RESULTS: Out of 64 pancreatic cancer tissues, 21 were marked as Vimentin methylation-positive, and 43 were marked as Vimentin methylation-negative. The location of pancreatic carcinoma was related to the Vimentin methylation state. The pathological T staging ($P < 0.001$), adjuvant chemotherapy ($P = 0.003$) and the Vimentin methylation state ($P = 0.037$) were independent prognostic factors.

CONCLUSION: In our study, Vimentin methylation status can predict the prognosis of pancreatic cancer patients. However, additional experiments and clinical trials are needed to accurately validate this observation.

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Key words: Vimentin; Methylation; Pancreatic carcinoma; Prognosis

Core tip: Vimentin is reported to be an important mesenchymal marker, and plays an important role in epithelial-mesenchymal transition in malignant tumors with regard to cellular adhesion, migration and signaling. In our study, we found that pathological T staging ($P < 0.001$), adjuvant chemotherapy ($P = 0.003$) and the Vimentin gene methylation state ($P = 0.037$) were independent prognostic factors. However, additional experiments and clinical trials are needed to accurately validate this observation.

Zhou YF, Xu W, Wang X, Sun JS, Xiang JJ, Li ZS, Zhang XF. Negative methylation status of Vimentin predicts improved prognosis in pancreatic carcinoma. World J Gastroenterol 2014; 20(36): 13172-13177  Available from: URL: http://www.wjg.net/1007-9327/full/v20/i36/13172.htm  DOI: http://dx.doi.org/10.3748/wjg.v20.i36.13172

INTRODUCTION

Pancreatic cancer is one of the most lethal malignan-
Vimentin is reported as an important mesenchymal marker, and plays an important role in epithelial-mesenchymal transition in malignant tumors with regard to cellular adhesion, migration and signaling. Several investigators have previously shown that Vimentin is an important marker for the early detection of cancer, such as bladder cancer, hepatocellular carcinoma and colorectal cancer. In addition, methylation of the Vimentin gene is described as a marker in several malignant tumors, including gastric carcinoma, colorectal carcinoma, cervical cancer and bladder cancer. In our current study, we have attempted to identify the relationship between the methylation state of Vimentin and pancreatic cancer.

**MATERIALS AND METHODS**

**Sample collection and DNA preparation**

Sixty-four primary tumor specimens and normal tissues were collected consecutively from pancreatic cancer patients undergoing surgery at Hangzhou First People’s Hospital and Affiliated Hospital of the Logistics University of the Chinese People’s Armed Police Force. All specimens were confirmed by histopathology. Written informed consent was obtained from all patients. All the collected samples were stored at -80°C. DNA from the samples was extracted by QIAamp DNA Mini Kit (Catalog number: 51306, Qiagen, Hilden, Germany). The clinicopathological characteristics of the patients who were enrolled in our study are shown in Table 1.

**Sodium bisulfite modification**

Genomic tumor DNA (1 μg) and the corresponding normal pancreatic tissue specimens were subjected to bisulfite treatment using an Epitect Bisulfite Kit (Catalog No. 59104, Qiagen, Hilden, Germany). The clinicopathological characteristics of the patients who were enrolled in our study are shown in Table 1.

**Quantitative methylation-specific polymerase chain reaction**

The bisulfite-treated DNA was amplified using a quantitative methylation-specific polymerase chain reaction and a Thermal Cycler Dice® Real-time System TP800 (Takara Bio Inc., Otsu, Japan). Thermo-cycling was carried out in a final volume of 25 μL containing 1.0 μL of the DNA sample, 100 nmol/L each of the Vimentin or β-actin primers (forward and reverse sequences), and 12.5 μL of SYBR Premix Ex Taq™ (Takara Bio Inc., Otsu, Japan), which consists of Taq DNA polymerase, 

