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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 cancer tumorigenesis 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 RNeasyTMmini 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 β -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 β -actin gene in tumour and marginal samples of patients with colon cancer, β -actin gene as a housekeeping gene was used. Gene-Specific PCR method indicated that there is a positive result in polyA cDNA for β -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.

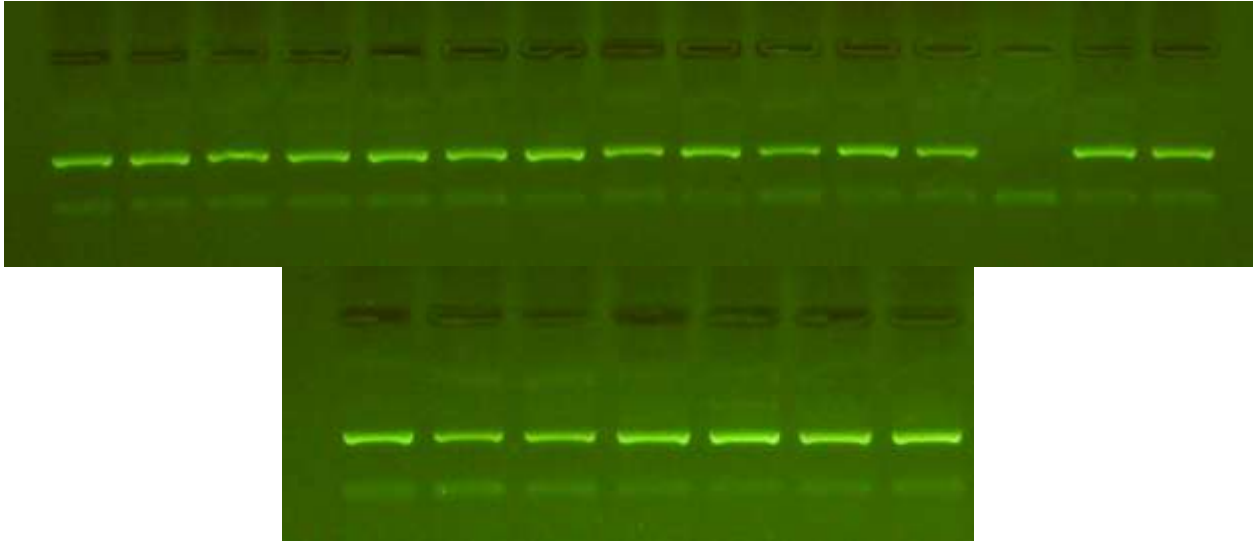


Fig 1: Expression of β -actin gene in some of samples

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).

