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RESEARCH ARTICLE

MIR-378 TARGET GENE TGFB2 IN THE STAGE II COLON CANCER TISSUE.

Cigdem gungormez.
Central Research Laboratory, Harran University, Sanliurfa, Turkey.

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Abstract

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths worldwide. The sensitivity of markers used in the early and painless diagnosis of various cancers, including CRC, remains low. Recent studies have shown that non-protein coding small RNA molecules, called microRNAs (miRNA), play a key role in the mechanism of both the development of cancer and its treatment. In the development of certain types of cancer, including CRC, miRNAs have tumor suppressing and oncogenic effects. Therefore, the aim of the present study was to determine the oncogenic and tumor suppressing miRNAs profiles affecting cancer development and miRNA target gene in the tumor tissue as well as in normal tissues of patients with Stage II CRC. This study array analysis have demonstrated been for the first time in colorectal tumor tissues, 6 of the 8 miRNAs of the miR-378 family (hsa-miR-378i, hsa-miR-378c, hsa-miR-378d, hsa-miR-378e, hsa-miR-378f and hsa-miR-378g) showed that targets TGFB2. As a result; it is possible that early diagnosis of mir-378 colon cancer may have an important role in early diagnosis by investigating expression with TGFB2.

Corresponding Author: Cigdem gungormez
Address:Central Research Laboratory, Harran University, Sanliurfa, Turkey.

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Introduction:

Colorectal cancers arise in the colon and rectum. A normal cell progressively evolves into a premalignant tumor state, malignant tumor, and finally a metastatic spreading tumor, as a result of the accumulation of a number of molecular changes (Hermsen et al., 2002; Grady et al., 2004; Michor et al., 2005).

The incidence of colorectal cancer varies according to the different societies in the world, and is reported to be higher in developed countries including Turkey, when compared to developing countries (Corte et al., 2012). Colorectal cancer is the third most common cancer type worldwide. Siegel et al. (2017) reported that 157700 deaths from 600920 cancer-related deaths in the United States in 2017 were due to colorectal cancer. The incidence of colorectal cancer among all cancers in the US is reported as 9% in males and 8% in females (Siegel et al., 2017).
According data of the Ministry of Health, the incidence of colorectal cancer in females and males ranks third among all cancer types in Turkey (Url-1).

The sensitivity of markers used in the early and painless diagnosis of all cancer types, including colorectal cancer is reported to be poor. Recent researches in cancer have demonstrated that miRNAs have a great influence in the mechanisms of cancer development and treatment steps.
MicroRNAs (miRNAs) are molecules with a size of 18-24 nucleotides, found in eukaryotic cells. They are coded from the conserved DNA regions and there is no protein translation. It plays a role in the regulation of gene expression, after transcription. miRNAs, which make up approximately 3% of the human genome, regulate 30% of protein coding genes (Lim et al., 2005). Non-protein coding RNA molecules bind complementarily to the 3' end of the target mRNAs which is complementary to their nucleotide sequences and perform translational suppression or regulation of post-transcriptional gene expression by mRNA degradation (Gregory et al., 2005). They rearrange the target gene expression by base matching with specific binding sites in the 3' untranslated region (3'-UTR) of target miRNA. miRNAs can be found in the exonic and intronic regions of genes encoding proteins and in the intergenic regions. It has been demonstrated that miRNAs can regulate a single target gene through suppression, as well as being capable of affecting more than one target gene on their own (Baek et al., 2008; Selbach et al., 2008). Therefore, miRNAs play an important role in homeostatic processes such as cell proliferation, differentiation, or cell death.

This gene-targeted regulation is involved in many biological processes such as cell differentiation, apoptosis, aging, diseases, and cancer. The regulatory functions of miRNAs in gene expression and their important physiological roles in pathological processes make them important components of the genome (Grosshans et al., 2006; Filipowicz et al., 2008; Kutte and Svoboda, 2008). Some studies show that miRNA expressions are also regulated at the post-transcriptional level

TGF-β (transforming growth factor-β) are 25-kDa cytokines that play an important role such as homeostasis, angiogenesis, carcinogenesis and differentiation of the cell. TGF- β and its signalling effectors influence cancer biological behavior. The TGF-β signalling pathway has been considered as both a tumor suppressor and a cancer promoter (Lampropoulos et al., 2012).

