Expression of DNA-repair proteins and their significance in pancreatic cancer and non-cancerous pancreatic tissues of Sprague–Dawley rats

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Abstract

**Background:** To establish a model of pancreatic cancer induced by 7,12-dimethylbenzanthracene (DMBA) in Sprague–Dawley (SD) rats, and detect the expression of DNA-repair proteins (MGMT, ERCC1, hMSH2, and hMLH1) and their significance in pancreatic cancer and non-cancerous pancreatic tissues of SD rats.

**Methods:** DMBA was directly implanted into the parenchyma of rat pancreas (group A and group B), and group B rats were then treated with trichostatin A (TSA). The rats in both groups were executed within 3 to 5 months, and their pancreatic tissues were observed by macrography and under microscopy. Meanwhile, the rats in the control group (group C) were executed at 5 months. Immunohistochemistry was used to assay the expression of MGMT, ERCC1, hMSH2, and hMLH1.

**Results:** The incidence of pancreatic cancer in group A within 3 to 5 months was 48.7% (18/37), including 1 case of fibrosarcoma. The incidence of pancreatic cancer in group B was 33.3% (12/36), including 1 case of fibrosarcoma. The mean of maximal diameters of tumors in group A was higher than that in group B (P <0.05). No pathological changes were found in pancreas of group C and other main organs (except pancreas) of group A and group B. No statistical differences were found among the positive rates of MGMT, ERCC1, hMSH2, and hMLH1 in ductal adenocarcinoma and non-cancerous pancreatic tissues of group A (P >0.05). The positive rates of MGMT, ERCC1, hMSH2, and hMLH1 were significantly lower in ductal adenocarcinoma than those in non-cancerous tissues of group B (P ≤0.05). All pancreas of group C had positive expression of MGMT, ERCC1, hMSH2, and hMLH1 and two cases of fibrosarcoma showed a negative expression.

**Conclusions:** DMBA, directly implanted into the parenchyma of pancreas, creates an ideal pancreatic cancer model within a short time. TSA might restrain DNA damage related to the genesis and growth of pancreatic cancer in rats. The DNA-repair proteins, including MGMT, ERCC1, hMSH2, and hMLH1, might play an important role in the genesis of pancreatic cancer induced by DMBA in rats.

**Keywords:** 7,12-dimethylbenzanthracene, Animal model, DNA-repair proteins, Immunohistochemistry, Pancreatic neoplasms, Sprague–Dawley rats, Trichostatin A
Background
Pancreatic cancer is a solid malignancy characterized by its rapid growth and propensity to invade adjacent organs and metastasize. Worldwide, pancreatic cancer causes approximately 213,000 deaths each year. The 1-year survival rate is around 20% and 5-year survival rate is less than 5% in spite of aggressive therapies [1].

Within the last two decades, research has shown that pancreatic cancer is fundamentally a genetic disease caused by inherited germline and acquired somatic mutations in cancer-associated genes, and more and more investigation of molecular pathogenesis has been used in the diagnosis and treatment of pancreatic cancer. To build useful models studying the pathological molecular mechanisms of pancreatic cancer, Rivera et al. directly implanted dimethylbenzanthracene (DMBA) into the parenchyma of the rat pancreas and found a pancreatic cancer incidence of 39% within 10 months [2]; Bockman et al. reported similar studies [3].

Trichostatin A (TSA) is a histone deacetylase inhibitor with a broad spectrum of epigenetic activities. It can up-regulate the expression of several genes and restrain other genes’ expression, thus intervening in the genesis and development of tumors. In vivo or in vitro experiments have confirmed that TSA could restrain the genesis of some tumors and control tumor progression by restraining tumor angiogenesis and changing the tumor microenvironment [4]. Some studies have shown that TSA acts as a tumor suppressor in human pancreatic cancer cell lines [5,6].

The DNA mismatch repair (MMR) system is an inbuilt security system that can repair DNA mismatch in human cells, and plays an important role in retaining the integrity and stability of genes. The main MMR genes are hMSH1-6, hMLH1-5 and others, and the methylation integrality and stability of genes. The main MMR genes under microscopy. It is made with these specimens. Finally the sections were dyed by hematoxylin and eosin staining, and observed under microscopy.

Immunohistochemical staining of EnVision™
Immunostaining was conducted by use of the ready-to-use, peroxidase-based EnVision™ Detection kit (Dako Laboratories, CA, USA) according to the user manual.

Table 1 Incidences of pancreatic tumors in groups A and B (case number ratio (%))

| Group | n  | 3rd month | 4th month | 5th month | Total cases |
|-------|----|-----------|-----------|-----------|-------------|
| A     | 37 | 2/7 (28.6)| 4/10 (40.0)| 12/20 (60.0)| 18/37 (48.7) |
| B     | 36 | 1/6 (16.7)| 3/10 (30.0)| 8/20 (40.0) | 12/36 (33.3) |
Four-micrometer-thick sections were cut from routinely paraffin-embedded tissues. Rabbit anti-rat MGMT, ERCC1, hMSH2, and hMLH1 monoclonal antibodies were obtained from Cell Signaling Technology, Inc. (Danvers, MA, USA). EnVision™ detection kit was from Dako Laboratories, CA, USA. Cytoplasm and (or) cell nuclei containing brown-yellow granules were defined as positive cells. The percentage of positive cells was calculated from 10 random fields. Cases with ≥25% positive cells were considered positive and cases were otherwise considered negative [22,23]. The positive controls were the positive pancreatic cancer biopsies provided by CST (Cell Signaling Technology, Inc.) while the negative controls were prepared by 5% fetal bovine serum substituting the primary antibody.