| Table 1 Clinicopathological features of pancreatic carcinoma (n) (%) |
|-----------------------------------------------|
| **Data** | **Vimentin methylated negative group (n = 43)** | **Vimentin methylated positive group (n = 21)** | **P value** |
| Sex | | | 0.582 |
| Male | 27 (62.8) | 15 (71.4) | |
| Female | 16 (37.2) | 6 (28.6) | |
| Tumor position | | | 0.007 |
| Head | 11 (25.6) | 13 (61.9) | |
| Body and tail | 32 (74.4) | 8 (38.1) | |
| Preoperative CEA level | | | 0.294 |
| Normal | 20 (46.5) | 13 (61.9) | |
| Elevated | 23 (53.5) | 8 (38.1) | |
| Preoperative CA19-9 level | | | 0.600 |
| Normal | 24 (55.8) | 10 (47.6) | |
| Elevated | 19 (44.2) | 11 (52.4) | |
| Pathological N staging | | | 0.426 |
| N0 | 21 (48.8) | 13 (61.9) | |
| N1 | 22 (51.2) | 8 (38.1) | |
| Pathological T staging | | | 0.753 |
| T1 | 14 (32.6) | 5 (23.8) | |
| T2 | 19 (44.2) | 11 (52.4) | |
| T3 | 10 (23.3) | 5 (23.8) | |
| Adjuvant chemotherapy | | | 0.791 |
| No | 20 (46.5) | 11 (52.4) | |
| Yes | 23 (53.5) | 10 (47.6) | |

The pathological T and N staging was based on the UICC staging systems for pancreatic cancer; 1Median age 54 years, range: 36-71 years; 2Median age 53 years, range: 41-68 years.
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**Table 2 Univariate analysis of overall survival in pancreatic carcinoma**

| Variables                      | n  | Median survival (mo) | P value |
|--------------------------------|----|----------------------|---------|
| Sex                            |    |                      |         |
| Male                           | 42 | 13.45                | 0.819   |
| Female                         | 22 | 10.84                |         |
| Preoperative CEA               |    |                      |         |
| Normal                         | 33 | 15.15                | 0.260   |
| Elevated                       | 31 | 13.61                |         |
| Preoperative CA19-9            |    |                      |         |
| Normal                         | 40 | 14.12                | 0.947   |
| Elevated                       | 24 | 15.09                |         |
| Tumor position                 |    |                      |         |
| Body and tail                  | 40 | 16.20                | 0.007   |
| Head                           | 24 | 11.25                |         |
| Pathological T stage           |    |                      | < 0.001 |
| T1                             | 19 | 21.01                |         |
| T2                             | 30 | 13.10                |         |
| T3                             | 15 | 8.00                 |         |
| Pathological N stage           |    |                      | 0.311   |
| N0                             | 34 | 14.28                |         |
| N1                             | 30 | 16.20                |         |
| Adjuvant chemotherapy          |    |                      |         |
| Yes                            | 33 | 16.12                | 0.015   |
| No                             | 31 | 13.07                |         |
| Vimentin methylation state     |    |                      | 0.013   |
| Positive                       | 21 | 11.09                |         |
| Negative                       | 43 | 16.03                |         |

**RESULTS**

**Vimentin methylation in pancreatic cancer and corresponding pancreatic tissues**

We detected Vimentin methylation in pancreatic cancer and corresponding pancreatic tissues. Of the 64 pancreatic cancer tissues, 21 of them had a high-level methylation status and 45 of the corresponding pancreatic tissues had a high level of methylation. There were 9 pancreatic cancer tissues and 5 normal corresponding pancreatic tissues without methylation of the Vimentin gene. In addition, Vimentin methylation scores were recorded and informed that 43 of them were marked as Vimentin methylation-negative, and the remaining 21 were Vimentin methylation-positive.

**Vimentin methylation state was related to the age and the diameter of the tumor**

The clinicopathological factors seen between these two groups are summarized in Table 1. Moreover, we found that the location of the pancreatic carcinoma was associated with the status of Vimentin methylation. However, patient gender, preoperative serum tumor markers, lymph node metastasis and pathological T-stage were found not to be associated with the Vimentin methylation state.

