БІОЛОГІЧНІ НАУКИ

Borsuk M.M.
student,
Taras Shevchenko Kiev National University

DETERMINATION OF METHYLATION STATUS OF FOXP1 GENE PROMOTOR IN HUMAN COLORECTAL CANCER SAMPLES

Colorectal cancer is the third most widespread form of cancer and the fourth most widespread reason of death in the world [1]. The most common subtype of CRC is adenocarcinoma with variation in structure and amount of glandular tissue. As adenocarcinomas are about 90-95% of all CRC cases, it is important to investigate causes of this type of colorectal cancer. Exploring molecular genetic mechanisms of this type of cancer is necessary for development of methods of early diagnosis of this disease [2]. It was shown FOXP genes somatic misregulation and/or mutation are associated with cancer because of their central role in signalling pathways and in the regulation of homeostasis [3]. One of the types of somatic misregulation during the pathogenesis of CRC is a violation of epigenetical regulation in cells, leading to abnormal methylation of regulatory regions of onco-suppressor genes and block of their functions [4]. One of these genes-candidates for onco-suppressor genes is FOXP1, which role in cancerogenesis is still not clear nowadays.

The aim of this research was to determine a methylation status of FOXP1 gene promotor region in human colorectal cancer in comparison with normal surrounding tissues.

22 CRC samples of all stages and their adjacent normal tissues were collected from the National Institute of Cancer and annotated with appropriate clinicopathologic data. Fresh frozen tissues were stored at -80^{o}C. DNA was extracted from the tumours using Sigma-Aldrich™ TRI reagent Kit according to the manufacturer's instructions. Estimating the amount and quality of extraced DNA was performed by measurement of the ratio of the optical densities at wavelengths A260 and A280 and performing agarose 0,8% gel electrophoresis.

The analysis of methylated promotor regions of FOXP1 gene was completed using MSP – methylation specific PCR. Previous bisulphate processing of DNA was performed using EZ DNA Methylation Kit. Then PCR with specific primers to the methylated and to unmethylated region was performed. Products of MSP with primers to the methylated promotor region were analyzed with 8% polyacrylamidic gel electrophoresis and products of MSP with primers to the unmethylated promotor region were analyzed with 1,5% agarose gel electrophoresis.

Quality of extracted DNA from tumour sample pairs was similar among the samples and adequate for analysis – the ratio of the optical densitities was among 1,7 and 1,9, which has testified about purity of the extracted DNA.

Performed MSP with primers to the methylated region of the promotor of FOXP1 gene showed that PCR was successful, the length of products corresponded to the desired size and that in 8 of 22 tumour samples promotor region was methylated. It has also shown that in all the normal tissue samples and 14 tumour samples, where weren't any PCR products, was the absence of

methylation or presence of deletions, which could cause the same result.

MSP with primers to the unmethylated region of the promotor of FOXP1 gene showed that PCR was successful, the length of the products corresponded to the desired size. Positive result was obtained in all unmethylated tumour samples and in all samples from normal tissue. This has shown the absence of deletions in those samples.

One of the causes of CRC development is an abnormal methylation of regulatory regions of suppressor genes, (that) leads to the decrease of their expression. To these onco-suppressor genes belongs FOXP1 gene, which is located in the locus 3p14.1.

In the analysed samples we have found that the methylation of the promotor region of FOXP1 gene was present in 8 of 22 CRC samples, which constituted 36,4%. This indicator co-ordinates with the analysis of the NotI microchips of the human colorectal cancer (33,3%). This data can be used for the early diagnosis of human colorectal cancer.

References:

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