The analysis of polymorphisms in the *TLR4* gene and their associations with the pathomorphological characteristics of cervical cancer and the course of the disease

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Objective

The risk of cancer is increased by additional factors that activate the immune system and cause inflammation. Toll-like receptors (TLRs) family plays essential role in the pathway of activating the immune response associated with autoimmune diseases, inflammation, and tumor-associated diseases. The signaling pathway of TLRs begins in the cytoplasmic TIR domain, contributes to the recognition of antigenic molecules such as lipopolysaccharides, nucleic acids, and activates the protein complex (NF-kB, IRFs MAP kinases) via the MyD88-dependent or MyD88-independent pathway. In this way, it regulates the production of cytokines, chemokines, type I interferon, thus eliminating antigens. Negative regulation of the signal path helps protect the host from inflammatory damage. TLRs can produce the desired antitumor effects by inducing the expression of inflammatory cytokines and the response of cytotoxic T lymphocytes. Changes in *TLR4* gene expression are involved in carcinogenesis and tumor process progression through chronic inflammation, forming a tumor microenvironment. However there is very little information on the impact of *TLR4* gene polymorphisms on cervical cancer as one of the most common oncological diseases.

We performed a study to determine the distribution of *TLR4* gene functional polymorphisms (rs7276633, rs2838342, rs2051407, rs4986791) in a group of patients with cervical cancer. Then we analyzed the correlations between genotypes and alleles with tumor pathomorphological parameters and course of the disease.

Methods

- Ninety-two patients with cervical cancer were enrolled in the retrospective study.
- Subjects were recruited from October 2014 to January 2019.
- Clinical data on patients and peritheral blood sample were collected.
- Genomic DNA was extracted from leucocytes. Molecular genetic studies were performed using the real time polymerase chain reaction (RT-PCR) method (Figure from 1 to 4).
- The obtained SNPs were used in further statistical analysis in genotype and allelic models.
- The statistical analysis was performed using SPSS.
- The associations between the genotypes and alleles with tumor characteristics were assessed using Pearson's Chi-square or Fisher's Exact tests. Univariate and multivariate analysis to present odds ratios with 95% confidence intervals (CIs) and p-values were calculated with logistic regression. Differences in PFS and OS were assessed using hazard ratios (HRs) from univariate and multivariate Cox proportionate hazard models. p-value of <0.05 was considered statistically significant for all analysis.

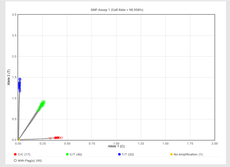


Figure 1. RT-PCR SNP assay (rs7276633).

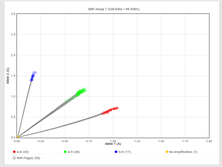


Figure 2. RT-PCR SNP assay (rs2838342).

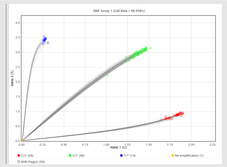


Figure 3. RT-PCR SNP assay (rs2051407).

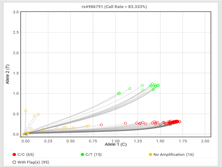
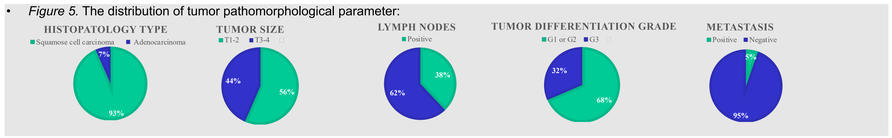


Figure 4. RT-PCR SNP assay (rs4986791).

Results

- 92 patients (mean (+/- SD age, 56.6 (+/- 11.7), 69.9% ≥50 years) were involved in the study.
- *Figure 5.* The distribution of tumor pathomorphological parameter:



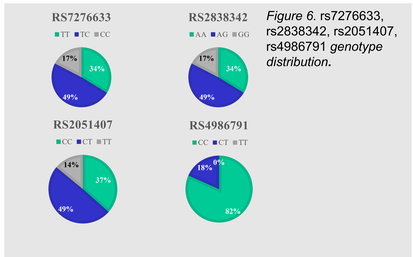


Figure 6. rs7276633, rs2838342, rs2051407, rs4986791 *genotype distribution.*

- The distribution of **rs7276633** genotypes was as follows: TT-33.7%, TC-48.9%, CC-17.4%.
  Rs7276633 CC genotype compared to TT genotype increased chance for T3-4 tumors (OR = 5.455, 95% CI: 1.414-21.035, p = 0.014).
  T allele was significantly associated with less chance for T3-4 tumors (OR = 0.194, 95% CI: 0.057-0.661, p = 0.009).
  Borderline significant association detected between CC genotype and stage III-IV (OR = 4.062, 95% CI: 0.963-17.138, p = 0.056).
  Patients with T allele were less likely to have stage III-IV cancer (OR = 0.197, 95% CI: 0.052-0.748, p = 0.017).
  Carrying the T allele statistically significantly reduced the chance of having a worse prognosis (T3-4 and G3) for cancer (OR = 0.118, 95% CI: 0.024-0.574, p = 0.008).

- **Rs2838342** genotypes are distributed as follows: AA-33.7%, AG-48.9%, GG-17.4%.
  GG genotype compared to AA genotype increased chance for T3-4 tumors (OR = 5.455, 95% CI: 1.414-21.035, p = 0.015) and likely chance for a higher stage (III-IV) of cancer (OR = 4.062, 95% CI: 0.963-17.138, p = 0.056).
  Patients with GG genotype are more likely to have a worse prognosis (T3-4 and G3) cancer (OR = 10.0, 95% CI: 1.558-64.198, p = 0.015).

- In cox regression analysis of **rs2051407** the links between C allele and PFS or OS were detected. The carriers of C allele had an decreased risk for shorter PFS than non-carriers (OR = 0.428, 95% CI: 0.183-1.000, p = 0.042).
  In a multivariate analysis this association remained significant when the adjustment for tumor differentiation (G) was done (OR = 0.401, 95% CI: 0.169-0.952, p = 0.038).
  C allele decreased chance to have shorter OS (OR = 0.416, 95% CI: 0.176-0.981, p = 0.045).

- In case of **rs4986791**, no significant link between genotypes or alleles and tumor phenotype or patient survival was detected.

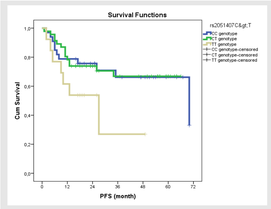


Figure 7. Kaplan_Meier curves for PFS according to **rs2051407** *genotypes.*
Patiens with TT genotype showed a trend for shorter PFS.
Log Rank, p-value = 0.08.

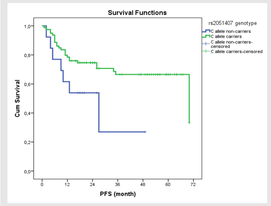


Figure 8. Kaplan_Meier curves for PFS according to **rs2051407** *alleles.*
Log Rank, p-value = 0.042.

Conclusions

The study suggests that SNPs rs7276633, rs2838342 may have the potential to be markers contributing to the assessment of the cervical cancer phenotypes. Rs2051407 may have implications for survival prognosis.

Key words

Cervical cancer, SNPs, *TLR4,* associations