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Methylation of PTCH1 Gene in Pancreatic Cancer: A Study in Kashmiri Population

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Abstract

Cancer results from a series of molecular events that fundamentally alter the normal properties of cells. In cancer cells the normal control systems that prevent cell overgrowth and the invasion of other tissues are disabled. As these cells grow they develop new characteristics, including changes in cell structure, decreased cell adhesion, and production of new enzymes. These heritable changes allow the cell and its progeny to divide and grow, even in the presence of normal cells that typically inhibit the growth of nearby cells. Such changes allow the cancer cells to spread and invade other tissues (Gibbs et al., 2003). The abnormalities in cancer cells usually result from mutations in protein-encoding genes that regulate cell division. Over time more genes become mutated. This is often because the genes that make the proteins that normally repair DNA damage are themselves not functioning normally because they are also mutated. Consequently, mutations begin to increase in the cell, causing further abnormalities in that cell and the daughter cells. Some of these mutated cells die, but other alterations may give the abnormal cell a selective advantage that allows it to multiply much more rapidly than the normal cells.

KEY WORDS,gene,cancer metastasis,proliferation,exocrine.

INTRODUCTION

Cancer can be defined as a disease in which a group of abnormal cells grow uncontrolled by disregarding the normal rules of cell division. Cancer cell results in uncontrolled growth and proliferation. If this proliferation is allowed to continue and spread, it can be fatal. Cancer involves change or mutation in the cell genome these changes produces proteins that disrupt the delicate cellular balance between cell division and quiescence resulting in cells that keep dividing to form cancer. The Signs and symptoms of cancer are Double vision, Skin irritation, Fatigue, nausea, weight loss, Loss of appetite, Chest pain, Abnormal lumps close to the surface of the body, Coughing or trouble breathing.Pancreatic cancer is one

of the most dangerous solid tumors with a 5-year survival rate. Pancreatic cancer develops when cells in the pancreas grow out of control, forming a lump (tumor). This can happen in the head, body or tail of the pancreas. Pancreatic cancers are divided into two main groups. Exocrine tumors start in the exocrine cells. These cells make enzymes. About ninety-five out of a hundred pancreatic cancers (95%) are exocrine tumors. The most common type is pancreatic ductal adenocarcinoma – about eighty out of a hundred of all pancreatic cancers (80%). Endocrine tumors (also called neuroendocrine tumors) start in the cells that produce hormones. Less than five in a hundred (5%) of all pancreatic cancers are endocrine tumors.

Pancreatic cancer often starts with pre-malignant lesions that then progress to malignant tumor by accumulating mutations in key genes involved in cell proliferation control, like *K-RAS, p16/CDKN2A, TP53* and *SMAD4* (Feldman et al., 2007). There are three types of pancreatic precursor lesions: intraepithelial pancreatic lesion (Pan IN), intraductal mucinous neoplasm (IPMNs) and mucinous cystic neoplasm (MCN). The most common form of pancreatic tumors, are the PanIN. Pancreatic cancer is also characterized by the alteration of pathways normally involved in development like Hedgehog, Wnt and Notch pathways.

The Hedgehog (Hh) signal transduction pathway controls numerous processes during embryonic development and adult homeostasis, including tissue/organ patterning, cellular proliferation and differentiation, path finding, left/right asymmetry, and stem cell maintenance. Hh signaling is dysregulated in several congenital defects and many types of tumors. Activation of the hedgehog pathway has been implicated in the development of cancers in various organs, including brain, lung, mammary gland, prostate and skin. The Hh signaling cascade is initiated by Hh binding to the Patched 1 protein (*PTCH1*) on the target cell. The regulation of Hh pathway expression is quite complex and can occur at many different levels. *PTCH1* exert a negative feedback on the Hh pathway in order to balance the expression of the pathway gene targets. *PTCH1* negative feedback prevents its own overexpression which would otherwise result in inhibition of the Hh pathway with severe consequences during development.

Pancreatic cancer is the most common occurring form of cancer in Kashmir valley and the frequency of pancreatic cancer has significantly increased among the population of Kashmir from past 10-20 years (Ayub et al., 2011). Also owing to the fact that there is very less data on genetic alterations in pancreatic cancer available in our population this study was designed to establish status of *PTCH1* gene in patients with pancreatic cancer.

MATERIAL AND METHODS

Population Studied

All the study subjects were born in the Kashmir division of J&K state India. Sixteen (n=16) confirmed cases of Pancreatic Cancer attending Department of Surgical Gastroenterology, Sher-I-Kashmir Institute of Medical Sciences (SKIMS), Srinagar were included in the study. A pre informed consent was obtained from each patient were recorded in a questionnaire. This study was approved by Ethical committee of the SKIMS.

Sample Collection /Storage

Pancreatic cancer tissues along with their adjacent normal tissues (controls) were taken for the analysis of *PTCH1* gene. Tissue samples were stored in liquid nitrogen (-176ºC) till further processing. Patients for study had first time diagnosis and did not receive any chemo or radiotherapy.

Extraction and Quantitation of Genomic DNA

DNA was isolated from the pancreatic cancer tissues of all individuals sampled using DNA extraction kit. The concentration of the extracted DNA was measured in a spectrophotometer at 260nm wavelength by using the formula:
DNA µg/ml = A260 ×50×dilution factor.