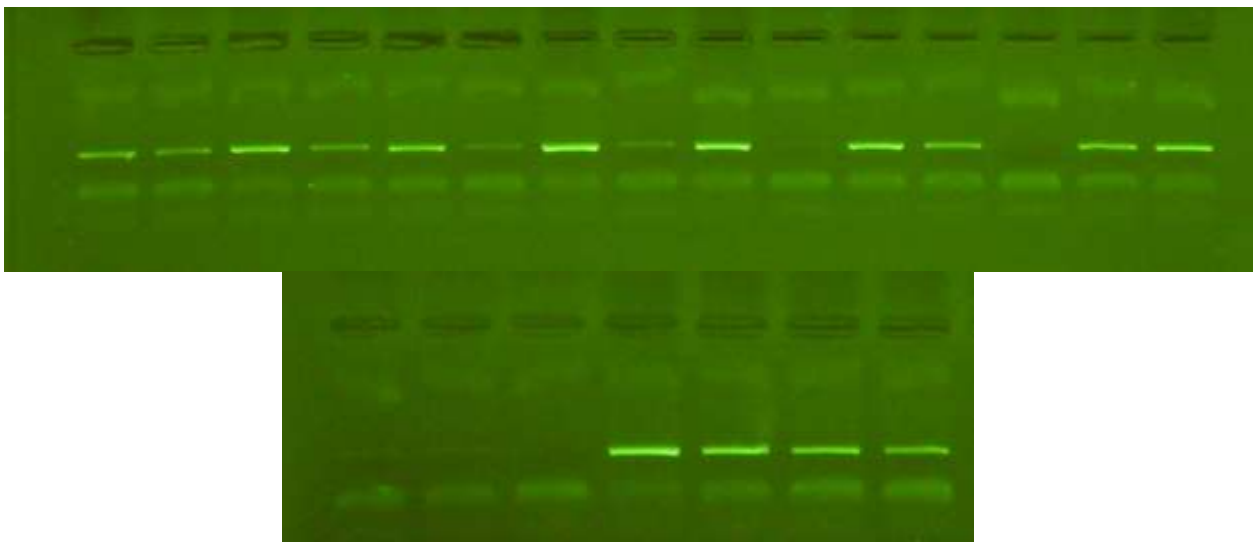


Fig 2: Expression of STC2 gene in some of samples.

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 mammals, 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.

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References:

1. Jemal, A., et al., Cancer statistics, 2005. CA: a cancer journal for clinicians, 2005. 55(1): p. 10-30.
2. Potter, J.D., et al., Colon cancer: a review of the epidemiology. Epidemiologic reviews, 1993. 15(2): p. 499-545.
3. Flik, G., et al., Rainbow trout corpuscles of Stannius: stanniocalcin synthesis in vitro. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 1990. 258(5): p. R1157-R1164.
4. Sterba, T., et al., In situ detection and distribution of stanniocalcin mRNA in the corpuscles of Stannius of sockeye salmon, *Oncorhynchus nerka*. Molecular and cellular endocrinology, 1993. 90(2): p. 179-185.
5. Honda, S., et al., Regulation by 1 α , 25-dihydroxyvitamin D₃ of expression of stanniocalcin messages in the rat kidney and ovary. FEBS letters, 1999. 459(1): p. 119-122.

6. [Wagner, G.F., E.M. Jaworski, and M. Haddad, Stanniocalcin in the seawater salmon: structure, function, and regulation. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 1998. 274\(4\): p. R1177-R1185.](#)
7. [Ishibashi, K., et al., Molecular cloning of a second human stanniocalcin homologue \(STC2\). Biochemical and biophysical research communications, 1998. 250\(2\): p. 252-258.](#)
8. [Nguyen, A., A. Chang, and R. Reddel, Stanniocalcin-1 acts in a negative feedback loop in the prosurvival ERK1/2 signaling pathway during oxidative stress. Oncogene, 2009. 28\(18\): p. 1982-1992.](#)
9. [Lai, K.P., et al., Induction of stanniocalcin-1 expression in apoptotic human nasopharyngeal cancer cells by p53. Biochemical and biophysical research communications, 2007. 356\(4\): p. 968-975.](#)
10. [Moore, E., et al., Stanniocalcin 2: characterization of the protein and its localization to human pancreatic alpha cells. Hormone and metabolic research, 1999. 31\(07\): p. 406-414.](#)
11. [Tamura, K., et al., Stanniocalcin 2 overexpression in castration-resistant prostate cancer and aggressive prostate cancer. Cancer science, 2009. 100\(5\): p. 914-919.](#)
12. [Law, A. and C.K. Wong, Stanniocalcin-2 promotes epithelial–mesenchymal transition and invasiveness in hypoxic human ovarian cancer cells. Experimental cell research, 2010. 316\(20\): p. 3425-3434.](#)
13. [Volland, S., et al., Stanniocalcin 2 promotes invasion and is associated with metastatic stages in neuroblastoma. International Journal of Cancer, 2009. 125\(9\): p. 2049-2057.](#)
14. [Fazio, E.N., et al., Stanniocalcin 2 alters PERK signalling and reduces cellular injury during cerulein induced pancreatitis in mice. BMC cell biology, 2011. 12\(1\): p. 17.](#)
15. [Ito, D., et al., Characterization of stanniocalcin 2, a novel target of the mammalian unfolded protein response with cytoprotective properties. Molecular and cellular biology, 2004. 24\(21\): p. 9456-9469.](#)
16. [Wagner, G.F. and E. Jaworski, Calcium regulates stanniocalcin mRNA levels in primary cultured rainbow trout corpuscles of stannius. Molecular and cellular endocrinology, 1994. 99\(2\): p. 315-322.](#)
17. [Fenwick, J.C. and Y.P. So, A perfusion study of the effect of stanniectomy on the net influx of calcium 45 across an isolated eel gill. Journal of Experimental Zoology, 1974. 188\(1\): p. 125-131.](#)
18. [Wagner, G.F., et al., Studies on the regulation and characterization of plasma stanniocalcin in rainbow trout. Molecular and cellular endocrinology, 1991. 79\(1\): p. 129-138.](#)
19. [Lu, M., G.F. Wagner, and J.L. Renfro, Stanniocalcin stimulates phosphate reabsorption by flounder renal proximal tubule in primary culture. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 1994. 267\(5\): p. R1356-R1362.](#)
20. [Sundell, K., et al., Chum salmon \(*Oncorhynchus keta*\) stanniocalcin inhibits in vitro intestinal calcium uptake in Atlantic cod \(*Gadus morhua*\). Journal of Comparative Physiology B, 1992. 162\(6\): p. 489-495.](#)
21. [Chang, A.C.-M., et al., A novel human cDNA highly homologous to the fish hormone stanniocalcin. Molecular and cellular endocrinology, 1995. 112\(2\): p. 241-247.](#)

