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The impact of XPC gene single nucleotide polymorphism rs2228001 on head and neck cancer patients' response to radiotherapy treatment

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ABSTRACT

Background: Head and neck squamous carcinoma (HNSC) is the sixth most common neoplasm, with a 40–50% overall survival rate. HNSC standard treatment depends on tumor size, metastasis or human papillomavirus (HPV) status including surgery, chemotherapy, and radiotherapy. The last two may lead to defects in the tumor microenvironment and cancer cell biology as disorders in DNA damage repair systems. Here, we evaluate the correlation between single nucleotide polymorphism (SNP) rs2228001 in the *XPC* gene with the early and late adverse effects of radiotherapy, determine the distribution of the SNP and post-treatment follow-up in HNSC patients.

Materials and methods: Head and neck cancer tissues and clinical data were obtained from 79 patients. The SNP of the *XPC* gene (rs2228001) was evaluated with polymerase chain reaction — restriction fragment length polymorphism (PCR-RFLP). The chi-square test was used to determine the correlation between mutation and adverse effects occurrence.

Results/Conclusion: Single nucleotide polymorphism rs2228001 in the *XPC* gene is correlated with the early adverse effect of skin reaction and the late adverse effect of elevated C-reactive protein (CRP) levels in the HNSC patients.

Key words: head and neck cancer; XPC; damage repair systems; radiotherapy; adverse effects

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Introduction

Head and neck squamous carcinomas (HNSC) are a group of neoplasms occurring in the oral cavity, pharynx, salivary glands and larynx. HNSC risk factors include tobacco usage, alcohol abuse, inefficient oral hygiene, or human papillomavirus (HPV) type 16 or 18 infection [1]. The disease's first alarming symptoms may be an irregular and painless protrusion, ulcerations, or leukoplakia in the head and neck area. Prediction data suggests a rise in the number of incidents, especially in younger populations, with a 30% annual increase in incidence by 2030 [2]. Nevertheless, HNSC affects more than 600,000 people per year worldwide [3]. Treatment of HNSC is complex and depends on

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primary site of tumor, TNM staging, and individual patient performance status. In general, surgery, chemotherapy (CT), radiotherapy (RT), and immunotherapy (IO) in various combinations are used [4]. Patients after treatment require a long time for recovery, including time after treatment and rehabilitation [5]. Postoperative RT is recommended if a patient has one or more risk factors of relapse, such as a positive or close surgical margin, multiple positive lymph nodes, extranodal extension of cancer and often if in advanced local stage. After completion of radiotherapy course, patients may suffer from adverse effects (AE) such as skin or mucosa fibrosis or even ulceration. Moreover, xerostomia, leukopenia, higher C-reactive protein (CRP) levels, among others, are observed after radiotherapy [6, 7]. Radiotherapy impacts the tumor microenvironment (TME) since it can induce both immune suppressive and proinflammatory effects. The seriousness of changes in TME depends on many factors, and it is likely correlated with chronic inflammation of the irradiated site [8, 9]. Despite many studies on patients' clinical material, such as tumor and blood, we still cannot identify direct specific markers of disease progression and response to treatment. SNPs are promising candidates for markers due to quick and simple analysis and reported influence on cancer prognosis [10, 11]. The *XPC* (XPC complex subunit, DNA damage recognition and repair factor) gene participates in the global genome nucleotide excision repair (GG-NER) system that repairs the mismatched nucleotides. To initiate the GG-NER process, XPC protein forms a complex, which recognizes the DNA point mutations [12]. Scientific reports suggest that disturbances in the NER system may influence carcinogenesis in the premalignant state as oral lesions [13] and promote tumor growth in the cervix [11], genitourinary system [10] and breast [14]. The SNP rs2228001 that occurs in the *XPC* gene causes the substitution of adenine to cytosine on at least one of the chromosome arms. This point mutation yields to of exchanging the 939th amino acid lysine to glutamine. Previous reports suggest that SNP rs2228001 can be responsible for higher morbidity and worse AE during and after the treatment [11]. Hence, this study aims to assess the correlation of the SNP rs2228001 occurrence with adverse late effects after radiotherapy to indicate genetic markers for monitoring AE's progression and predict the treatment's outcome. Here, we investigate whether the SNP of the *XPC* gene may be a biomarker for predicting radiotherapy treatment response and adverse events after radiotherapy.

Materials and methods

Patient material

Head and neck squamous carcinoma tissues were collected from 79 patients from Greater Poland who underwent surgical tumor resection in the Department of Head and Neck Surgery, Poznan University of Medical Sciences, The Greater Poland Cancer Centre. Radiotherapy and AEs data was collected retrospectively from patients' medical records. Samples were immediately frozen and stored at –80°C until DNA isolation. The inclusion criteria involved diagnosed squamous cancer of oral cavity or larynx. The exclusion criteria for this study involved a distant metastasis, a second primary tumor, and HPV infection. The procedures were approved by the Local Ethical Committee of Poznan University of Medical Sciences (Consent no. 121/23). The characteristics of the study group are presented in Table 1.