**Vimentin methylation state was an independent prognostic factor in pancreatic cancer**

Univariate analysis showed that tumor position (P = 0.002), pathological T-staging (P < 0.001), adjuvant chemotherapy (P = 0.015) and the Vimentin methylation state (P = 0.013) were prognostic factors in pancreatic carcinoma (Table 2 and Figure 1). Multivariate analysis showed that the pathological T-staging (P < 0.001), adjuvant chemotherapy (P = 0.003) and the Vimentin methylation state (P = 0.037) were independent prognostic factors (Table 3).

**DISCUSSION**

An estimated 44030 people were diagnosed with pancreatic cancer and approximately 37660 people died of pancreatic cancer in the United States in 2011[19]. Although the technology of radiotherapy and chemotherapy has been developed, the incidence and mortality rates have remained approximately the same over the past two decades. A mutation in the CDKN2A (p16) gene has been reported...
found that of 64 pancreatic cancer tissues, 21 of them displayed a high level of methylation status and 45 of the corresponding pancreatic tissues displayed a high level of methylation. Moreover, survival analysis showed that the Vimentin methylation status was an independent prognostic factor as well as a prognostic marker in T-staging and adjuvant chemotherapy. We are also aware that a low methylation status is always associated with high Vimentin expression levels. Additionally, Vimentin has been shown to be associated with several pathways, including cell adhesion, cytoplasmic microtubule assembly, and cytoskeleton remodeling. Higher Vimentin expression in pancreatic cancer cells may imply a higher state of malignancy of these cells, with an associated higher metastatic ability. The detailed mechanism of Vimentin and its gene methylation status requires further study.

Our observations only covered 64 pancreatic cancer patients, which is a small population sample. Although our results showed that the Vimentin methylation status could be used to predict prognosis in pancreatic cancer, more studies and clinical trials are needed to validate this result. In summary, our study showed that pancreatic cancer patients exhibiting a negative Vimentin methylation directly modulating transcription factor function or by triggering the formation of inactive chromatin. We found that of 64 pancreatic cancer tissues, 21 of them displayed a high level of methylation status and 45 of the corresponding pancreatic tissues displayed a high level of methylation. Moreover, survival analysis showed that the Vimentin methylation status was an independent prognostic factor as well as a prognostic marker in T-staging and adjuvant chemotherapy. We are also aware that a low methylation status is always associated with high Vimentin expression levels. Additionally, Vimentin has been shown to be associated with several pathways, including cell adhesion, cytoplasmic microtubule assembly, and cytoskeleton remodeling. Higher Vimentin expression in pancreatic cancer cells may imply a higher state of malignancy of these cells, with an associated higher metastatic ability. The detailed mechanism of Vimentin and its gene methylation status requires further study.

Our observations only covered 64 pancreatic cancer patients, which is a small population sample. Although our results showed that the Vimentin methylation status could be used to predict prognosis in pancreatic cancer, more studies and clinical trials are needed to validate this result. In summary, our study showed that pancreatic cancer patients exhibiting a negative Vimentin methylation
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status displayed a poorer prognosis as compared with those with a positive status. The role of Vimentin methylation in pancreatic cancer warrants further empirical exploration.

COMMENTS

Background

Vimentin is reported as an important mesenchymal marker, and plays an important role in epithelial-mesenchymal transition in malignant tumors with regard to cellular adhesion, migration and signaling. In their current study, authors have attempted to identify the relationship between the methylation state of the Vimentin gene and pancreatic cancer.

Research frontiers

Several investigators have previously shown that Vimentin is an important marker for the early detection of cancer, such as bladder cancer, hepatocellular carcinoma and colorectal cancer. In addition, methylation of Vimentin is described as a marker in several malignant tumors, including gastric carcinoma, colorectal carcinoma, cervical cancer and bladder cancer.

Innovations and breakthroughs

The location of pancreatic carcinoma was related to the methylation state. The pathological T staging (P < 0.001), adjuvant chemotherapy (P = 0.003) and the Vimentin methylation state (P = 0.037) were independent prognostic factors.

Applications

This result showed that the Vimentin methylation status could be used to predict prognosis in pancreatic cancer.

Peer review

The manuscript is very interesting. The authors try to determine the existence of a potential relationship between the methylation state of Vimentin and the pathological T staging. The authors found that Vimentin methylation status can predict the prognosis of pancreatic cancer patients.

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