Pattern of Dipkoff-2 gene expression in tumoral tissues of colorectal cancer and two tumor tissues of colorectal cancer and three cell lines (HUVEC, SW480 and HCT116)

Farnaz Khodarahmi[1,2], Mohammad Sadegh Fazeli[3], Habibollah Mahmoodzadeh[4], Amirhosein Mehrtash[2], Narges Keikhosravi[2,5], Mahdis Ghadir[2], Ladan Teimoori-Toolabi[2,*]

1- Department of Genetics, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran
2- Molecular Medicine Department, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran
3- Colorectal Surgery ward, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran
4- Cancer Institute, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran
5- East Tehran Branch, Islamic Azad University, Tehran, Iran
*Corresponding author

Objectives:

Colorectal cancer (CRC) is the third most frequent cancer in men, after lung and prostate cancer, and is the second most frequent cancer in women after breast cancer. It is also the third cause of death in men and women separately, and is the second most frequent cause of death by cancer if both genders are considered together. CRC represents approximately 10% of death by cancer (1, 2). The Wnt signaling pathway, which plays important roles in embryogenesis, development and homeostasis , is also closely linked to carcinogenesis (3). There are different modulators of this pathway such as Dickokopf 2 (*DKK2*) which is assumed to play a dual role as a tumor suppressor gene or oncogene (4). In this study we studied the expression of *DKK2* in 10 patients affected with colorectal cancer also we examine expression status of this gene in three cell lines including SW480, HCT116 (colon cancer cell lines) and HUVEC, in addition to two tumor tissue of colorectal cancer.

Methods:

Total RNA was extracted using Trizol reagent thereafter cDNA was synthesized by Mu-MLV reverse transcriptase. The studied cell lines had been obtained from ATCC. Fresh tumor tissues of colorectal cancer were obtained from Imam Khomeini Hospital (Tehran, Iran). Total RNA of samples and cells were extracted usingTrizol and converted to cDNA using Mu-MLV reverse transcriptase. The expression of *DKK2* and *GAPDH* (as the reference gene) in tumoral tissues was studied by using primers, probe, Taqman master mix (TAKARA) while in cell lines the expression was studied by Sybr Green MasterMix.

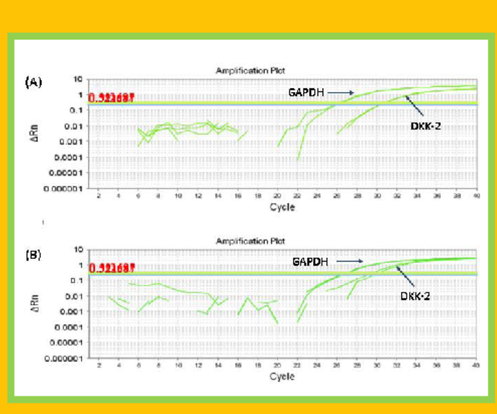

Figure A) The amplification plot of tumor tissue. Figure B) The amplification plot of normal tissue. ΔCT of *DKK2* in comparison to reference gene in tumor sample in proportion to the ΔCT of normal sample have been increased. It means *DKK2* shows decreased expression in tumor tissue in comparison to normal adjacent tissue.

Results:

The age of patients was from 37 to 84 years old. Tumor and normal adjacent samples were obtained from 5 females (who suffered from colorectal cancer in colon, rectum and sigmoid sites) and 5 male patients (who suffered from colorectal cancer in colon, rectum and rectosigmoid sites) from Imam Khomeini Hospital (Tehran, Iran). In 100% of cases *DKK2* was expressed in normal and tumoral tissues. In 90% of patients downregulation of this gene in tumoral tissue relative to adjacent normal tissues was seen (ΔΔCTs in males: -0.64, -1, -4.32, -8.27, -1.93 and in females: -1.46, -4.32, -1.1, -6.73) while in one tumor tissue (female, rectal cancer) overexpression of *DKK2* was seen (ΔΔCT: 0.29). ΔΔCTs of *DKK2* in tumor tissues (obtained from a 49 year old female and 70 year old male) in comparison to HUVEC were -0.88 and -0.97 while ΔΔCTs of *DKK2* in SW480 and HCT116 in comparison to HUVEC were -4.65 and -3.35

Conclusions:

While *DKK2* gene was observed to be downregulated in malignant melanoma and human ovarian carcinoma, it had been claimed that it was upregulated in primary colorectal cancers and even in adenomas (4). Our observations showed that *DKK2* expression was downregulated in tumoral and to a greater extent in HCT116 and SW480 cancer cell lines in comparison to normal ones. While in the previous studies it was claimed that *DKK2* expression was absent or barely detectable in SW480 and HCT116 cell lines and it is upregulated in primary colorectal cancers and even in adenomas, our study showed that generally expression of this gene was downregulated in colorectal cancer tissues and cells which should be validated more.

References:

1. López PJT, Albero JS, Rodríguez-Montes JA. Primary and Secondary Prevention of Colorectal Cancer. Clinical medicine insights Gastroenterology. 2014;7:33.
2. Pericleous M, Mandair D, Caplin ME. Diet and supplements and their impact on colorectal cancer. Journal of gastrointestinal oncology. 2013;4(4):409.
3. Sato H, Suzuki H, Toyota M, Nojima M, Maruyama R, Sasaki S, et al. Frequent epigenetic inactivation of DICKKOPF family genes in human gastrointestinal tumors. Carcinogenesis. 2007;28(12):2459-66.
4. Matsui A, Yamaguchi T, Maekawa S, Miyazaki C, Takano S, Uetake T, et al. DICKKOPF-4 and -2 genes are upregulated in human colorectal cancer. Cancer science. 2009;100(10):1923-30. Epub 2009/08/08.

WCGC 2015
214--P
Basic Colon Cancer
Farnaz Khodarahmi
DOI: 10.3252/pso.eu.17wcgc.2015
Poster presented at: ESMO Imedex 17th World Congress on Gastrointestinal Cancer 1–4 JULY 2015 BARCELONA, SPAIN