Material homogenization and DNA isolation

Tumor tissues were homogenized with mortar and pestle with the liquid nitrogen and subsequently used for DNA extraction. Genomic DNA was extracted using a DNA Mammalian Genomic Purification Kit from Sigma-Aldrich Co. (St. Louis, USA). The concentration and purity of the isolated DNA was assessed using the spectrophotometric method. Quality Control (QC) metrics for DNA purity means are $\bar{x}_{A260/280} = 1.74 \pm 0.16$ and $\bar{x}_{A260/230} = 1.64 \pm 0.54$.

Restriction fragments length polymorphism (RFLP)

The KAPA HiFi HotStart ReadyMix (Roche, Switzerland) was used to perform polymerase chain reaction (PCR) amplification of the *XPC* gene containing the rs2228001 fragment (281 bp). Each 25 µL reaction contains 12.5 µL 2X KAPA HiFi HotStart Ready Mix, 10 µM of forward and reverse primers, 100 ng of the DNA template and PCR-grade water. The amplification started with an initial denaturation at 95°C for 3 min, 35 cycles contain-

Table 1. The clinical characteristics of the study cohort

Characteristic	Total number	%
Patients number	79	
Age at the time of surgery (years)		
Mean	64	
Median	65	
Range	36-90	
Gender		
Female	24	30%
Male	55	70%
TNM classification		
T1	3	4%
T2	17	21%
T3	31	40%
T4	28	35%
N0	28	35%
N1	19	24%
N2	22	28%
N3	10	13%
M0	79	100%
Histologic grade		
G1	13	16%
G2	52	66%
G3	14	18%
Anatomical site		
Oral cavity	58	74%
Larynx	21	26%
Smoking		
Yes	54	68%
No	24	32%
Alcohol		
Yes	17	22%
No	61	78%
SNP Variant		
AA	27	34%
AC	46	58%
CC	6	8%
Adjuvant treatment		
None	19	24%
Radiotherapy	25	32%
Chemoradiotherapy	35	44%

TNM — tumor–node–metastasis; SNP — single nucleotide polymorphism

ing denaturation 95°C for 20s, annealing 60°C for 15 s, and elongation at 72°C for 30s, followed by final extension in 72°C for 30s. The primer sequences are presented in Supplementary Table 1.

For XPC amplicon restriction digestion, we used *PvuII* (ThermoFisher, USA). Each 31 µL reaction contains 10 µL of PCR reaction products, 2 µL 10X buffer G, 1 µL *PvuII* and an appropriate volume of PCR-grade water. Incubation lasts for 2 hours. To inactivate the enzyme, 0.5 M EDTA pH 8.0, with a final concentration of 20 mM, was used. To determine the presence of SNP rs2228001 in the *XPC* gene, we performed electrophoresis in 2% agarose gel with the addition of the ethidium bromide in 1X TAE buffer; DNA bands were visualized using UV light in the ChemiDoc™ Touch Imaging System (Bio-Rad, USA). The result of gel electrophoresis is presented in the Supplementary Figure 1. The expected fragment size of the AA genotype was 281 bp, CA 131, 150, 281 bp, and CC 131, 150 bp, respectively.

Adverse effects grading

The grade of early adverse effects occurring during radiotherapy was assessed by radiotherapy specialists, based on standardized scales Common Terminology Criteria Adverse Events (CTCAE) v5 and World Health Organization (WHO) (Supplementary File — Tab. S2). Late adverse effects were classified using the CTCAE v5.0 scale. Additionally, CRP concentration was tested. CRP level > 5 mg/L was assumed elevated, according to laboratory normal range.

Statistics

The distribution of genotypes was tested for the Hardy-Weinberg equilibrium (HWE) using the χ^2 test (HWE asymptotic significance = 0.134). The association between SNPs and early and late adverse effects was estimated using the χ^2 test with Fisher's exact test (the observed numbers was ≤ 10 or one of the expected numbers was < 5). A two-sided $p < 0.05$ was regarded as significant.

