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# The Role of rs2232365 Polymorphism of FOXP3 Gene in Cervical Cancer

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## Abstract

Despite significant advances in oncological therapies and diagnostical approaches, cancer remains a major cause of morbidity and mortality. Cervical cancer is the second common malignancies in women and significantly threatens the health and life of women. In the general population, the incidence of cervical cancer shows a decreasing tendency, but the morbidity and mortality of cervical cancer are still increasing in the developing countries and the cervical cancer patients becomes younger. Cervical cancer is an immunogenic malignancy, and high-risk human papilloma virus (HPV) subtypes may cause cervical cancer through several steps. Persistent infection of high-risk HPV subtypes may significantly facilitate the development of cervical intraepithelial neoplasia, it has been confirmed as a major risk factor of cervical cancer. Regulatory T cells (Treg cells) are a group of mature T cells generated in the thymus following the induction of peripheral naïve T cells. They are essential for the inhibition of immune overreaction induced damage, but over-production of Treg cells may block the protective immune response to infection and tumors. FOXP3 (Forkhead box protein P3) is a key transcription factor in regulatory T cells (Tregs), and has important roles in the immunosuppressive functions in Tregs. The role of FOXP3 gene polymorphisms in cancer patients is not determined till now. In the present study we have investigated the association of rs2232365 (A to G) of FOXP3 gene with cervical cancer. It has been revealed that rs2232365A/G polymorphism is detected in cervical cancer (25 cases, 73.53% of cancer cases, p=0.03) and CIN (36 cases, 64.29% of CIN cases, p=0.02). We assume that rs2232365 polymorphism of the Foxp3 gene may contribute to the cervical cancer development.

**Keywords:** Cervical Cancer, FOXP3, Single Nucleotide Polimorphism

## Introduction

Despite significant advances in oncological therapies and diagnostical approaches, cancer remains a major cause of morbidity and mortality. The incidence of cancer had increased both in developed and in developing countries because of increasing exposure to risk factors and life expectancy **[1]**. In many cancer cases, surgical resection to de-bulk and remove the primary tumor is the mainstay of treatment; however, metastatic recurrence is common.

Cervical cancer is the second common malignancies in women and significantly threatens the health and life of women. In the general population, the incidence of cervical cancer shows a decreasing tendency, but the morbidity and mortality of cervical cancer are still increasing in the developing countries and the cervical cancer patients becomes younger. Cervical cancer is an immunogenic malignancy, and high-risk human papilloma virus (HPV) subtypes may cause cervical cancer through several steps: cervical intraepithelial neoplasia (CIN), carcinoma in situ of cervix, invasive carcinoma of cervix and cervical cancer metastasis. Persistent infection of high-risk HPV subtypes (such as HPV16) may significantly facilitate the development of CIN (especially the CIN2 and CIN3) and has been confirmed as a major risk factor of CIN2, CIN3 and cervical cancer. For healthy female with HPV infection confirmed by cervical cytology, more than 50% of them are diagnosed with transient HPV infection by re-examination at 12 months. Whether HPV infection occurs after exposure to HPV and the outcome after HPV infection is closely related to the immune response of the host **[2]**.

Regulatory T cells (Treg cells) are a group of mature T cells generated in the thymus following the induction of peripheral naïve T cells. Treg cells are indispensable for the maintenance of non-response of host to autoantibodies and the inhibition of immune overreaction induced damage. On the other hand, over-production of Treg cells may block the protective immune response to infection and tumors. FOXP3 (Forkhead box protein P3) is a key transcription factor in Tregs, and has important roles in the immunosuppressive functions in these cells **[3]**. It has been demonstrated, that tumor cells express FOXP3 **[4-6]**. Its expression in cancer cells is an important mechanism of tumor escape **[7]**. Both tumor cells and Tregs express FOXP3, making the interactions between these cellular types complicated. Tumor cells can transform T cells into Treg, resulting in immune escape **[8-9]**; the presence of Treg cells in tumor microenvironment has been associated with poor prognosis and worst clinical outcomes **[10-11]**. The molecular features of FOXP3 should be noted. This gene is located at the short arm of X chromosome and presents complex mechanisms of expression regulation, in which conserved non-coding sequences (CNS) in intronic regions are capable to bind transcriptional factors and actuate in concert with FOXP3 promoter **[12]**. Polymorphisms in these regions could affect binding capability for transcriptional factors and, hence, FOXP3 expression and Treg function **[13]**. Among the polymorphisms described in regulatory regions of FOXP3 rs2232365 (A to G) has been associated with immunologic diseases **[14]**, highlighting its importance in Treg function, but it is not investigated in cancers. Investigation of probable association of FOXP3 gene polymorphisms in cancers may shed light on the molecular pathogenesis of cancer and open new windows to screening of susceptible individuals. To date, few studies, have investigated FOXP3 gene polymorphisms in cancer patients **[15]**. The table **(Table 1)** presenting the FOXP3 gene polymorphisms associated with oncology diseases is given below. In the present study we have investigated the association of rs2232365 (A to G) of FOXP3 gene with cervical cancer.