22. [Olsen, H.S., et al., Human stanniocalcin: a possible hormonal regulator of mineral metabolism. Proceedings of the National Academy of Sciences, 1996. 93\(5\): p. 1792-1796.](#)
23. [Wagner, G.F., et al., Human stanniocalcin inhibits renal phosphate excretion in the rat. Journal of Bone and Mineral Research, 1997. 12\(2\): p. 165-171.](#)
24. [Madsen, K.L., et al., Stanniocalcin: a novel protein regulating calcium and phosphate transport across mammalian intestine. American Journal of Physiology-Gastrointestinal and Liver Physiology, 1998. 274\(1\): p. G96-G102.](#)
25. [Varghese, R., et al., Overexpression of human stanniocalcin affects growth and reproduction in transgenic mice. Endocrinology, 2002. 143\(3\): p. 868-876.](#)
26. [Chang, A.C., D. Jellinek, and R. Reddel, Mammalian stanniocalcins and cancer. Endocrine-related cancer, 2003. 10\(3\): p. 359-373.](#)
27. [McCudden, C.R., et al., Characterization of mammalian stanniocalcin receptors Mitochondrial targeting of ligand and receptor for regulation of cellular metabolism. Journal of Biological Chemistry, 2002. 277\(47\): p. 45249-45258.](#)
28. [Welch, P.L., et al., BRCA1 transcriptionally regulates genes involved in breast tumorigenesis. Proceedings of the National Academy of Sciences, 2002. 99\(11\): p. 7560-7565.](#)
29. [Esseghir, S., et al., Identification of NTN4, TRA1, and STC2 as prognostic markers in breast cancer in a screen for signal sequence encoding proteins. Clinical cancer research, 2007. 13\(11\): p. 3164-3173.](#)
30. [Buckanovich, R.J., et al., Tumor vascular proteins as biomarkers in ovarian cancer. Journal of clinical oncology, 2007. 25\(7\): p. 852-861.](#)
31. [Meyer, H.-A., et al., Identification of stanniocalcin 2 as prognostic marker in renal cell carcinoma. European urology, 2009. 55\(3\): p. 669-678.](#)
32. [Staehler, M., Editorial comment on: Identification of stanniocalcin 2 as prognostic marker in renal cell carcinoma. European urology, 2009. 55\(3\): p. 678.](#)