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

Xian-Min Bu, Cheng-Hai Zhao, Ning Zhang, Feng Gao, Shuai Lin, Xian-Wei Dai

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 ($\chi^2 = 28.23, P < 0.0001$ for SFRP gene 1; $\chi^2 = 10.71, P = 0.001$ for SFRP gene 2; $\chi^2 = 32.97, P < 0.0001$ for SFRP gene 4; $\chi^2 = 19.55, P < 0.0001$ 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 ($\chi^2 = 19.39, P < 0.0001$ for SFRP gene 1; $\chi^2 = 9.40, P = 0.002$ for SFRP gene 2; $\chi^2 = 17.37, P < 0.0001$ for SFRP gene 4; $\chi^2 = 19.22, P < 0.0001$ 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.

© 2008 The WJG Press. All rights reserved.

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

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

The methylation status of SFRPs was determined 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).
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 [20–23]. Over-expression of many members of the Wnt family, such as Wnt1 [24], Wnt5a [23], Wnt5b [20], Wnt7a [24], 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 status and expression of SFRPs in pancreatic cancer and explored their role in pancreatic carcinogenesis. For this reason, demethylated agents may be used to treat cancer in clinical practice.

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

|          | SFRR1 | SFRR2 | SFRP4 | SFRP5 |
|----------|-------|-------|-------|-------|
| Pancreatic cancer samples | 60    | 42    | 29    | 36    | 46    |
| Adjacent tissue samples    | 60    | 13    | 12    | 6     | 22    |
| $\chi^2$                   | 28.25 | 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

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

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 hypermethylation 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.

**REFERENCES**

1. Finch PW, He X, Kelley MJ, Uren A, Schaudies RP, Popescu NC, Rudikoff S, Aaronson SA, Varums HE, Rubin JS. Purification and molecular cloning of a secreted, Frizzled-related antagonist of Wnt action. Proc Natl Acad Sci USA 1997; 94: 6770-6775
2. Melkonyan HS, Chang WC, Shapiro JP, Mahadevappa M, Fitzpatrick PA, Kiefer MC, Tomei LD, Umansky SR. SARPs: a family of secreted apoptosis-related proteins. Proc Natl Acad Sci USA 1997; 94: 13636-13641
3. Rattner A, Hsieh JC, Smallwood PM, Gilbert DJ, Copeland NG, Jenkins NA, Nathans J. A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. Proc Natl Acad Sci USA 1997; 94: 2859-2863
4. Ugolini F, Charafe-Jauffret E, Bardou VJ, Geneix J, Adalde J, Labat-Molleur F, Penault-Llorca F, Longy M, Jacquier M, Birnbaum D, Pebusque MJ. WNT pathway and mammary carcinogenesis: loss of expression of candidate tumor suppressor gene SFRP1 in most invasive carcinomas except of the medullary type. Oncogene 2001; 20: 5810-5817
5. Caldwell GM, Jones C, Genberg K, Jan S, Hardy RG, Byrd P, Chughtai S, Wallis Y, Matthews GM, Morton DG. The Wnt antagonist sFRP1 in colorectal tumorigenesis. Cancer Res 2004; 64: 883-888
6. Takada T, Yagi Y, Maekita T, Imura M, Nakagawa S, Tsao SW, Miyamoto K, Yoshino O, Yasugi T, Taketani Y, Ushijima T. Methylation-associated silencing of the Wnt antagonist SFRP1 gene in human ovarian cancers. Cancer Sci 2004; 95: 741-744
7. Zou H, Molina JR, Harrington JI, Osborn NK, Klatt KK, Romero Y, Burgart LJ, Ahlquist DA. Aberrant methylation of secreted frizzled-related protein genes in esophageal adenocarcinoma and Barrett's esophagus. Int J Cancer 2005; 116: 584-591
8. Lodigyn D, Ephapintsev A, Menssen A, Diebold J, Hermeking H. Functional epigenomics identifies genes frequently silenced in prostate cancer. Cancer Res 2005; 65: 4218-4227
9. Zhao CH, Bu XM, Zhang N. Hypermethylation and aberrant expression of Wnt antagonist secreted frizzled-related protein 1 in gastric cancer. World J Gastroenterol 2007; 13: 2214-2217

