Alteration of somatostatin receptor subtype 2 gene expression in pancreatic tumor angiogenesis

Ren-Yi Qin, Ru-Liang Fang, Manoj Kumar Gupta, Zheng-Ren Liu, Da-Yu Wang, Qing Chang, Yi-Bei Chen

AIM: To explore the difference of somatostatin receptor subtype 2 (SST2R) gene expression in pancreatic cancerous tissue and its adjacent tissue, and the relationship between the change of SST2R gene expression and pancreatic tumor angiogenesis related genes.

METHODS: The expressions of SST2R, DPC4, p53 and ras genes in cancer tissues of 40 patients with primary pancreatic cancer, and the expression of SST2R gene in its adjacent tissue were determined by immunohistochemical LSAB method and EnVision™ method. Chi-square test was used to analyze the difference in expression of SST2R in pancreatic cancer tissue and its adjacent tissue, and the correlation of SST2R gene expression with the expression of p53, ras and DPC4 genes.

RESULTS: Of the tissue specimens from 40 patients with primary pancreatic cancer, 35 (87.5%) cancer tissues showed a negative expression of SST2R gene, whereas 34 (85%) a positive expression of SST2R gene in its adjacent tissues. Five (12.5%) cancer tissues and its adjacent tissues simultaneously expressed SST2R. The expression of SST2R gene was markedly higher in pancreatic tissues adjacent to cancer than in pancreatic cancer tissues (P<0.05). The expression rates of p53, ras and DPC4 genes were 50%, 60% and 72.5%, respectively. There was a significant negative correlation of SST2R with p53 and ras genes (χ²=9.33, χ²=15.43, P<0.01), but no significant correlation with DPC4 gene (χ²=2.08, P>0.05).

CONCLUSION: There was a significant difference of SST2R gene expression in pancreatic cancer tissues and its adjacent tissues, which might be one cause for the different therapeutic effects of somatostatin and its analogs on pancreatic cancer patients. There were abnormal expressions of SST2R, DPC4, p53 and ras genes in pancreatic carcinogenesis, and moreover, the loss or decrease of SST2R gene expression was significantly negatively correlated with the overexpression of tumor angiogenesis correlated p53 and ras genes, suggesting that SST2R gene together with p53 and ras genes may participate in pancreatic cancerous angiogenesis.
and incubated at 37 °C for 1 hour, followed by incubation with peroxidase conjugated streptavidin (1:400 dilution) at 37 °C for 30 minutes. For p53 and ras staining, peroxidase conjugated streptavidin was replaced by EnVision. DAB chromogen was applied for 10 to 20 minutes and rinsed for visualization under microscope, followed by slight counterstaining with haematoxylin, dehydration, and finally cover slips were sealed with permount. Negative control was obtained by replacing the primary antibody with PBS.

**Evaluation of immunohistochemical staining**

Positive staining was located in cell membrane/cytoplasm for SST2R, and the cytoplasm for DPC4 and ras, and the nucleus for p53. At least 1000 cells were counted per 40 X 10 field. The expressions were graded, as follows. Negative (-) if <10% of the cancerous cells in a given specimen were positively stained. Weak positive (+) if 10% to 20% of the cancerous cells in a given specimen were positively stained. Positive (+++) if 20% to 50% of the cancerous cells in a given specimen were positively stained, and strongly positive (++++) if 75% of the cancerous cells in a given specimen were positively stained.

**Statistical analysis**

Chi-square test was used to analyze the results. $P<0.05$ was considered statistically significant.

**RESULTS**

**Expression of SST2R gene in pancreatic cancer tissue and its adjacent tissue**

The expression rate of SST2R gene was markedly higher in the adjacent non-cancerous tissue (85%) than in cancer tissue (12.5%) ($P<0.05$). Twenty-nine patients (72.5%) expressed SST2R gene only in the adjacent non-cancerous tissue. For SST2R gene expression in pancreatic cancer tissue, strong positive was only 10%, weak positive 2.5%, and negative 87.5%. 12.5% pancreatic cancer tissue and its adjacent non-cancerous tissue expressed SST2R simultaneously (Table 1 and Figures 1, 2).

**Expression of p53 gene in pancreatic cancer tissue**

For p53 gene in pancreatic cancerous tissue, 19 patients (47.5%) had strong positive expression, 20 patients (50%) negative expression, and 1 patient (2.5%) weak positive expression. The SST2R gene expression in 17 patients with strong positive expression of p53 was negative. Three patients with strong positive expression of SST2R were negative for p53 gene expression. The SST2R and p53 gene expressions in 1 patient were strong positive. One patient with weak positive expression of p53 gene was negative for SST2R gene expression. The SST2R gene expression of 1 patient with strong positive expression of p53 gene was weak positive (Table 1 and Figure 3).
Expression of ras gene in pancreatic cancer tissue
For expression of ras gene, 14 patients (35%) were strong positive, 10 patients (25%) weak positive, and 16 patients (40%) negative. Eleven patients with strong positive expression of ras gene showed negative expression of SST2R gene, 1 patient with negative expression of ras gene showed strong positive expression of SST2R gene. The SST2R and ras genes in 4 patients showed positive expression of different grades correspondingly. The SST2R and ras genes in 15 patients were negative. Nine patients with weak positive expression of ras gene were negative for SST2R gene expression (Table 1 and Figure 4).