TGF-β signaling is initiated by the binding of TGF-β ligands to type II TGF-β receptors (TGFBR2). Three TGF-β isoforms (TGFB1, TGFB2 and TGFB3) are expressed in mammalian epithelium, and each is encoded by a unique gene and expressed in both a tissue-specific and developmentally. regulated manner (Xu and Pasche, 2007).

It has been reported that miRNAs with different expression levels are associated with tumor stage, molecular subtype, and clinical characteristics and as a result, expression changes in colorectal tumors can be used as biomarkers in cancer diagnosis and treatment (Schetter et al., 2012).

The expression profiles of miRNAs have been examined in some colorectal cancer cell lines, normal and tumor tissues, and various studies have been conducted to detect changes in the expression levels of miRNAs in colon carcinoma, and to use these miRNAs as diagnostic biomarkers (Mazeh et al., 2013; Shen et al., 2013)

Materials and methods:-

Patients and Samples:-

This study was conducted with tumor and normal (control-clean surgical margin) colon tissues of five patients diagnosed with colorectal cancer according to their clinical evaluation based on the laboratory and pathological findings obtained from the Department of General Surgery, Gaziantep University Faculty of Medicine, Research and Practice Hospital. Colorectal carcinoma cases which were in line with Stage II (T3N0M0) criteria according to specific stages of differentiation for tumor samples were included in the study. Ethics Committee approval for the study was obtained from the Ethics Committee of Harran University Faculty of Medicine, with decision numbered 16/05/2014.

Sample processing and microarray analysis:-

Microarray data analysis and Statistical analysis:-

Target genes were identified using the Transcriptome Analysis Console (TAC) program after the previous study profiles of the miRNA of normal and tumor tissues of five patients with Stage II Colorectal cancer were mapped-out.

Furthermore, TGFB2 was identified as the target gene of hsa-miR-378i, hsa-miR-378c, hsa-miR-378d, hsa-miR-378e, hsa-miR-378f and hsa-miR-378g, which demonstrated significant expression changes when compared to the control group of our study based on the results of bioinformatics target gene analysis performed using the miRanda, Targetscan and miRbase database.

Results:-

In this study, we investigated the miRNA target gene pathway of tumor and normal colon tissues from patients (n = 5) with colon cancer (stage II).

Clinicopathological data was collected prospectively and is summarised in Table 1.

Specific miRNAs and target pathways were identified using software (Transcriptome Analysis Console Software). Difference between groups was compared using student's t-test. P value less than 0,05 was considered as statistically significant. After it was determined that there was no similarity between different samples by correlation analysis, Principal Component Analysis (PCA) was used to group the samples. According to the result of the bioinformatic analysis, Figure 1 shows that two groups (control and tumor) can be created for one patient with a similarity ratio of 71,4% (Figure 1).

This study array analysis have demonstrated been for the first time in colorectal tumor tissues, 6 of the 8 miRNAs of the miR-378 family (hsa-miR-378i, hsa-miR-378c, hsa-miR-378d, hsa-miR-378e, hsa-miR-378f and hsa-miR-378g) showed that targets TGFB2 (Table 2).

Discussion:-

The biomarkers currently used in the diagnosis and treatment of colorectal cancers frequently encountered in Turkey, which are known to have a high mortality rate, are reported to be inadequate. Moreover, although few studies have been conducted on the PCR analysis of the expression levels of miRNAs in Turkish population, no studies have been carried out to determine prominent miRNA array analysis in the literature.

In a study investigating the relationship between TNM stages and miRNA expression levels, the expression quantity of hsa-miR-378a-3p and hsa-miR-378a-5p was demonstrated by RT-PCR analysis, to vary in relation to the clinicopathological conditions of the tumor (Li et al., 2014). In other studies conducted on miRNA-378, it was also demonstrated that miR-378, which was detected to have decreased expression compared to normal tissues, had tumor suppressing properties in patients with colorectal cancer (Li et al., 2014; Faltejskova et al., 2010; Wang et al., 2010).

miR378 was shown to be a possible tumor suppressor biomarker both in array studies and RT-PCR research studies. It was further reported that it could affect various cellular processes such as cell proliferation and differentiation and

apoptosis (Wang et al., 2010; Gattoliat et al., 2015; Zhang et al., 2014). Our results and those of literature studies suggest that this miRNA family can be used as a biomarker for patients with Stage II colorectal cancer.

