

Coagulation-related Factors, Thrombomodulin and Protein Z, are not Associated with Risk for Oral Cancer

ELEFThERIOS VAIRAKTARIS¹, ZOE SEREFOGLOU¹, CHRISTOS YAPIJAKIS¹,
EMEKA NKENKE², STAVROS VASSILIOU¹, SOFIA SPYRIDONIDOU¹,
ANTONIS VYLLIOTIS¹, ALEXANDER MICHAEL NIXON¹,
FRIEDRICH WILHELM NEUKAM² and EFSTRATIOS PATSOURIS³

¹Department of Oral and Maxillofacial Surgery, University of Athens Medical School,
Vas. Sofias 93 and Dim. Soutsou 1, Athens 11521, Greece;

²Department of Oral and Maxillofacial Surgery, Universität Erlangen, Klinik und Poliklinik für Mund-, Kiefer-,
Gesichtschirurgie, Glueckstrasse 11, Erlangen D-91054, Nurnberg, Germany;

³Department of Pathology, University of Athens Medical School, Mikras Asias 75, Athens 11527, Greece

Abstract. *Background: The link between thrombosis and cancer has been well established. Levels of protein Z and thrombomodulin indirectly regulate thrombin production and therefore may affect cancer susceptibility. Patients and Methods: The functional polymorphisms -13A/G and -33G/A in protein Z and thrombomodulin genes (respectively) influence transcription. The two polymorphisms were investigated in 160 oral cancer patients and 168 controls of equivalent age, gender and ethnicity using restriction fragment length polymorphism typing. Results: The frequency of the -13G allele, which results in lower expression of protein Z gene, was not significantly elevated in patients compared to controls (8.1% and 6.3%, respectively; odds ratio 1.35, 95% confidence interval 0.72-2.56). No carriers of the thrombomodulin low expression -33A allele were identified, underscoring the rarity of this allele in Caucasians. Conclusion: Inherited predisposition affecting protein Z or thrombomodulin levels does not modulate susceptibility to oral cancer. Any possible contribution of thrombin to oral oncogenesis may involve other factors.*

The link between thrombosis and cancer has been well established (1). Recently, some coagulation-related agents have been shown to influence the risk for oral squamous cell carcinoma (OSCC) and, therefore, the study of factors

Correspondence to: Dr. Eleftherios Vairaktaris, MD, DDS, Ph.D., Department of Oral and Maxillofacial Surgery, University of Athens Medical School, Vas. Sofias 93 and Dim. Soutsou 1, Athens 11521, Greece. Tel: +302106443035, Fax: +302106443803, e-mail: lvairakt@med.uoa.gr

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regulating thrombin production and activity may offer new insights in oral oncogenesis (2-4). Protein Z (PZ) acts as a cofactor for the activation of a PZ-dependent protease inhibitor, which ends the coagulation cascade by inhibiting activated factor X and reducing formation of thrombin (5). Another significant modulator of thrombin function is thrombomodulin (TM) that interacts with thrombin, activating the anticoagulant protein C pathway, which in turn reduces both activity and production of thrombin (6).

Since thrombin has been implicated in many processes enhancing tumor development, such as cell proliferation, increased cell motility and angiogenesis (1), expression levels of PZ and/or TM may influence oncogenesis as indirect regulators of thrombin production. To our knowledge, the possible role of PZ in oncogenesis has not previously been investigated. In contrast, several studies have investigated the role of TM expression in cancer (6). Interestingly, reduced TM expression has been demonstrated in severe oral mucosa dysplasia and well-differentiated OSCC (7).

Production of PZ and TM has been shown to be influenced by functional polymorphisms in their respective genes (8, 9). In the case of the PZ gene, the -13A/G polymorphism in the promoter region influences its expression (9). The less frequent G allele (range 6-12% in Caucasians) is associated with lower PZ levels (9). On the other hand, transcriptional activity in the TM gene is thought to be regulated by the -33G/A polymorphism, which is located within its promoter region (8). The frequency of the low-expression A allele is 8-10% in Asians but seems to be significantly rarer in Caucasians (<1%) (8).

In light of the above, the possible association of PZ and TM levels with oral oncogenesis was investigated by studying the prevalence of the -13A/G and -33G/A polymorphisms in patients with OSCC and in healthy subjects.

Table I. Characteristics of patients with OSCC and healthy controls.

