Association of *TIRAP* rs8177376 and rs8177374 Polymorphisms with Cervical Cancer Phenotype and Prognosis

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Objective

One of the most common cancers in woman worldwide is cervical cancer, which is usually caused by HPV, mostly high-type risk HPV16 and HPV18. Toll-like receptors (TLRs) signaling pathways are responsible for removing virus from the body. However, in some cases persistent infection can develop. Although the mechanism is still not fully understood, studies have been shown that signal transduction between TLRs and other components could be disrupted and become uncontrollable due to single nucleotide polymorphisms (SNPs) in genes that are involved in TLRs signaling pathways.

Therefore, the aim of this study was to analyze associations between *TIRAP* rs8177376, rs8177374 polymorphisms and cervical cancer phenotype and prognosis.

Methods

- **Patients' group**: A total of 153 female patients with cervical cancer were enrolled in this study.
- **Inclusion criteria**: Age at the time of diagnosis, tumor size (T) and grade (G), lymph node involvement (N), distant metastasis (M), disease progression and death.
- **Exclusion criteria**: Incomplete medical documentation, other malignancies and significant comorbidities.
- **Data collection:** The data was collected from medical records.
- **Methods:** For SNPs analysis blood samples were collected and subsequently used for genomic DNA extraction. PCR-RFLP assay was done.
- **The approval:** the study was approved by Kaunas Regional Biomedical Research Ethical Committee (protocols No. BE-2-10 and No. P1-BE-2-10/2014).

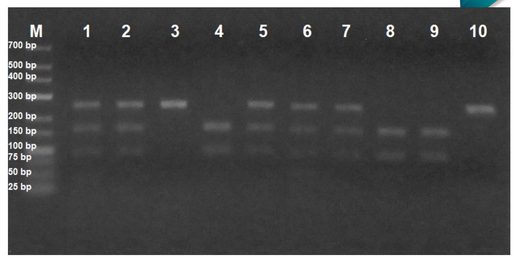

Figure 1. ***PCR-RFLP products of TIRAP rs8177376.***
From left to right. Lane M indicates DNA molecular marker GeneRuler Low Range DNA ladder (Thermo Fisher Scientific Baltics, Lithuania); TT genotype (158 and 87 bp fragments) are shown in lines 4, 8 and 9; TG genotype (245, 158 and 87 bp fragments) – lines 1, 2, 5, 6 and 7; GG genotype (245 bp fragments) – lines 3 and 10.

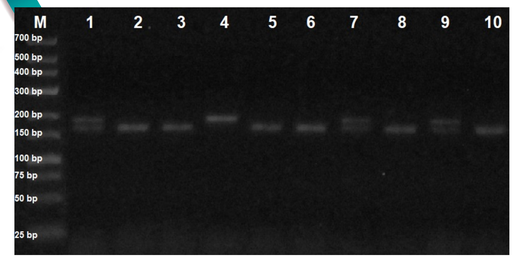

Figure 2. ***PCR-RFLP products of TIRAP rs8177374.***
From left to right. Lane M indicates DNA molecular marker GeneRuler Low Range DNA ladder (Thermo Fisher Scientific Baltics, Lithuania); CC genotype (141 and 20 bp fragments) are shown in lines 2, 3, 5, 6, 8 and 10; CT genotype (161, 141 and 20 bp fragments) – lines 1, 7 and 9; TT genotype (161 bp fragments) – line 4.

Results

In our study, the distribution of clinicopathological features of patients with cervical cancer and genotypes of *TIRAP* polymorphisms were investigated. The results are shown in Table 1 and 2, respectively.

Clinicopathological features	Subgroups and frequencies *n* (%)
Age at the time of diagnosis	≤ 50 years - 60 (39.2); > 50 years - 93 (60.8)
Tumor size (T)	T1 + T2 - 97 (63.4); T3 + T4 - 56 (36.6)
Lymph node involvement (N)	N0 (negative) - 84 (54.9); N1 (positive) - 69 (45.1)
Distant metastasis (M)	Absent - 145 (94.8); Present - 8 (5.2)
Tumor grade	G1 + G2 - 114 (74.5); G3 - 39 (25.5)
Disease progression	Absent - 108 (70.6); Present - 45 (29.4)
Death	Absent - 117 (76.5); Present - 36 (23.5)

Table 1. The distribution of clinicopathological features of patients with cervical cancer.

Polymorphism	Genotype *n* (%)		
rs8177376	TT	TG	GG
	77 (50.3)	61 (39.9)	15 (9.8)
rs8177374	CC	CT	TT
	116 (75.8)	35 (22.9)	2 (1.3)

Table 2. The distribution of genotypes of TIRAP polymirphisms.

In association analysis, the results by Pearson Chi-square test showed that rs8177376 T allele was associated with death ($p = 0.023$), however the significance was not found after logistic regression analysis ($p > 0.05$).
The analysis also revealed that an association between rs8177374 and death was significant ($p = 0.029$). The carriers of CT genotype had a 2.85 times higher risk for death (95% CI 1.254-6.469, $p = 0.029$) than those with CC genotype. Unfortunately, after multivariate logistic regression analysis an association was in loss of significance, suggesting that other factors, such as age at the time of diagnosis, tumor size, tumor grade and lymph node involvement could be more important for an association.
On the contrary, some associations were observed after logistic regression analysis even though a significant association was not determined by Pearson Chi-square test. Univariate logistic regression analysis revealed that rs8177376 T allele was more prevalent in the group of patients older than 50 years (OR = 1.654, 95% CI 1.172-2.334, $p = 0.004$). Moreover, the carriers of rs8177376 T allele had a lower risk for G3 tumor grade (OR = 0.340, 95% CI 0.232-0.499, p = 0.000) compared with non-carriers. In contrast, patients having CT genotype of rs8177374 had a higher probability for G3 tumor grade (OR = 2.427, 95% CI 1.081-5.445, p = 0.032) in comparison to CC genotype. The significance between mentioned associations were observed even after multivariate logistic regression analysis.
In survival analysis, statistically significant associations between *TIRAP* polymorphisms and OS or PFS were not determined.

Conclusions

In conclusion, the results of our study showed that *TIRAP* rs8177376 T was associated with tumor size and tumor grade, while rs8177374 CT was associated with tumor grade. Thus, we suggest that T allele of rs8177376 and CT genotype of rs8177374 might be useful in the future as genetic biomarker for prognosis of cervical cancer.

Key words

TIRAP, SNP, cervical cancer, phenotype, survival, prognosis, PCR – RFLP