter does not appear to be associated with the development of BCC, a role of TLR7 cannot be excluded. Further genetic and functional studies of this receptor and its ligands may increase the knowledge in the mechanisms underlying the carcinogenic process of BCC and other UV-related skin cancers.

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