Table 2 Pathological types of pancreatic tumors in groups A and B (case number)

| Group | n  | Fibrosarcoma | Pancreatic ductal adenocarcinoma |
|-------|----|--------------|----------------------------------|
|       |    |              | Well-differentiated | Moderately-differentiated | Poorly-differentiated |
| A     | 18 | 1            | 6                      | 7                      | 4                     |
| B     | 12 | 1            | 6                      | 4                      | 1                     |

Results

Macrography

The incidence of pancreatic tumors in groups A and B are shown in Table 1; the incidence of tumors in group A was higher than that in group B (P >0.05). Both groups A and B had one case of fibrosarcoma that developed liver metastasis and epiploon metastasis. The distribution of diameter of tumor mass in group A was 0.5–1.0 cm (7 cases), 1.0–2.0 cm (10 cases), and >2.0 cm (1 case); and the distribution of diameter of tumor mass in group B was 0.5–1.0 cm (9 cases), 1.0–2.0 cm (2 cases), and >2.0 cm (1 case). The mean of maximal diameter of tumors in group A was higher than that in group B (P <0.05). No pathological changes were found by macrography in pancreas of group C and other main organs (except pancreas) of groups A and B.

Pathological observation

Pathological results of pancreatic tumors in groups A and B are shown in Table 2 and Figure 1A. Both non-cancerous pancreatic tissues and peritumoral pancreatic tissues in groups A and B showed hyperplasia to atypical-hyperplasia. Non-cancerous pancreatic tissues in group A which showed mild atypical-hyperplasia were found in 5 cases (26.3%) and moderately to severely atypical-hyperplasia in 10 cases (52.6%). The same tissues were found in group B in 10 cases (41.6%) and 8 cases (33.3%), respectively; therefore, no statistical differences were found in the two groups (P >0.05). No pathological changes were found by microscopy in pancreas of other main organs (except pancreas) of groups A and B.

Figure 1 Pathological observation of rat pancreatic lesions induced by DMBA. Hematoxylin-eosin stain, original magnification × 200. (A) Poorly-differentiated ductal adenocarcinoma (group A); (B) Well-differentiated ductal adenocarcinoma (group B); (C) Fibrosarcoma (group B); (D) Severe atypical hyperplasia in ductal epithelia (group A).
group C and other main organs (except pancreas) of groups A and B.

Expression of MGMT, ERCC1, hMSH2, and hMLH1 in pancreatic ductal adenocarcinoma and non-cancerous pancreatic tissues

The positive rates of MGMT, ERCC1, hMSH2, and hMLH1 (Figure 2) were significantly lower in ductal adenocarcinoma than those in non-cancerous pancreatic tissues in group A + group B (P <0.01 or P <0.05). No statistical differences were found among the positive rates of MGMT, ERCC1, hMSH2, and hMLH1 in ductal adenocarcinoma and non-cancerous pancreatic tissues of group A (P >0.05). The positive rates of MGMT, ERCC1, hMSH2, and hMLH1 were significantly lower in ductal adenocarcinoma than those in non-cancerous tissues of group B (P <0.05). The ductal epithelium of non-cancerous pancreas which had negative expression of MGMT, ERCC1, hMSH2, and hMLH1 in groups A and B all showed moderately or severe atypical-hyperplasia. The fibrosarcoma had negative expression of MGMT, ERCC1, hMSH2, and hMLH1, while pancreas of group C had positive expression of MGMT, ERCC1, hMSH2, and hMLH1 (Table 3). Expression of MGMT, ERCC1, hMSH2, and hMLH1 had no obvious correlation with the size of tumor mass and differentiation degree of ductal adenocarcinoma (P >0.05).

Discussion

Establishing of a pancreatic cancer model can be achieved through three kinds of methods [24-27]: 1) exposing canine animal to carcinogen, 2) activating the oncogenes of transgenic mice, and 3) transplanting the xenogenic pancreatic cancer tissues to athymic mouse.

