ORIGINAL PAPERS

Adv Clin Exp Med 2006, **15**, 4, 595–598
ISSN 1230-025X

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Lack of Association Between *CD28* Gene Polymorphism and Cervical Cancer in Lower Silesian Population*

Brak zależności między polimorfizmem genu *CD28* a występowaniem raka szyjki macicy w populacji Dolnego Śląska

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Abstract

Background. The mechanisms of abnormal immune function in patients with cervical cancer are objects of several investigations. There is strong evidence that altered immunological function entails an increased risk of neoplastic disease. The *CD28* gene encodes the main T-cell costimulatory molecule. Dysregulated CD28 expression has been reported in several neoplastic diseases, among them cervical cancer.
Objective. Estimation of the association between *CD28* gene polymorphism and cervical cancer.
Material and Methods. Fifty patients with cervical cancer and 72 healthy subjects were examined. The T/C transition at position 17 in intron 3 was genotyped by polymerase chain reaction followed by labeling with a SNaPshot kit and detection using a capillary genetic analyzer.
Results. The genotype, allele, and phenotype frequencies did not differ significantly between cervical cancer patients and controls.
Conclusions. The present study was unable to reveal any association between *CD28* gene polymorphism and cervical cancer (**Adv Clin Exp Med 2006, 15, 4, 595–598**).

Key words: *CD28* gene polymorphism, cervical cancer.

Streszczenie

Wprowadzenie. Mechanizmy zaburzeń funkcji immunologicznych u chorych na raka szyjki macicy są przedmiotem wielu badań. Wiele dowodów wskazuje na istnienie zależności między ryzykiem wystąpienia choroby nowotworowej a nieprawidłowościami funkcji układu odpornościowego. Gen *CD28* koduje cząsteczkę odgrywającą kluczową rolę w kostymulacji komórek T. Zaburzenie ekspresji CD28 stwierdzono w wielu chorobach nowotworowych, między innymi w raku szyjki macicy.
Cel pracy. Ocena zależności między polimorfizmem genu *CD28* a występowaniem raka szyjki macicy.
Materiał i metody. Badania przeprowadzono u 50 chorych na raka szyjki macicy i 72 zdrowych osób. Wymiana T/C w pozycji 17 w intronie 3 była genotypowana z użyciem reakcji PCR. Następnie produkty PCR znakowano techniką SNaPshot i identyfikowano z użyciem sekwenatora kapilarnego.
Wyniki. Częstość poszczególnych genotypów, alleli i fenotypów nie różniła się między grupą chorych na raka szyjki macicy i grupą kontrolną.
Wnioski. Nie stwierdzono zależności między polimorfizmem genu *CD28* a występowaniem raka szyjki macicy (**Adv Clin Exp Med 2006, 15, 4, 595–598**).

Słowa kluczowe: polimorfizm genu *CD28*, rak szyjki macicy.

* This work was supported by the State Committee of Scientific Research (KBN, Poland) Grant No 2P05C01128.

Cervical cancer is the third most common cancer among women worldwide. Epidemiological evidence has long suggested an etiology of sexual transmission. Numerous studies have addressed the association between infection agents (mainly human papillomavirus – HPV) and cervical neoplasia [1]. The high prevalence of HPV infection in young healthy women compared with the low incidence of cervical neoplasia and the low progression rate of untreated pre-invasive lesions support the existence of other cofactors in cervical carcinogenesis [2, 3]. Several studies have reported high rates of cervical cancer among women with defects of the immune system [4].

Recently there has been a growing appreciation of the importance of the costimulatory and inhibitory regulation pathways in normal and disease-related cellular immune function. An optimal T-cell activation requires two signals, the first through the ligation of the TCR/CD3 complex and the second, costimulatory signal delivered by direct interaction between T-cell costimulatory molecules and their ligands on antigen-presenting cells (APCs). Triggering TCR/CD3 alone, in the absence of the costimulatory signal, not only fails to induce proliferation, but also leads to a state of hyporesponsiveness, anergy, or apoptosis.

The pivotal costimulatory molecule, CD28, is expressed on CD3 thymocytes, 95% of CD4+ T cells, and approximately 50% of CD8+ T cells, malignant plasma cells, and γδ T cells. Binding of CD28 by its ligands B7 (CD80 or CD86) costimulates T-cell proliferation, cytokine production, and the generation of cytotoxic T lymphocytes. It is known that both TCR/CD3 and CD28 ligation upregulate IL-2 mRNA and increase IL-2 secretion and T-cell proliferation [5]. Targeted deletion of *CD28* gene in mice results in pronounced immune deficiency and impaired lymphokine secretion after stimulation with concavalin A or superantigen. Furthermore, CD28-deficient mice exhibit lower levels of certain isotypes of immunoglobulins, and germinal centers are not formed in response to immunization. Abnormal expression of CD28 antigen on peripheral blood T lymphocytes was reported in several autoimmune and neoplastic diseases [6]. The aim of present study was to estimate the association between *CD28* gene polymorphism and susceptibility to cervical cancer.

Material and Methods

The study included 50 Polish patients with cervical cancer and 72 healthy subjects living in the Lower Silesian area aged 35–60 (mean: 42 ± 10 years). All cases of cervical cancer were histologically defined as squamous cell cancer.

