Vol. 104, No. 2; April 2014

NATIONALPARK-FORSCHUNG
IN DER SCHWEIZ
(Switzerland Research Park Journal)

Identification of Stanniocalcin 2 as Marker in Colorectal Cancer

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Abstract: The pathogenesis of colon cancer involves both genetic and environmental factors. During colon cancer tumorigenesis and progression, specific alterations in tumor suppressor-genes and oncogenes are required. Stanniocalcin (STC) is a glycoprotein hormone that is secreted by the corpuscle of Stannius, an endocrine gland of bony fish, and is involved in calcium and phosphate homeostasis. STC2 involvement in tumor progression is unclear, as its expression seems to correlate with type of cancer. In this study we examined the expression status of STC2 in colon cancer patients in Iran. We investigated STC2 expression in tumour and marginal samples from 48 patients by Gene-Specific PCR. Results revealed a positive correlation between STC2 gene expression and colon cancer. This study identified STC2 as a biomarker of colon cancer.
Keywords: Pathogenesis, Colon cancer, Stanniocalcin

1- Introduction

Colon cancer is the second leading cause of cancer-related death in the United States and it is after lung and prostate cancers in men, and lung and breast cancer in women [1, 2].During colon cancertumorigenesis and progression, specific alterations in tumor suppressor-genes and oncogenes are required. Hence identifying novel oncogenes as colon cancer therapeutic agents is a focal point of anti-cancer research. Stanniocalcin (STC) is a family of secreted hormones that originally found in the corpuscles of Stannius, an endocrine gland of fish [3, 4]. STC1 and STC2, two homologues of STC family, are reported to have role in phosphate and calcium homeostasis [5, 6]. Both two proteins are different at C-terminal half but share similar hydropathy profiles at the N-terminal half [7]. STC1 is the first discovered STC family member and has been implicated

to involve in different physiological functions [8, 9]. STC2 is expressed in human tissues with high transcripts levels in skeletal muscle, heart and pancreas [7, 10]. Several reports have declared that STC2 overexpression could promote tumor cell proliferation, invasion and metastasis in some malignant[11-13]. Also, STC2 is vital for cytoprotective properties when exposed to hypoxia and ER stress [14, 15]. Although STC2 is well-known in other tumor types, the relationship between STC2 and colon cancer has not yet been reported so far except one study. So, in this study we examined the expression status of STC2 in colon cancer patients in Iran.

2- Materials and Methods

A total of 48 tumor samples and paired tumor margins samples were obtained during surgery.All patients underwent resection of the tumor. All patients were clearly identified as having colon cancer based on the clinicopathological findings. All patients have given an informed written consent.

The median age of patients with CRC was 56 years. None of the patients had received preoperative chemotherapy or radiotherapy and have no other malignancies. **All samples were separated in to two groups including 48 tumors, 48 marginal samples tissues which were all pathologically diagnosed.**All samples immediately after surgery, incubated overnight at 4C in RNAlater and were **subjected to total RNA extraction using RNeasy**TM**mini kit, as recommended by the manufacturer, then a cDNA library was constructed using polyA RT-PCR.PCR primer pairs were designed for mRNA sequence of each gene. Specific PolyA PCR for** ß-actin **and STC2 genes was carried out with all samples.**

Study Group	Type of Samples	Number of Samples
1	Tumor	48
2	Margin	48

Table 1: Study samples

3- Results

We investigated STC2 expression in tumour and marginal samples from 48 patients by Gene-Specific PCR.The median age of patients with colon cancer was 56 years (range, 23 to 76 years), and this subset comprised 25 males and 23 females.

For cDNA quality confirmation of ß-actin gene in tumour and marginal samples of patients with colon cancer, ß-actin gene as a housekeeping gene was used. Gene-Specific PCR method indicated that there is a positive result in polyA cDNA for ß-actin gene in tumour and marginal samples. There is a clear band was seen (Fig 1) that confirms the existence of target transcript in polyA cDNA and high quality of tumour and marginal samples of colon cancer patients.

