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doi:10.3748/wjg.14.3421

World J Gastroenterol 2008 June 7; 14(21): 3421-3424
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Hypermethylation and aberrant expression of secreted fizzled-related protein genes in pancreatic cancer

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Supported by The Fund for University Science and Technology Research from Education Office of Liaoning Province, No. 05L557
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Received: November 7, 2007 Revised: January 23, 2008
Accepted: January 30, 2008
Published online: June 7, 2008

Abstract

AIM: To determine the methylation status and aberrant expression of some secreted frizzled-related protein (SFRP) genes in pancreatic cancer and explore their role in pancreatic carcinogenesis.

METHODS: Methylation status and expression of SFRP genes were detected by methylation-specific PCR (MSPCR) and reverse-transcription PCR (RT-PCR) respectively.

RESULTS: The frequencies of methylation for SFRP genes 1, 2, 4, 5 were 70%, 48.3%, 60% and 76.7% in pancreatic cancer samples, and 21.7%, 20%, 10% and 36.7% in matched cancer adjacent normal tissue samples, respectively (χ^2 = 28.23, P < 0.0001 for SFRP gene 1; χ^2 = 10.71, P = 0.001 for SFRP gene 2; χ^2 = 32.97, P < 0.0001 for SFRP gene 4; χ^2 = 19.55, P < 0.0001 for SFRP gene 5). Expression loss of SFRP genes 1, 2, 4 and 5 was found in 65%, 40%, 55% and 71.7% of 60 pancreatic cancer samples, and 25%, 15%, 18.3% and 31.7% of matched cancer adjacent normal tissue samples, respectively (χ^2 = 19.39, P < 0.0001 for SFRP gene 1; χ^2 = 9.40, P = 0.002 for SFRP gene 2; χ^2 = 17.37, P < 0.0001 for SFRP gene 4; χ^2 = 19.22, P < 0.0001 for SFRP gene 5). SFRP gene 1 was methylated but not expressed in PC-3 and PANC-1, SFRP gene 2 was methylated but not expressed in PANC-1 and CFPAC-1, SFRP gene 4 was methylated but not expressed in PC-3, and SFRP gene 5 was methylated but not expressed in CFPAC-1.

CONCLUSION: Hypermethylation and aberrant expression of SFRP genes are common in pancreatic cancer, which may be involved in pancreatic carcinogenesis.

Key words: Hypermethylation; Secreted frizzled-related protein; Pancreatic cancer

Peer reviewer: Kazuhiro Hanazaki, MD, Professor and Chairman, Department of Surgery, Kochi Medical School, Kochi University, Kohasu, Okohcho, Nankoku, Kochi 783-8505, Japan

Bu XM, Zhao CH, Zhang N, Gao F, Lin S, Dai XW. Hypermethylation and aberrant expression of secreted fizzled-related protein genes in pancreatic cancer. *World J Gastroenterol* 2008; 14(21): 3421-3424 Available from: URL: http://www.wjgnet.com/1007-9327/14/3421.asp DOI: http://dx.doi.org/10.3748/wjg.14.3421

INTRODUCTION

Secreted frizzled-related proteins (SFRPs) are a group of negative regulators of the Wnt signaling pathway[1-3]. These proteins contain a cysteine-rich domain (CRD) which shares a sequence similarity of 30%-50% with Wnt receptor frizzled proteins. Through the CRD, SFRPs can antagonize Wnt signaling by interacting with Wnt ligand. As the Wnt signaling pathway plays an important role in cell proliferation, differentiation and apoptosis in adult tissues, aberrant activation of the Wnt pathway caused by down-regulation of SFRPs may induce tumorigenesis. It was recently reported that some members of the SFRP family are down-regulated by hypermethylation in a series of human cancers[4-9].

The prognosis of pancreatic cancer, one of the most malignant tumors, is usually very poor. The pathogenesis of pancreatic cancer is still not very clear. It has been found that hypermethylation and subsequent expression loss of some tumor suppressor genes and tumor-related genes, such as p16[10], RASSF1A[11], SOCS-1[12], and hMLH1[13] occur frequently in pancreatic cancer.

This study was designed to determine the methylation status and aberrant expression of some members of the SFRP family in pancreatic cancer and explore their role in pancreatic carcinogenesis.

MATERIALS AND METHODS

Cell lines, cancer and matched adjacent tissue samples

Human pancreatic cancer cell lines PC-3, PANC-1 and CFPAC-1 (from KEYGEN, Nanjing, China) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 100 μg/mL penicillin, and 100 μg/mL streptomycin, at 37℃ in a humid incubator containing 50 mL/L CO_2. Pancreatic cancer and matched adjacent tissue samples were obtained from patients who underwent operation at the Second Affiliated Hospital of China Medical University. The samples were frozen in liquid nitrogen immediately after surgery. Haematoxylin and eosin staining was used to assure that cancer samples were consisted mostly of tumor cells with no tumor cells in the tumor adjacent tissue samples.