Results

Single nucleotide polymorphism rs2228001 distribution and HNSC patients' follow-up

A total group of 79 patients with HNSC was recruited for the study. Figure 1. presents the distribution of SNP in a group of patients included in the study. 34% of patients represented unchanged variant of nucleotides (AA), and 66% had at least

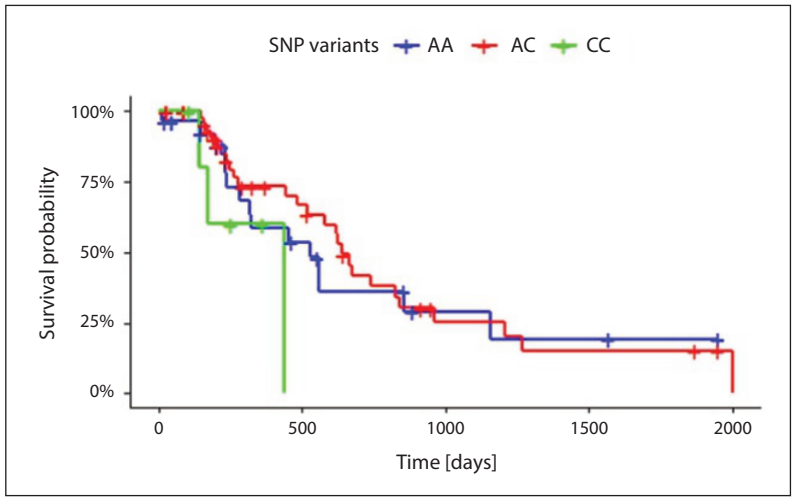

Figure 1. Kaplan-Meier curve representing the survival of the patients with single nucleotide polymorphism (SNP) rs2228001. There were 27 patients with AA variant, 46 with AC and 6 with CC, respectively

one mutation (58% AC; 8% CC). Moreover, we determined that tumor and paired-matched margin tissue had identical mutations (Supplementary File — Fig. S2).

To test whether the presence of the mutation affects HNSC patients' survival, we performed a Kaplan-Meier analysis. The follow-up was measured by the period from surgery to the last check-up or death, whatever came first. The Kaplan-Meier curve showed no significant difference ($p > 0.05$) between groups, the mean survival rate for patients with the AA variant equaled 764 days, 781days for the AC variant and 311 days for the CC variant (Fig. 1).

SNP rs2228001 occurrence has an impact on HNSC patients' post radiotherapy response effect adverse effects

Adverse effects of radiation can be divided according to the time of their occurrence. Early AEs present as dermatitis of skin of the neck and mucositis of the oral cavity and /or throat. Late AEs include chronic pain, fibrosis, xerostomia, lymphopenia, and CRP concentration. The chi-square test was performed, and it confirmed a correlation between mutation occurrence and early AE on patients' skin ($p = 0.033$) and late AE in elevated CRP levels ($p = 0.030$). The rest of the parameters measured were not correlated significantly ($p > 0.05$). The entire analysis is presented in Table 2.

Amongst patients with early AE on the skin (n = 24), 75% (n = 18) had the mutation. Late adverse effects of elevated CRP levels amongst the research group (n = 16) were presented by 44% (n = 7) of patients, and 29% (n = 2) of them had mutation (Fig. 2).

Discussion

Head and neck cancer morbidity is still rising year by year. Oncological treatment, such as radiotherapy, is very effective but also induces tissue, cellular and molecular damage in healthy tissues. Ionizing radiation directly damages the DNA helix by creating DNA breaks such as single or double strand breaks, generation of reactive oxygen species [15]. Surgery and RT are often considered as equal [3]. Technological upgrades of radiotherapy systems contributed to more beneficial outcome for patients due to scoring lower grades during AEs assessment [16].

Numerous studies prove increasing importance of DNA repair systems, especially in cancers therapy [17–19]. A better understanding of DNA repair systems may be crucial to describe novel biomarkers of morbidity or treatment response. Some studies suggest that overexpression of DNA repair systems related genes such as XPC (that participate in NER mechanism) may be a cause of platin-based drugs resistance [20]. Moreover, the higher expression level of various NER-related genes leads to a decrease in the efficiency of platinum-based therapies in stomach [21], colon [22] and lung [23] can-

Table 2. Association between single nucleotide polymorphism (SNPs) and early and late adverse effects in the Chi-square analysis

Radiotherapy	Chi-square test		Chemoradiotherapy	Chi-square test	
	Value	Fisher exact test asymptotic significance (2-sided)		Value	Fisher exact test asymptotic significance (2-sided)
Early adverse effects					
Skin	8.505	0.033*	Skin	5.309	0.659
Mucosa	3.644	0.458	Mucosa	3.341	0.557
Late adverse effects					
Fibrosis	0.751	1.000	Fibrosis	2.548	1.000
Pain	3.543	1.000	Pain	8.105	0.188
Xerostomia	2.978	1.000	Xerostomia	1.360	1.000
LLN decreased	6.320	0.436	Lymphopenia decreased	5.448	0.198
Elevated CRP	5.864	0.030*	Elevated CRP	1.343	0.784