Genomic DNA was isolated using the NucleoSpin®Blood kit (Marchery-Nagel, Germany) from whole frozen blood. To amplify the target sequence of DNA in intron 3 in *CD28* gene from chromosome 2, the primers F: 5' – CCT GTA TCA TTT AAT CCA CT – 3' and R: 5' – TGG AAA AGT TAC ATA AAA CC – 3' were designated. The designation was based on the complete *CD28* gene sequence derived from the NCBI Sequence Viewer. Allele identification was achieved by polymerase chain reaction (PCR) amplification of 0.5–1.0 ng of genomic DNA using the Biometra UNO-Thermoblock (Biometra, Germany). PCR was carried out in a total volume of 10 µl, containing 0.1 µM of each primer and TaqMasterMix (Qiagen, Germany) with 1 unit Taq DNA polymerase, 1 × PCR buffer (containing 15 mM of $MgCl_2$), and 200 µM of each dNTP. The PCR profile was as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles denaturation at 95°C for 1 min, annealing for 1 min at 58°C, extension for 1 min at 72°C, and a final extension for 10 min at 72°C. The amplified DNA was 198 in length.

The amplified product for SNP loci was purified and minisequenced using the commercial kit SnapShot (PE Applied Biosystems) using the primer F: 5' – TCT GGG TAA GAG AAG CAG CAC – 3'. The products of the SNaPshot reaction were analyzed on an ABI PRISM 310 Genetic Analyzer (ABI PRISM 310 capillary electrophoresis system) equipped with GeneScan Analysis Software version 3.1 of PE Applied Biosystems (USA).

Statistical Analysis

Allele, phenotype and genotype frequencies were compared between groups using Fisher's exact test. A p value of < 0.05 was considered significant.

Results

The distribution of *CD28* T17int3C genotypes, alleles, and phenotypes did not differ between patients with cervical cancer and healthy subjects (Table 1).

Discussion

A decreased expression of CD28 antigen on peripheral blood T lymphocytes was reported in patients with several neoplastic diseases, such as ovarian cancer [7], malignant melanoma [8], gastric cancer [9], chronic lymphocytic leukemia

Table 1. Genotype, phenotype, and allele frequencies of the *CD28* T17int3C gene polymorphism in patients with cervical cancer and the control group

Tabela 1. Częstości genotypów, fenotypów i alleli polimorfizmu genu *CD28* TI7int3C u pacjentek chorych na raka szyjki macicy i w grupie kontrolnej

	Patients with cervical cancer (Pacjentki chore na raka szyjki macicy) n = 50 (%)	Control group (Grupa kontrolna) n = 72 (%)
Genotype (Genotype)		
CC	2 (4)	2 (2,8)
CT	9 (18)	18 (25,0)
TT	39 (78)	52 (72,2)
Allele (Allele)		
C	13 (13)	22 (15,3)
T	87 (87)	122 (84,7)
Phenotype (Fenotyp)		
C-positive	11 (22)	20 (27.8)
C-negative	39 (78)	52 (72.2)

n – the number of studied subjects.
The values in parentheses are percentages.
The genotype frequencies were in Hardy-Weinberg equilibrium.

n – liczba badanych.
Wartości w nawiasach – %.
Częstości genotypów były w stanie równowagi Hardy'ego-Weinberga.

[10], and hairy leukemia [11]. Cosinsky et al. found decreased expression of CD28 antigen in cervical cancer patients [12]. They found also that the peripheral blood CD8+CD28+ cell count may be used as a predictive factor of clinical response to chemotherapy in patients in advanced stages of the disease. Similarly, the authors reported a reduced level of costimulatory CD28 molecule on peripheral blood T lymphocytes in cervical cancer patients. The levels of the expression of this molecule did not normalize after *ex vivo* anti-CD3 stimulation [13]. Since genetic differences may affect the level of protein expression, we estimated the association between *CD28* gene polymorphism and susceptibility to cervical cancer.

The gene encoding CD28 is located on the chromosome region 2q33. A *CD28* gene polymorphism with a thymine/cytosine substitution at intron 3 position +17 has been recently identified [14]. Up to now, *CD28* gene polymorphism was mainly studied in patients with autoimmune diseases. The studies were performed in lupus erythematous [15], multiple sclerosis [16], autoimmune thyroid diseases [14], autoimmune hepatitis [17], and atopic asthma [18, 19]. The polymorphic frequencies did not differ in studied groups of patients and controls. To the best of authors' knowledge, there is only one report regarding *CD28* gene polymorphism and susceptibility to neoplastic diseases. Piras et al. performed studies on 100 non-Hodgkin's lymphoma patients and 128 healthy controls, both groups originating in Sardinia. No association between *CD28* gene polymorphism and non-Hodgkin's lymphoma was observed [20].

The results of carried out study on cervical cancer patients did not demonstrate any differences in genotype, allele, and phenotype frequencies between patients and controls. Therefore, the abnormal CD28 molecule expression on peripheral blood T cells in cervical cancer patients which the authors recently reported could not be related to *CD28* gene polymorphism. It cannot be excluded that the abnormal CD28 expression on T cells could be due to a transcriptional block resulting from the loss of nuclear transcription factors binding to two distinct regulatory motifs in the promoter region of the *CD28* gene or abnormalities in the translation process.

It might prove useful to investigate other immune system gene polymorphisms in neoplastic diseases to make it possible to diagnose at-risk individuals in the population.

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Conflict of interest: None declared

Received: 17.03.2006
Revised: 8.05.2006
Accepted: 24.05.2006

Praca wpłynęła do Redakcji: 17.03.2006 r.
Po recenzji: 8.05.2006 r.
Zaakceptowano do druku: 24.05.2006 r.