Characteristic	Controls	Patients	Characteristics of patients (number)
Total number	168	160	Cancer Stage I, II 88
Age range (years)	31-83	40-84	Cancer Stages III&IV 72
Mean age (years)	54.7	58.6	Positive family history of cancer 60
Gender Ratio (Males/Total)	0.75	0.80	Positive family history of thrombosis 32
Males total	126	128	Tobacco abuse 150
Females total	42	32	Alcohol Abuse 52

Patients and Methods

A total of 328 individuals of Greek and German origin were enrolled, after informed consent. The groups of healthy controls and patients were comparable in regard to gender, age and ethnicity. Their overall demographic characteristics are presented in Table I. The patients with OSCC had undergone surgery within the last decade and biopsies were available confirming the diagnosis and identifying tumor stages. In addition, a family history regarding cancer and thrombophilia was obtained from all patients.

Blood samples were collected from patients and controls and DNA was isolated using the NucleoSpin™ kit (Macherey-Nagel GmbH & Co, Düren, Germany). Molecular detection of the -13A/G and -33G/A polymorphisms in the *PZ* and *TM* genes respectively was performed by restriction fragment length polymorphism typing, as described elsewhere (8, 9). In the case of *PZ*, the generated PCR product of 272 bp was cleaved by restriction enzyme Hha I into two fragments of 157 bp and 115 bp only if the G allele was present. In the case of *TM*, the PCR product is digested by endonuclease Stu I into two fragments of 235 bp and 24 bp only when the mutant A allele is present.

The genotype distribution and allelic frequencies of the whole group or subgroups of patients in regard to cancer stage, family history of cancer or thrombosis, smoking or alcohol abuse were compared to the respective ones of the control group by Fisher's exact test, using SAS® software (version 9.0; SAS Institute Inc Chicago, Illinois, USA). The age criterion for the adjustment of odds ratios (OR) has been set at 60 years. The Maentel-Haenzel method was used for the calculation of all OR with a 95% confidence interval (CI). A *p*-value less than 0.05 was considered statistically significant.

Results

The prevalence of the detected *PZ* genotypes in healthy controls and patients is shown in Table II. Neither the Greek nor the German studied populations exhibited any significant difference in allele frequencies of the -13A/G polymorphism and therefore their respective data were analyzed together. The genotype distributions were compatible with Hardy-Weinberg equilibrium in the control

Table II. Prevalence of *PZ* (-13A/G) polymorphism in healthy controls and patients with oral cancer.

Genotype	Controls (%)	Patients (%)	<i>P</i> -value	OR ^a (CI)
G/G	0 (0%)	0 (0%)	1.00	-
A/A	147 (87.5%)	134 (83.8%)		1 (reference)
A/G	21 (12.5%)	26 (16.2%)	0.08	1.35 (0.72-2.56)
Total	168 (100%)	160 (100%)		
Prevalence of G allele				
G allele frequency	6.3%	8.1%	0.08	
Carrier frequency of G allele	12.5%	16.3%	0.08	

^aAge-adjusted odds ratios.

group, as well as the whole group and all subgroups of patients.

The detected frequency of the low-expression G allele was 6.3%. No homozygotes for the G allele were observed either in the control or the patient groups, a finding in accordance with the rarity of G/G individuals in Caucasian populations (9). The G allele frequency was slightly elevated when considering all patients (8.1%), as well as in most of their subgroups (ranging 6.0-9.7%), but these findings were not statistically significant.

The A allele of the -33G/A *TM* polymorphism was not detected in any of the studied controls, underlining its rarity in Greeks and Germans in accordance with other Caucasian populations (8). Moreover patients were G/G homozygotes.

Discussion

Despite the small number of studied individuals, clearly no association between these polymorphisms and risk for oral cancer was revealed. Therefore, our findings suggest that these two thrombin-related factors are not implicated in oral oncogenesis.

Nevertheless, the role of thrombin in oral cancer has not been fully elucidated. We have previously shown that there is no association of prothrombin conversion to thrombin with risk for oral cancer (4). On the other hand, experiments on murine OSCC models have shown increased expression of protease-activated receptor-1, an important thrombin-dependent signaling mediator, which induces cell proliferation and motility, suggesting that thrombin might contribute to oral oncogenesis (10). Other mechanisms

provide both amplification and negative feedback loops that regulate thrombin-related coagulation, such as the plasminogen/plasmin feedback regulation of thrombin-activatable fibrinolysis inhibitor. A strong association of plasminogen activator inhibitor-1 with increased risk for OSCC was previously detected by our group (3). Further genetic association studies of coagulation-modulating factors will be necessary to elucidate the possible role of certain pathways in oral oncogenesis.

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