DNA and RNA extraction

DNA was extracted by a standard phenol/chloroform extraction and ethanol precipitation procedure. RNA was isolated using Tri reagent (Takara, Dalian, China) according to its manufacturer's instructions.

Reverse transcription-PCR (RT-PCR)

RT-PCR was performed using a RNA PCR 3.0 kit (Takara, Dalian, China). cDNA was synthesized from 1 μg RNA using a random 9 primer and AMV reverse transcriptase. One cycle was performed at 30℃ for 10 min, at 42℃ for 25 min, at 99℃ for 5 min, and at 5℃ for 5 min. The primer sequences used in PCR are described elsewhere[7]. PCR was performed for one cycle at 94℃ for 2 min, followed by 30 cycles at 94℃ for 30 s, at 60℃ for 30 s and at 72℃ for 2 min.

Methylation-specific PCR (MSPCR)

Methylation of SFRP1 was detected with a MSPCR kit (GENMED, Shanghai, China) according to its manufacturer's instructions. The primer sequences are described elsewhere[7]. MSPCR was performed for one cycle at 95℃ for 5 min, followed by 35 cycles at 95℃ for 30 s, at 60℃ for 30 s and at 72℃ for 30 s.

Statistical analysis

Methylation and expression of SFRP1 in primary pancreatic cancer and its adjacent tissue samples were compared by chi-square test. $P < 0.05$ was considered statistically significant.

RESULTS

Hypermethylation and expression of SFRPs in pancreatic cancer cell lines

The methylation status of SFRPs was detected by MSPCR. SFRP1 was methylated in PC-3 and PANC-1, SFRP2 in PANC-1 and CFPAC-1, SFRP4 in PC-3 and SFRP5 was methylated in CFPAC-1, respectively (Figure 1). The mRNA expression of SFRPs was determined by RT-PCR. No expression of SFRP1, SFRP2, SFRP4 and SFRP5 was found in PC-3 and PANC-1, PANC-1 and CFPAC-1, PC-3 and in CFPAC-1, respectively (Figure 2).

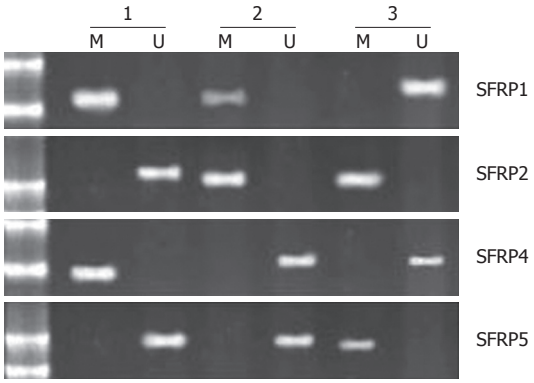

Figure 1 Hypermethylation of SFRP genes in pancreatic cancer cell lines detected by MSPCR. 1: PC-3; 2: PANC-1; 3: CFPAC-1; M: Methylated; U: Unmethylated.

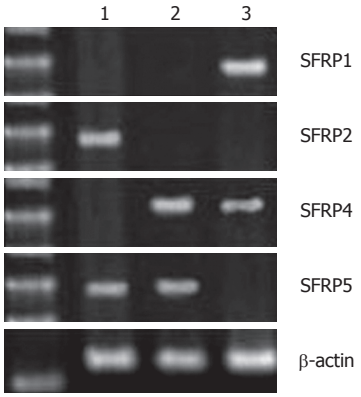

Figure 2 Expression of SFRPs in pancreatic cancer cell lines detected by RT-PCR. 1: PC-3; 2: PANC-1; 3: CFPAC-1.

The expression loss of SFRPs was correlated with the methylation status.

Hypermethylation and expression of SFRPs in pancreatic cancer and its adjacent tissue samples

Hypermethylation of SFRP1, SFRP2, SFRP4 and SFRP5 was detected in 42 (70%), 29 (48.3%), 36 (60%) and 46 (76.7%) of 60 pancreatic cancer samples, and 13 (21.7%), 12 (20%), 6 (10%) and 22 (36.7%) of its adjacent tissue samples, respectively. The hypermethylation of each SFRP gene differed significantly in cancer and its adjacent tissue samples ($\chi^2 = 28.23$, $P < 0.0001$ for SFRP1; $\chi^2 = 10.71$, $P = 0.001$ for SFRP 2; $\chi^2 = 32.97$, $P < 0.0001$ for SFRP 4; $\chi^2 = 19.55$, $P < 0.0001$ for SFRP 5; Table 1). Expression loss of SFRP1, SFRP2, SFRP4 and SFRP5 was found in 39 (65%), 24 (40%), 33 (55%) and 43 (71.7%) of 60 pancreatic cancer samples, and 15 (25%), 9 (15%), 11 (18.3%) and 19(31.7%) of its adjacent tissue samples, respectively. The expression loss of each SFRP gene differed significantly in cancer and its adjacent tissue samples ($\chi^2 = 19.39$, $P < 0.0001$ for SFRP1; $\chi^2 = 9.40$, $P = 0.002$ for SFRP2; $\chi^2 = 17.37$, $P < 0.0001$ for SFRP4; $\chi^2 = 19.22$, $P < 0.0001$ for SFRP5; Table 2).