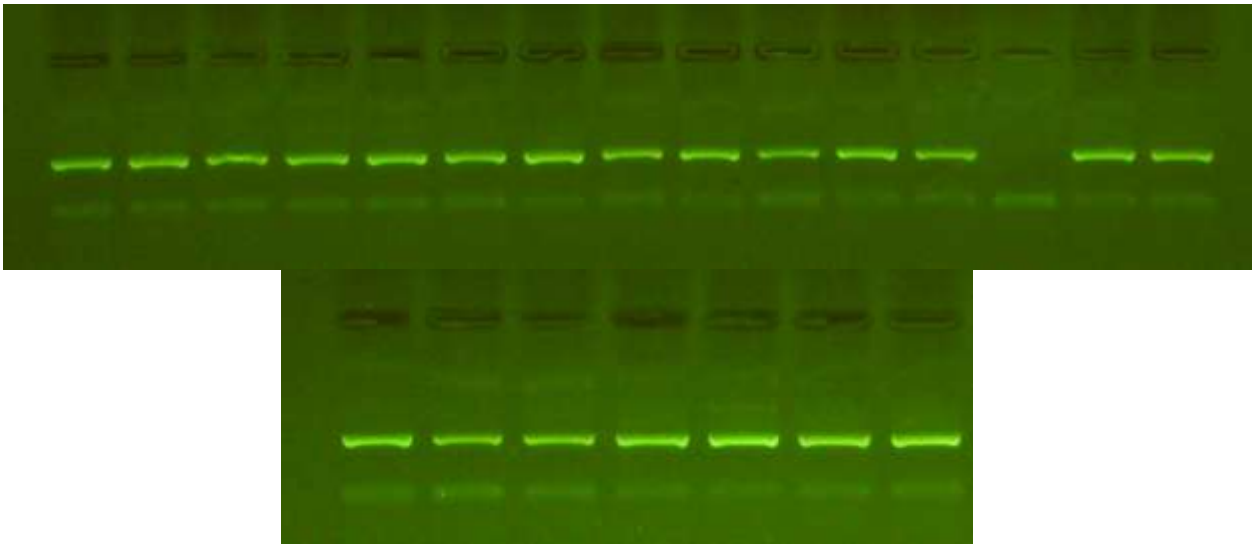

Fig 1: Expression of ß-actin gene in some of samples

For cDNA quality confirmation of STC2 gene in tumour and marginal samples of patients with colon cancer, Gene-Specific PCR method was used and products run in electrophoresis gel. Ethidium bromide was used for post-run staining of gels. Difference in size of bands confirms our result (Fig 2).

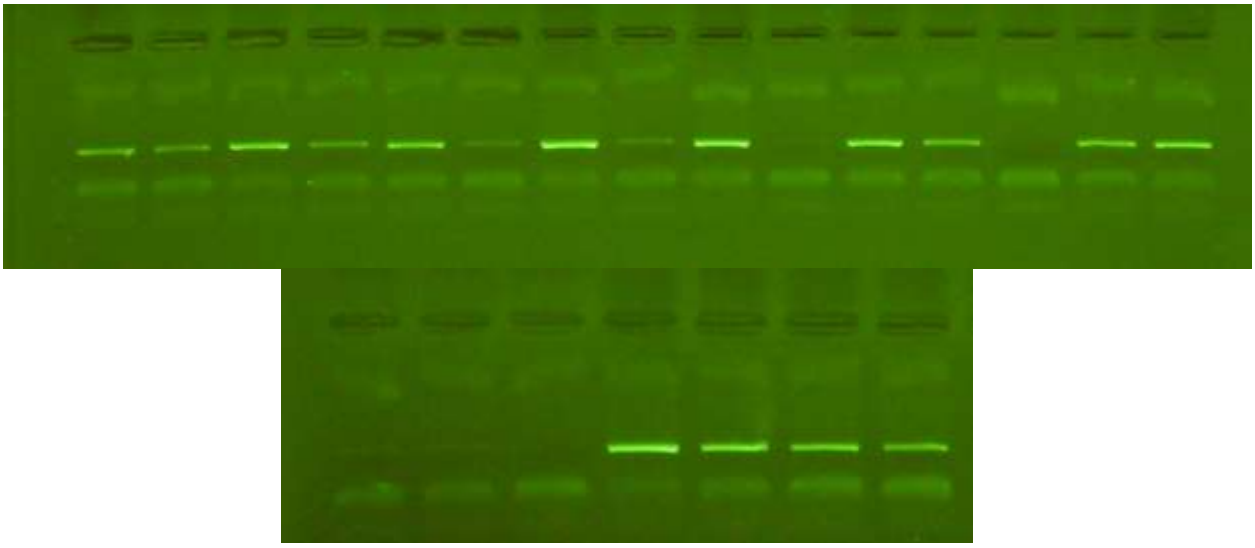

Fig 2: Expression of STC2 gene in some of samples.

4- Discussion and Conclusions

The outcome of patients with colon cancer depends on tumor stage at the time of diagnosis. The feasibility and cost effectiveness of invasive diagnostic methods for colon cancer in most countries remains questionable. A simple diagnostic test, such as a serum biomarker assay, could facilitate screening for colon cancer. Currently, however, there are no serum biomarkers

that are sufficiently sensitive and specific for the routine diagnosis of colon cancer. Stanniocalcin is a polypeptide hormone that was first found in specific endocrine glands (corpuscle of Stannius) in the kidney region of bony fish. STC is released into the blood in response to rising serum calcium levels to regulate the calcium and phosphate uptake in different organs. Fish STC thereby reduces the inward transport of calcium in the gill and in the gut, and stimulates the resorption of inorganic phosphorus in proximal renal tubuli[16-20]. In mammalians, which lack a specific corpuscle of Stannius gland, two Stanniocalcin homologue genes named STC1 and STC2 have been identified [7, 21, 22]. Human STC1 mRNA is mainly expressed in the kidney, ovary and pituitary [21]. Like fish STC, human STC1 involves in Ca2+ and mineral homeostasis [23-25]. The human STC2 gene encodes a 302 amino acid long protein. The corresponding mRNA is mainly expressed in the kidney, pancreas, heart, and spleen [26]. Interestingly, STC2 has tissue-specific antagonistic effects towards STC1. Honda *et al*[5] have indicated that calcitriol stimulates STC1 mRNA expression and decreases STC2 mRNA levels in rat kidneys. With understanding with this particular, Ishibashi*et al*[7] found that STC2 decreases phosphate uptake in opossum kidney cells by reducing the promoter activity of the renal type II Na-Pi co-transporter from the apical membrane of kidney proximal tubules. Different effects of the two STC homologues have also been mentioned during neuronal differentiation of neuroblastoma cells [27].

STC2 involvement in tumor progression is unclear, as its expression seems to correlate with breast cancer, ovarian cancer, prostate cancer, renal-cell carcinoma, and neuroblastoma[11, 13, 28-32]. In this study, analyzes revealed a positive correlation between STC2 expression and colon cancer.This study identified STC2 as a novel target molecule for therapy development or a biomarker of colon cancer.

5-Acknowledgments

This work was supported by Tabriz University of Medical Sciences. This article extracted from Dr Amir Ahmad Arabzadeh thesis. The authors wish to express their sincere thanks to all the participants who assisted in this study.

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