**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 hypermethylation 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.
10 Attraj, J, Srinivasan R, Majumdar S, Radotra BD, Wig J. Alterations of tumor suppressor gene p16INK4a in pancreatic ductal carcinoma. BMC Gastroenterol 2005; 5: 22
11 Dammann R, Schagdarsurengin U, Liu L, Otto N, Gimm O, Drale H, Boehm BO, Pfeifer GP, Hoang-Vu C. Frequent RASSF1A promoter hypermethylation and K-ras mutations in pancreatic carcinomas. Oncogene 2003; 22: 3806-3812
12 Komazaki T, Nagai H, Emi M, Terada Y, Yabe A, Jin E, Kawanami O, Konishi N, Moriyama Y, Naka T, Kishimoto T. Hypermethylation-associated inactivation of the SOCS-1 gene, a JAK/STAT inhibitor, in human pancreatic cancers. Jpn J Clin Oncol 2004; 34: 191-194
13 House MG, Herman JG, Guo MZ, Hooker CM, Schulick RD, Cameron JL, Hruban RH, Maitra A, Yeo CJ. Prognostic value of hMLH1 methylation and microsatellite instability in pancreatic endocrine neoplasms. Surgery 2003; 134: 902-908; discussion 909
14 Blavier L, Lazaryev A, Dorey F, Shackleford GM, DeClerck YA. Matrix metalloproteinases play an active role in Wnt1-induced mammary tumorigenesis. Cancer Res 2006; 66: 2691-2699
15 Katoh M. WNT2 and human gastrointestinal cancer (review). Int J Mol Med 2003; 12: 811-816
16 Verras M, Brown J, Li X, Nusse R, Sun Z. Wnt3a growth factor induces androgen receptor-mediated transcription and enhances cell growth in human prostate cancer cells. Cancer Res 2004; 64: 8860-8866
17 Byun T, Karimi M, Marsh JL, Milovanovic T, Lin F, Holcombe RF. Expression of secreted Wnt antagonists in gastrointestinal tissues: potential role in stem cell homeostasis. J Clin Pathol 2005; 58: 515-519
18 Katoh Y, Katoh M. Comparative genomics on DKK2 and DKK4 orthologs. Int J Mol Med 2005; 16: 477-481
19 Tanaka Y, Kato K, Notohara K, Hojo H, Ijiri R, Miyake T, Nagahara N, Sasaki F, Kitagawa N, Nakatani Y, Kobayashi Y. Frequent beta-catenin mutation and cytoplasmic/nuclear accumulation in pancreatic solid-pseudopapillary neoplasm. Cancer Res 2001; 61: 8401-8404
20 Miao J, Kusafuka T, Kuroda S, Yoneda A, Zhou Z, Okada A. Mutation of beta-catenin and its protein accumulation in solid and cystic tumor of the pancreas associated with metastasis. Int J Mol Med 2003; 11: 461-464
21 Dessimoz J, Grapin-Botton A. Pancreas development and cancer: Wnt/beta-catenin at issue... Cell Cycle 2006; 5: 7-10
22 Zeng G, Germinario M, Micsenyi A, Monga NK, Bell A, Sood A, Malhotra V, Sood N, Midda V, Monga DK, Kokkinakis DM, Monga SP. aberrant Wnt/beta-catenin signaling in pancreatic adenocarcinoma. Neoplasia 2006; 8: 279-289
23 Lowy AM, Fenoglio-Preiser C, Kim OJ, Kordich J, Gomez A, Knight J, James L, Groden J. Dysregulation of beta-catenin expression correlates with tumor differentiation in pancreatic duct adenocarcinoma. Ann Surg Oncol 2003; 10: 284-290
24 Katoh M. Expression and regulation of WNT1 in human cancer: up-regulation of WNT1 by beta-estradiol in MCF-7 cells. Int J Oncol 2003; 22: 209-212
25 Saitoh T, Katoh M. Expression and regulation of WNT5A and WNT5B in human cancer: up-regulation of WNT5A by TNFalpha in MKN45 cells and up-regulation of WNT5B by beta-estradiol in MCF-7 cells. Int J Mol Med 2002; 10: 345-349
26 Kikukoshi H, Katoh M. Expression of WNT7A in human normal tissues and cancer, and regulation of WNT7A and WNT7B in human cancer. Int J Oncol 2002; 21: 895-900
27 Kikukoshi H, Katoh M. Expression and regulation of WNT10B in human cancer: up-regulation of WNT10B in MCF-7 cells by beta-estradiol and down-regulation of WNT10B in NT2 cells by retinoic acid. Int J Mol Med 2002; 10: 507-511
28 Taniguchi H, Yamamoto H, Hirata T, Miyamoto N, Oki M, Nosho K, Adachi Y, Endo T, Imai K, Shionomura Y. Frequent epigenetic inactivation of Wnt inhibitory factor-1 in human gastrointestinal cancers. Oncogene 2005; 24: 7946-7952

S- Editor Zhong XY L- Editor Wang XL E- Editor Liu Y