The profile of the miR-378 family of miRNA, which affects cancer development and progression in the normal and tumor colon/rectum tissues of patients with colorectal cancer (Stage II) living in the Southeast Anatolian Region of Turkey, has been mapped-out using microarray analysis and new target genes have been identified. Our study was conducted particularly using tissues obtained from Stage II colon cancer patients, whose nodal spread and metastasis has not yet developed. In this way, it was aimed to help the development of biomarkers which could be used to diagnose the disease at an early stage.

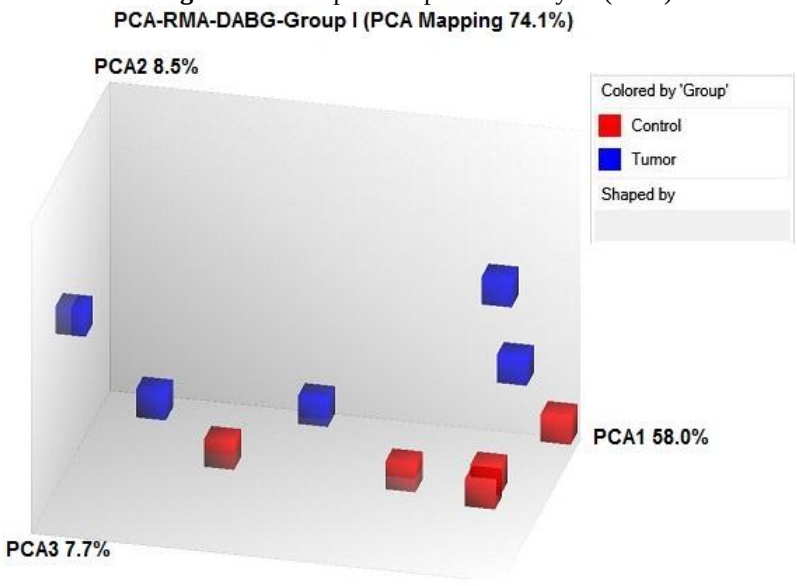
Table 1:-Clinicopathological data for 5 patients with colon cancer Stage II

Characteristics (n=5, control and tumor tissue)	Gender		Age (Mean)	Locations of Tumors		Pathologic T classification		Pathologic N classification		Methastatic classification		AJCC Classification
	Male	Female		Colon	Rectum	T2	T3	N0	N1	N2	M0	Stage II
n	5	0	43	5	0	0	5	5	0	0	5	5

Table 2:-Targeting TGFB miRNAs İn colon tissues including control and tumor by microarray

Transcript Cluster ID	**Transcript ID(Array Design)**	**Accession Number**	**Tumor Bi-weight Avg Signal (log2)**	**Control Bi-weight Avg Signal (log2)**	**Fold Change (linear) (Tumor vs. Control)**	**ANOVA p-value (Tumor vs. Control)***
20518936	hsa-miR-378i	MIMAT0019074	7,27	8,7	-2,7	0,041238
20501243	hsa-miR-378a-3p	MIMAT0000732	9,84	11,48	-3,13	0,006885
20517675	hsa-miR-378c	MIMAT0016847	7,73	9,69	-3,9	0,015631
20518788	hsa-miR-378f	MIMAT0018932	6,58	8,82	-4,72	0,014884
20504584	hsa-miR-378d	MIMAT0018926	4,26	7,21	-7,74	0,001238
20518783	hsa-miR-378e	MIMAT0018927	1,97	5,45	-11,2	0,009499
20501242	hsa-miR-378a-5p	MIMAT0000731	1,99	5,7	-13,1	0,019969
20518794	hsa-miR-378g	MIMAT0018937	1,73	6,54	-28,08	0,002622

Figure 1:-Principal Component Analysis (PCA)



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