Table 3 Expression of MGMT, ERCC1, hMSH2, and hMLH1 in ductal adenocarcinoma and non-cancerous pancreatic tissues (%)

| Group | n | MGMT | ERCC1 | hMSH2 | hMLH1 |
|-------|---|-------|-------|-------|-------|
| Pancreatic ductal adenocarcinoma | | | | | |
| A + B | 28 | 15 (53.6) | 13 (46.2) | 14 (50.0) | 12 (42.9) |
| A | 17 | 9 (52.9) | 8 (47.1) | 9 (52.9) | 8 (47.1) |
| B | 11 | 6 (54.6) | 5 (45.5) | 5 (45.5) | 4 (36.4) |
| Non-cancerous pancreatic tissues | | | | | |
| A + B | 43 | 35 (81.4) | 33 (76.7) | 32 (74.4) | 30 (70.0) |
| A | 19 | 14 (73.7) | 14 (73.7) | 12 (63.2) | 11 (57.9) |
| B | 24 | 21 (87.5) | 19 (79.2) | 19 (79.2) | 19 (79.2) |

Pancreatic ductal adenocarcinoma compared with non-cancerous pancreatic tissues in group A + B: χ²_MGMT = 6.30, P <0.05; χ²_ERCC1 = 6.83, P <0.01; χ²_hMSH2 = 4.43, P <0.05; χ²_hMLH1 = 5.08, P <0.05.

Pancreatic ductal adenocarcinoma compared with non-cancerous pancreatic tissues in group A: P_MGMT = 0.157, P_ERCC1 = 0.088, P_hMSH2 = 0.103, P_hMLH1 = 0.452.

Pancreatic ductal adenocarcinoma compared with non-cancerous pancreatic tissues in group B: P_MGMT = 0.042, P_ERCC1 = 0.050, P_hMSH2 = 0.050, P_hMLH1 = 0.018.
Rivera et al. directly implanted DMBA into the parenchyma of rat pancreas to establish a pancreatic cancer model of rats and the incidence of cancer of SD rats within 10 months was 39% [2]. Since then, a series of mouse and rat pancreatic cancer models using DMBA have been established [28-32]. TSA can increase intra-cellular histone levels and up-regulate the expression of several genes. Some experiments have confirmed that TSA can restrain the genesis of some tumors by restraining angiogenesis, inhibiting proliferative activity, and promoting apoptosis of tumor cells [33-37]. After we directly implanted a major dose of DMBA (9 mg) into the pancreas parenchyma of SD rats, the incidence of cancer in group A within 3 to 5 months was 48.7%, and that in group B was 33.3%; their pathological types were the same as those of human pancreatic ductal adenocarcinoma, except for two cases of fibrosarcoma. The incidence of cancer in group A was higher than that in group B, but the difference had no statistical significance ($P > 0.05$). The mean of maximal diameter of tumors in group A was higher than that in group B ($P < 0.05$). Our SD rat model of pancreatic cancer had some merits: 1) the period of tumor formation was short and the incidence of cancer was high; 2) the pathological type was mainly the same as human pancreatic ductal adenocarcinoma; 3) no pathological changes were found in main organs (except pancreas); 4) the inhibitive effect on carcinogenesis and growth of TSA was obvious; and 5) the cost was low.

MGMT is a high-performance DNA-repair enzyme that can protect cells from alkylating agent damage and can prevent cell carcinogenesis and death. The $MGM T$ gene is located in 10q26 and encodes 207 amino acids' proteins [7-11]. Normal cells all have MGMT expression, while some malignant tumors will lose MGMT expression which will induce the damage of DNA repair and the carcinogenesis of cells [7-11,38,39]. ERCC1 is a member of the exonuclease repair enzyme family and its low expression is always related to elevated cancer incidence, while its high expression is always related to resistance to platinum drugs [12-16]. Recent studies have confirmed that ERCC1 is the key enzyme of the DNA repair induced by cisplatin and it has been shown that ERCC1 expression of some malignant tumors played an important role in guiding chemotherapy [17-21,37]. The hMSH2 gene is located in 2P16 and is the first separated MMR. It can repair DNA mismatch and retain the integrity and stability of genes. Many recent papers have reported that the loss of hMSH2 protein expression was crucial to the genesis and progression of malignant tumors [7-11,40-42]. hMLH1 is also a type of MMR which can also inhibit carcinogenesis by repairing DNA mismatching. Mutation of the $hML H_1$ gene will induce the genesis of many malignant tumors [7-11,41].

Conclusions

Our data have shown that the positive rates of MGMT, ERCC1, hMSH2, and hMLH1 were significantly lower in pancreatic ductal adenocarcinoma than in non-cancerous pancreatic tissues of rats, and the ductal epithelia of non-cancerous pancreas which had negative expression of MGMT, ERCC1, hMSH2, and hMLH1 all showed atypical-hyperplasia. The results show that there was loss expression of MGMT, ERCC1, hMSH2, and hMLH1 in the course of genesis of pancreatic cancer induced by DMBA in rats, which might be the mechanism of carcinogenesis by DMBA. Therefore, testing the expression of MGMT, ERCC1, hMSH2, and hMLH1 in pancreatic cancer might play an important role in guiding the treatment of human pancreatic cancer.

Abbreviations

DMBA: Dimethylbenzanthracene; ERCC1: Excision repair cross-complementing gene 1; MGMT: O6-methylguanine DNA methyltransferases; MMR: Mismatch repair; SD: Sprague–Dawley; TSA: Trichostatin A.

Competing interests

The authors report no competing interests. No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

Authors’ contributions

TXG did most of the experiments and data acquisition, TXG and YZ participated in the design of experiments, interpretation of data, and writing of the manuscript. LY and XYM participated in the experiments and writing. All authors read and approved the final manuscript.

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