| FOXP3 gene polymorphism | Oncology disease |
|---|---|
| rs3761548 | Breast cancer, Colorectal cancer, Lung cancer, Thyroid cancer, Endometrial cancer |
| rs3761549 | Breast cancer, Lung cancer |
| rs2280883 | Hepatocellular cancer |
| rs3761549 | Hepatocellular cancer |
| rs2280883 | Lung cancer, Thyroid cancer, Breast cancer |
| rs5902434 | Endometrial cancer |

**Table 1:** FOXP3 gene polymorphisms associated with oncology diseases

## Methods

The study aimed performance of allele-specific polymerase chain reaction (AS-PCR) for analysis of FOXP3 gene rs2232365A/G polymorphism in collected plasma samples. For the study in May-August 2019 a total 100 plasma samples have been collected from the patients of the Research Institute of Clinical Medicine (Tbilisi, Georgia). 90 plasma samples were collected from patients with cervical cancer and cervical intraepithelial neoplasia (CIN); amongst the mentioned 90 plasma samples 34 plasma samples were collected from the patients with cervical cancer and 56 plasma samples from the patients with diagnosed CIN. 10 plasma samples of patients those were negative for intraepithelial lesion or malignancy was used as control group in the frames of our study. The responsible medical personnel of the Research Institute of Clinical Medicine have performed communication with patients as well as collection and labeling of plasma samples. Plasma samples have been provided to our research group anonymously, by labeling it wasn't possible to determine the sample category (i.e., patient with diagnosed cervical cancer / patient with diagnosed CIN / control group).

10 ml of peripheral blood were collected in EDTA syringe. The DNA has been extracted by using DNA extraction kit (Qiagen) as described by the manufacturer. The extracted DNA has been used for analysis of FOXP3 gene rs2232365A/G polymorphism by AS-PCR. The following AS-PCR primers (NorgenBiotek) were used:

FOXP3rs2232365A Allele:

Forward primer:

CCCAGCTCAAGAGACCCCA

Reverse primer:

GGGCTAGTGAGGAGGCTATTGTAAC.

FOXP3rs2232365G Allele:

Forward primer:

CCAGCTCAAGAGACCCCG

Reverse primer:

GCTATTGTAACAGTCCTGGCAAGTG.

The following parameters were used for amplification:
95˚C, 3 min
95˚C, 30 sec
66˚C, 45 sec
72˚C, 50 sec; 5 cycles
95˚C, 30 sec
61˚C, 50 sec
72˚C, 50 sec; 15 cycles
95˚C, 50 sec
61˚C, 1 min
72˚C, 1.5 min; 15 cycles
72˚C, 7 min.

Total volume was 20 µL with the following content: 10 µL Taq PCR Master Mix, 2 µL (10 pmol) of each primer, 4 µL Nuclease-Free Water, 2 µL (20 ng) of extracted DNA.

The results of AS-PCR were detected by gel electrophoresis. It has been performed by using 2% agarose gel, stained with ethidium bromide and photographed by UV transilluminator.

Statistical analysis has been performed by using SPSS v.21.0 software (SPSS Inc., Chicago, IL). A value of $p<0.05$ was considered as statistically significant.

## Results and Discussion

As it was mentioned above, the present study aimed determination of FOXP3 gene rs2232365A/G polymorphism in collected plasma samples of patients with diagnosed cervical cancer and CIN in comparison with control group. It has been revealed that rs2232365A/G polymorphism is detected in cervical cancer (25 cases, 73.53% of cancer cases, p=0.03) and CIN (36 cases, 64.29% of CIN cases, p=0.02). In control group FOXP3 gene rs2232365A/G polymorphism has not been detected (p=0.01).

Treg cells are important for cancer immunology and development of immune escape. Development of Treg cells requires continued expression of Foxp3 **[16]**, while attenuated Foxp3 expression results in its functional deficiency **[17]**. We assume that functional polymorphisms of the Foxp3 gene may contribute to the cervical cancer development.

## Conclusion

The present study revealed the probable link of rs2232365A/G polymorphism of FOXP3 gene with cervical cancer development. The limitation of the present study should not, however, be ignored. Increasing the sample size, simultaneous investigation of FOXP3 gene expression are required to further determine the role of FOXP3 gene polymorphism in cervical cancer.

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