CRP — C-reactive protein

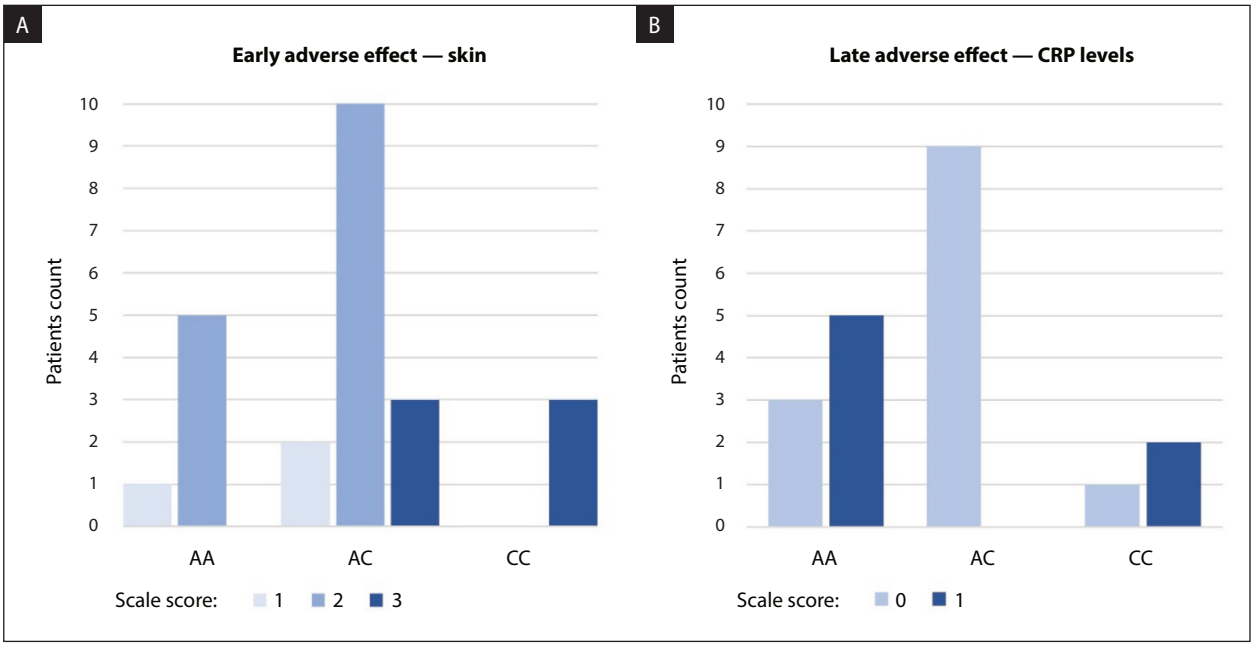

Figure 2. Cluster bars charts of significant associations between *XPC* gene SNP rs2228001 occurrence (AA — wildtype) and early (**A**) and late (**B**) adverse effects in radiotherapy-treated patients according to scale score a) assessing their skin condition (Supplementary File — Tab. S2) and **B**) 0 — C-reactive protein (CRP) < 5 mg/L; 1 — CRP > 5 mg/L

cers. However, head and neck cancers response to treatment have not yet been linked with *XPC* gene mutation occurrence. Cisplatin-based therapy has a similar molecular outcome as radiotherapy — both create DNA lesions; platin-based compounds have crosslinking properties, while irradiation creates bulks on DNA strands. XPC is responsible for DNA damage recognition, thus initiating the entire repair process [24].

Here, we assess the occurrence of *XPC* gene SNP rs2228001 in 79 patients with HNSC using the polymerase chain reaction — restriction fragment length polymorphism (PCR-RFLP) method and correlate the data with adverse effects during and after irradiation. Our results showed the correlation between the appearance of AC/CC mutation, early AE on the skin, and late AE of elevated CRP levels. However, difference in mean survival

rate in Kaplan-Meier should be considered with caution due to differences in sizes of groups (CC variant has only 6 patients).

The mechanism of radiation-induced dermatitis is also related to DNA damage and impaired mitosis. Combined with a defective NER system, patients with AC or CC rs2228001 *XPC* gene mutations have a higher probability of suffering from more advanced skin reactions after irradiation, which can deteriorate the quality of life. Due to irradiation, CRP levels may be higher due to the local inflammation process. Both cases suggest the DNA structure is damaged due to NER insufficient activity.

Conclusions

Our results indicate that XPC-deficient patients may have weakened DNA repair systems and, thus, have worse responses to radiotherapy treatment. An identical set of mutations in one patient in both types of material suggests that mutation is not gained during the carcinogenesis process. Yet, more studies are needed to confirm if these mutation symptoms apply to every cancer type. Both significant adverse effects are important factors in a patient's condition assessment during treatment.

In conclusion, this work contributes to understanding the impact of the XPC gene in radiotherapy treatment in HNSC patients. It presents the knowledge useful to work on future biomarkers of radiotherapy treatment response and personalised oncological approach.

Conflicts of interest

Authors declare no conflict of interest.

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