Expression of DPC4 gene in pancreatic cancer tissue
Seven patients (17.5%) were positive, 4 patients (10%) weak positive, and 29 patients (72.5%) negative for DPC4 gene expression. Twenty-six patients (65%) had negative expression of both DPC4 and SST2R gene. Of the 9 patients with negative expression of SST2R gene, 5 patients were strong positive, and 4 patients weak positive for DPC4 gene expression. Both SST2R and DPC4 genes expression of 2 patients were strong positive. One patient with negative expression of DPC4 gene was weak positive for SST2R gene expression (Table 1 and Figure 5).

Statistical analysis indicated that SST2R was significantly negatively correlated with the expression of p53 or ras genes (P<0.01), whereas not distinctly correlated with DPC4 gene (P>0.05).

Table 1 Expressions of SST2R and ras, DPC4 and p53 genes in 40 primary pancreatic cancerous tissues

| Expression                  | Strong positive (++++ n (%)) | Weak positive (+ n (%)) | Negative (- n (%)) |
|-----------------------------|-------------------------------|-------------------------|-------------------|
| SST2R                       | 4.0 (10)                      | 1.0 (2.5)               | 35.0 (87.5)       |
| ras                          | 14.0 (35)                     | 10.0 (25)               | 16.0 (4)          |
| DPC4                         | 7.0 (17.5)                    | 4.0 (10)                | 19.0 (47.5)       |
| P53                          | 19.0 (47.5)                   | 1.0 (2.5)               | 20.0 (50)         |
| Cancer          adjacent SST2R | 34.0 (85)                     | 0.0                     | 6.0 (15)          |

DISCUSSION
Somatostatin and its analogs can inhibit the growth of benign and malignant tumors, and it has been found that the inhibitory effects are directly correlated with the expression of SST2R on tumor tissues[11-13]. The mechanisms of the inhibition are the combined interaction of somatostatin and its analogs with SST2R in tumor tissues, either directly inhibiting division and proliferation of tumor cells or inhibiting the activities of growth factors such as vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)[16-21] and moreover, counteracting tumorigenesis and tissue proliferation[22,23]. In addition, somatostatin and its analogs could arrest pancreatic cancer cell cycle at G1-S phase by up-regulating p27, and thus induced apoptosis[24,25].

The expression of SST2R was decreased or lost in primary pancreatic cancerous tissue[26-29], but there is no report on the expression of SST2R in pancreatic cancerous adjacent tissue. Our study showed that 5 of 40 primary pancreatic cancer tissues showed positive expression of SST2R gene, suggesting that most of pancreatic cancerous tissues lack the expression of SST2R, which may be the major reason why somatostatin and its analogs had no marked therapeutic effects on pancreatic cancer. Interestingly, expression of SST2R was higher in most of the pancreatic cancerous adjacent tissues, which may be beneficial for somatostatin and its analogs to inhibit fast outward infiltration and growth of tumor cells. Our study indicated that if SST2R decreased in pancreatic cancerous tissue and its adjacent tissue, somatostatin and its analogs might not have therapeutic effect on pancreatic cancer. But when pancreatic cancerous tissue and its adjacent tissue obviously expressed SST2R, they had a definite therapeutic effect on patients with pancreatic cancer. Hence the differences in the expression of SST2R in pancreatic cancerous tissue and its adjacent tissue would certainly lead to different therapeutic effects. At present, this is the main possible cause for the different therapeutic effects of somatostatin and its analogs on pancreatic cancer. We consider that it is important to define the expression of SST2R in pancreatic cancerous tissue and its adjacent tissue before using somatostatin and its analogs. This will be beneficial to the ultimate definition of the exact therapeutic effects of somatostatin and its analogs on pancreatic cancer.

Mutations of DPC4, ras and p53 genes have been found to play crucial roles in tumor angiogenesis[30-33]. The mutation rates of these genes are higher in pancreatic cancerous tissue, indicating that the similar mechanism of pancreatic tumorigenesis with other tumorigenesis involving mutation and abnormal expression of multiple genes. Our study indicated that the expression of SST2R gene was significantly negatively correlated with p53 and ras genes, suggesting that the decrease or loss of SST2R gene expression in pancreatic cancerous tissue participates in tumor angiogenesis through a certain pathway, further investigation is required to probe into its the mechanism.

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Edited by Ren SY and Wang XL