The purity of DNA was checked by using A260/A280 ratio. The quality of DNA obtained from the tissue specimens and blood samples was analyzed on 1% agarose gel. *PTCH1* Methylation Analysis For methylation analysis methylation specific PCR was performed. Primers were as follows;

Primers for methylation-specific PCR (MSP) were designed with the assistance of *Meth primer software.* Sequences of the primers for MSP of the *PTCH1* promoter region were as following;
Methylated primers;

F: 5-AATTAAGGAGTTGTTGCGGTC-3
R: 5-GCTAAACCATTCTATCCCCGTA3 (125 bp).

Unmethylated primers;

F: 5-ATTAAGGAGTTGTTGTGGTTGT-3
R: 5-ACTAAACCATTCTATCCCCATA-3 (124 bp)

PCR cycling conditions for both unmethylated and methylated primers were 95ºC for 8 min, followed by 40 cycles of 95ºC for 1 min, 61ºC for 1 min and 72ºC for 50 sec, and a final elongation reaction at 72ºC for 7 min. PCR amplification was performed using Taq DNA polymerase. The amplification products were separated on a 2% agarose gel and visualized by ethidium bromide staining and ultraviolet transillumination. Water was used as a negative control and positive control was purchased from Sigma.

Statistical Analysis

Statistical analysis was carried out using SPSS version 14.0. Differences were considered statistically significant at P <0.05. The nonparametric correlations of *PTCH1* expression with methylation were analyzed with Spearman's test. Differences in the clinicopathological parameters between positive and negative *PTCH1* methylation were determined with the $\chi 2$ test.

RESULTS

In this study histologically confirmed pancreatic cancer (n=16) and their adjacent normal tissues where analyzed for *PTCH1* methylation. The cases included 11(68.75%) males and 5(31.25%) females. The clinicopathological characteristics of the studied subjects are given in *Table 2. To investigate the potential mechanism transcription of PTCH1 in pancreatic cancer the methylation status of the PTCH1 promoter in 16 pancreatic cancer tissues and their adjacent normal tissues was performed by MSP. PTCH1 methylation was present in*

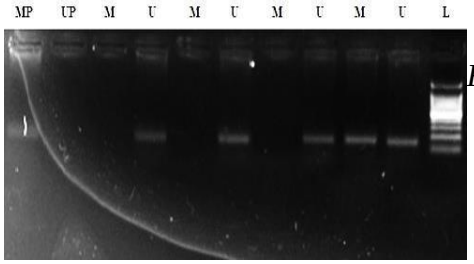

Fig Methylation of PTCH1 gene

We collected clinicopathological data from the patients and then analyzed statistically the relationship between the methylation of PTCH1 in pancreatic cancer tissues and clinical features. Patients with age of more than 50 years showed 75% (4/5) methylation of PTCH1 gene, whereas patients below the age of 50 years showed only 25%(3/11) methylation. Among males methylation was present in 54.54%(6/11), while as in females methylation was present in 20%(1/5). In smoker's methylation was present in 50%(6/12), while as in non- smokers only 25%(1/4) show methylation. Correlation of PTCH1 gene methylation status with clinicopathological characteristics Our results show methylation of PTCH1 was associated significantly with gender, age and smoking status of the patients.

Discussion

The Hedgehog (Hh) signaling pathway, which is essential for numerous processes during embryonic development, plays an integral role in the initiation and propagation of neoplastic diseases (Beachy et al., 2004). Extracellular hedgehog protein (Hh) binds to Patched homologue 1 (*PTCH1*/*PTCH*), a 12-transmembrane receptor, and prevents *PTCH1* mediated inhibition of signaling by smoothened

homologue (SMO). Therefore, aberrant function or activation of *PTCH1* is sufficient to induce the improper activation of the Hh pathway. Hh signaling is frequently activated in many cancers due to transcriptional up- regulation of the Hh ligands and epigenetic silencing of key genes (Fu et al., 2014)

In the present study, we evaluated the methylation status of *PTCH*, tumour suppressor in order to understand its possible role in the genesis of pancreatic cancer. Our data showed that the *PTCH1* promoter was methylated in 43.75 (7/16) of cancer tissues. No methylation was observed in any normal tissues. Our results indicate that methylation of *PTCH1* promoter region may be one of the mechanisms involved in pancreatic cancers. Furthermore, methylation of *PTCH1* was associated with and gender, age and smoking status of the patients. To the best of our knowledge ours is the first study demonstrating methylation of *PTCH1* gene in pancreatic cancers. Several studies have reported methylation profile of *PTCH1* gene in gastrointestinal tract. Our results are in line with study conducted by Zarah *et al.* who observed *PTCH* gene methylation in 84.37% of cervical and ovarian cancers (Zarah et al., 2010). Another study reported that methylation of *PTCH1* gene in gastric cancers (Zuo et al., 2013). A study by Peng et al reported *PTCH1* methylation was present in 64.8% (35/54) of colorectal cancers. (Peng et al., 2013). No study till date has suggested no role of *PTCH1* promoter methylation in carcinogenesis**.**

Conclusion

In conclusion, we found *PTCH1* gene was methylated in 43.75(7/16) of pancreatic cancers and it was significantly associated with smoke, age and gender. Our results suggest a role of *PTCH1* gene methylation in the pancreatic cancer progression. However further studies with increased sample size are needed to confirm the results**.**

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