Contribution of rs2232365 Polymorphism of FOXP3 Gene in Cervical Cancer Development

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Research note

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Abstract

Objective

Cervical cancer is the second common malignancy in women. This is an immunogenic malignancy, and high-risk human papilloma virus (HPV) subtypes may cause its development. Persistent infection of high-risk HPV subtypes may significantly facilitate the development of cervical intraepithelial neoplasia, which 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.

Results

We have investigated the association of rs2232365 (A to G) of FOXP3 gene with cervical cancer. rs2232365A/G polymorphism has been 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.

Introduction

In 21st century cancer still remains a major cause of morbidity and mortality. Cancer incidence is increased worldwide because of high exposure to risk factors and prolonged life expectancy [1]. Surgery is the main treatment approach for primary tumor removal; however it cannot diminish the risk of metastasis development.

Cervical cancer is the second most common malignancy of the female reproductive system. While the incidence of cervical cancer is decreasing, the morbidity and mortality rates of this oncologic disease are still high. Furthermore, cervical cancer patients become younger.

Cervical cancer is an immunogenic oncology disease which can be developed as a result of the persistent infection of the high-risk human papilloma virus (HPV) subtypes. The process may include the following 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) has been confirmed as a major risk factor of CIN (especially CIN2 and CIN3) and cervical cancer [2].

After exposure to HPV and as an outcome of HPV infection the immune response of the host is developed. A group of mature T cells generated in the thymus, regulatory T cells – Tregs, are responsible for the maintenance of non-response of host to autoantibodies and the inhibition of immune overreaction. However, overproduction of Tregs is inhibiting immune response during infection and

cancer. Immunosupressiv function of Tregs is regulated by the transcription factor FOXP3 (Forkhead box protein P3) [3]. The high levels of FOXP3 expression in cancer cells have been reported [4–6]; this is an important mechanism of tumor escape [7]. FOXP3 is expressed in tumor cells as well as Tregs. Furthermore, it has been reported, that tumor cells can transform T cells into Tregs and ensure immune escape [8–9]. The presence of Tregs in tumor microenvironment is associated with the worst clinical outcomes [10–11].

The molecular peculiarities of FOXP3 gene should be explained too. This gene is located at the short arm of X chromosome and is characterized by the complex mechanisms of regulation. The transcriptional factors bind to conserved non-coding sequences (CNS) in intronic regions; this mechanism ensures transcription of FOXP3 gene [12]. Polymorphisms of FOXP3 gene regulatory regions of FOXP3 gene may alter gene expression and Tregs function [13]. It has been reported, that rs2232365 (A to G) polymorphism, which is specific to the FOXP3 gene regulatory regions, is associated with immunologic diseases [14] and Treg function regulation. The role of this polymorphism in cancer has not been investigated till now. The determination of FOXP3 gene polymorphisms role in cancer is important for understanding of molecular mechanisms of cancer and for identification of the effective screening biomarker. Only several studies reported association of the FOXP3 gene polymorphisms with cancer [15]; this data are presented in table given below (Table 1). Our study has been focused on determination of the association of FOXP3 gene rs2232365 (A to G) polymorphism 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 allele-specific polymerase chain reaction (AS-PCR) has been used for determination of FOXP3 gene rs2232365A/G polymorphism in collected plasma samples. A total 100 plasma samples have been collected from the patients of the Research Institute of Clinical Medicine (Tbilisi, Georgia) in May-August 2019. 90 samples have been collected from the patients with diagnosed cervical cancer (34 plasma

samples) and cervical intraepithelial neoplasia (CIN, 56 plasma samples). 10 plasma samples have been collected from the patients negative for intraepithelial lesion or malignancy; these samples have been used as a control group. The communication with patients, collection and labelling of the plasma samples has been performed by the responsible medical staff of the Research Institute of Clinical Medicine. The plasma samples have been provided to the research group anonymously; determination of sample category (i.e., patient with diagnosed cervical cancer / patient with diagnosed CIN /control group) by labeling was impossible.

Samples, 10 ml of peripheral blood were collected in EDTA syringe. DNA has been extracted accordingly with the manufacturer's instruction by using DNA extraction kit (Qiagen). Extracted DNA has been used for AS-PCR based determination of FOXP3 gene rs2232365A/G polymorphism. The following AS-PCR primers (Norgen Biotek) 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 wich included: 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; 2% agarose gel, stained with ethidium bromide has been used. The results were photographed by UV transilluminator.

For statistical analysis SPSS v.21.0 software (SPSS Inc., Chicago, IL) has been used. A value of $p<0.05$ was considered as statistically significant.

Results And Discussion

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. rs2232365A/G polymorphism has been detected in 75.33% of cervical cancer (25 cases, $p = 0.03$) and 64.29% of CIN (36 cases, $p = 0.02$). FOXP3 gene rs2232365A/G polymorphism has not been detected in control group ($p = 0.01$).

Treg cells have the key role in the process of immune escape. Their development requires continued expression of Foxp3 [16]; attenuated Foxp3 expression results in its functional deficiency [17]. By analysis of the obtained results, we assume that polymorphisms of the Foxp3 gene may contribute to the cervical cancer development.

Conclusion

The present study revealed the link of rs2232365A/G polymorphism of FOXP3 gene with cervical cancer development.

Limitations

- This study only concentrates on the rs2232365A/G polymorphism of FOXP3 gene.
- Increasing of the target group size as well as the investigation of FOXP3 gene expression in case of rs2232365A/G polymorphism presence are required.
- The role of FOXP3 gene polymorphisms in cervical cancer development should be investigated.

Abbreviations

HPV – Human papillomavirus

Treg – Regulatory T cells

FOXP3 - Forkhead box protein P3

CIN – Cervical intraepithelial neoplasia

CNS - Conserved non-coding sequences

AS-PCR - Allele-specific polymerase chain reaction

EDTA - Ethylenediaminetetraacetic acid

DNA – Deoxyribonucleic acid

Declarations

Ethics approval and consent to participate

The present study was approved by the Bioethics Committee of Petre Shotadze Tbilisi Medical Academy (Tbilisi, Georgia). The design of the research and procedures performed in the present study were in accordance with the Helsinki Declaration of 1975, as revised in 2000. Written informed consent has been obtained for all collected samples.

Consent for publication

Not applicable

Availability of data and materials

All data generated and analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contribution

EK designed the experiment and analyzed obtained data. TM supervised collection of blood samples and performed the analysis. EK and TM equally contributed to the present article preparation. Both authors, EK and TM, read and approved the final manuscript.

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