DISCUSSION

The Wnt signaling pathway plays an important role not only in development of cancer but also in cell proliferation, differentiation and apoptosis in adult tissues.

Table 1 Hypermethylation of SFRPs in pancreatic cancer and its adjacent tissue samples

	n	SFRR1	SFRR2	SFRP4	SFRP5
Pancreatic cancer samples	60	42	29	36	46
Adjacent tissue samples	60	13	12	6	22
χ^2		28.23	10.71	32.97	19.55
P		< 0.0001	0.001	< 0.0001	< 0.0001

Table 2 Expression loss of SFRPs in pancreatic cancer and its adjacent tissue samples

	n	SFRR1	SFRR2	SFRP4	SFRP5
Pancreatic cancer samples	60	39	24	33	43
Adjacent tissue samples	60	15	9	11	19
χ^2		19.39	9.40	17.37	19.22
P		< 0.0001	0.002	< 0.0001	< 0.0001

Aberrant activation of Wnt signaling in tumorigenesis has been reported frequently, and some members of the Wnt family are over-expressed in breast cancer, gastrointestinal cancer and prostate cancer[14-16]. Down-regulation of the Wnt inhibitors DKKs and SFRPs also occurs frequently in human cancers[17,18]. Most of these reports show that expression loss of these inhibitors is mainly caused by promoter hypermethylation, an important epigenetic gene silencing mechanism.

Aberrant Wnt signals are also involved in pancreatic cancer. It was reported that activated mutation of β-catenin on exon 3, a downstream component in the Wnt signaling pathway, plays an important role in pancreatic tumorigenesis. This kind of mutation leads to excessive accumulation of β-catenin and aberrant activation of the Wnt pathway[19-23]. Over-expression of many members of the Wnt family, such as Wnt1[24], Wnt5a[25], Wnt5b[25], Wnt7a[26], Wnt10b[27] in pancreatic cancer, has been reported in recent years, further suggesting that the Wnt pathway plays a role in the pathogenesis of pancreatic cancer. It has recently been shown that epigenetic inactivation of Wnt inhibitory factor 1 by hypermethylation occurs frequently in pancreatic cancer[28].

The pathogenesis of pancreatic cancer, a very malignant carcinoma, has been poorly understood. In this study, we analyzed the hypermethylation and expression of SFRPs in pancreatic cancer and explored their role in pancreatic carcinogenesis, showing that hypermethylation and expression loss of SFRPs occur frequently in pancreatic cancer. The frequencies of hypermethylation and expression loss of SFRPs in pancreatic cancer samples were significantly higher than those in its adjacent normal tissue samples, suggesting that hypermethyaltion and subsequent expression loss of SFRPs occur early and play an important role in the pathogenesis of pancreatic cancer.

As we know, Wnt signaling can be divided into canonical Wnt/β-catenin pathway and non-canonical pathway which includes the planar cell polarity pathway and the Wnt/Ca^{2+} pathway. As we did not measure the level of β-catenin, we could not determine whether the pathway through which SFRP1 expression loss is involved in the pancreatic carcinogenesis. Further study is needed to elucidate its mechanism.

COMMENTS

Background

Secreted frizzled-related proteins (SFRPs) are a group of negative regulators of the Wnt signaling pathway. Aberrant activation of the Wnt pathway caused by down-regulation of SFRPs may induce tumorigenesis.

Research frontiers

The pathogenesis of pancreatic cancer, a very malignant carcinoma, is poorly understood. In this study, we found that hypermethylation status and expression of SFRPs played an important role in pancreatic carcinogenesis.

Innovations and breakthroughs

In this study, we analyzed the hypermethylation status and expression of SFRPs in pancreatic cancer and explored their role in pancreatic carcinogenesis.

Applications

Our study suggested that hypermethyaltion and subsequent expression loss of SFRPs play an important role in pancreatic carcinogenesis. For this reason, demethylated agents may be used to treat cancer in clinical practice.

Peer review

In this study, the authors reported that the expression loss of some SFRP genes caused by hypermethylation was common in pancreatic cancer, which may play an important role in pancreatic carcinogenesis. This paper is original and informative.

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