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# Histopathological Characteristics of Pancreatic Cancer Stroma Induced by Neoadjuvant Chemotherapy

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## ABSTRACT

Pancreatic cancer is considered as a chemoresistant tumor because of its abundant cancer stroma which contains cancer-associated fibroblasts (CAFs). Nab-paclitaxel (Nab-PTX, cytoskeletal anticancer drug) is expected as a key drug for pancreatic cancer. The patients of pancreatic cancer treated with Nab-Paclitaxel show remarkable tumor reduction in size. We analyzed total 20 surgically resected cases of pancreatic ductal adenocarcinoma; ten cases treated with Nab-PTX before surgical operation (treated group) and ten cases without neoadjuvant chemotherapy (untreated group). The treated group showed high density of aniline blue (AnB) positive collagen bundles in the stroma and low density of α-smooth muscle actin (α-SMA) positive CAFs. On the other hand, the untreated group exhibited low density of AnB positive collagen bundles and high density of CAFs. We speculated that the Nab-PTX neoadjuvant chemotherapy plays in roles of stromal collagenous fibrosis and inactivation of CAFs.

**Keywords:** Pancreatic ductal adenocarcinoma, Neoadjuvant chemotherapy, Nab-Paclitaxel, Cancer associated fibroblast

## INTRODUCTION

Pancreatic cancer shows one of the poorest patient prognoses among all types of human cancer [1]. The majority pancreatic cancer is invasive ductal carcinoma, i.e., pancreatic ductal adenocarcinoma [2-4]. Histopathologically, pancreatic cancer tissues have abundant cancer stroma and many cancer-associated fibroblasts (CAFs) which are considered to be one of the major factors contributing to the poor prognosis of pancreatic cancer [5,6]. Previously the pancreatic cancers were chemoresistance [7], but the recent chemotherapy has been improved the patient prognosis gradually. Nab-Paclitaxel (Nab-PTX) has been expected as a key drug for the pancreatic cancer treatment. The patients with pancreatic cancer performed neoadjuvant chemotherapy with Nab-PTX frequently showed remarkable tumor reduction in size. Paclitaxel (PTX) is one of cytoskeletal drugs, and its derivative Nab-PTX is developed to improve solubility binding to human serum albumin. The main mechanism of PTX is to inhibit the depolymerization of microtubules. A few studies have reported Nab-PTX controls tumor by reducing CAFs [8,9]. However, the anticancer mechanisms of Nab-PTX are still incompletely understood.

In this study we investigated why Nab-PTX has prominent anticancer effects, analyzing histopathological specimens of pancreatic cancer stroma which were performed neoadjuvant chemotherapy with Nab-PTX.

## MATERIALS AND METHODS

We investigated total 20 surgically resected cases of pancreatic ductal adenocarcinomas; ten cases treated with Nab-PTX before surgical operation (treated group) and ten cases without neoadjuvant chemotherapy (untreated group). Numbers of the cases were limited because the Nab-PTX treatment has recently established as the neoadjuvant chemotherapy for pancreatic cancer.

We evaluated the cancer stromal phenotypes using Heidenhain's AZAN trichrome stain (AZAN stain) [10,11] and immunohistochemical α-smooth muscle actin (α-SMA) stain. Collagen bundles in the cancer stroma are stained with aniline blue (AnB) of AZAN stain, while CAFs in the cancer stroma are immunohistochemically positive for α-SMA. We

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used imaging software "Image J" to analyze fibrosis of cancer stroma, i.e., quantification of collagen bundles (AnB) and CAFs (α-SMA). We selected three invasive foci which represented the most intense fibrosis, and took photographs with x40 objective lens for all cases. Then, we analyzed binarized/extracted images of collagen bundles (AnB) and CAFs (α-SMA) using the software. The images were represented as pixels on the computer screen. We calculated the average of three areas for all the cases.

## RESULTS

Pancreatic ductal adenocarcinoma was characterized by an invasive growth pattern with abundant fibrous stroma in both treated/untreated groups **(Figure 1)**. However, the treated/untreated groups revealed different distribution pattern of AnB positive collagen bundles and α-SMA positive CAFs. The treated group showed the very high density of collagen bundles and the low density of CAFs. On the other hand, the untreated group exhibited the low density of collagen bundles and high density of CAFs.

**Table 1** is the results of Image J analyzation. Student t-test proved that the treated group significantly increased AnB positive collagen bundle area ($P<0.01$) and significantly decreased α-SMA positive CAF area ($P<0.01$).

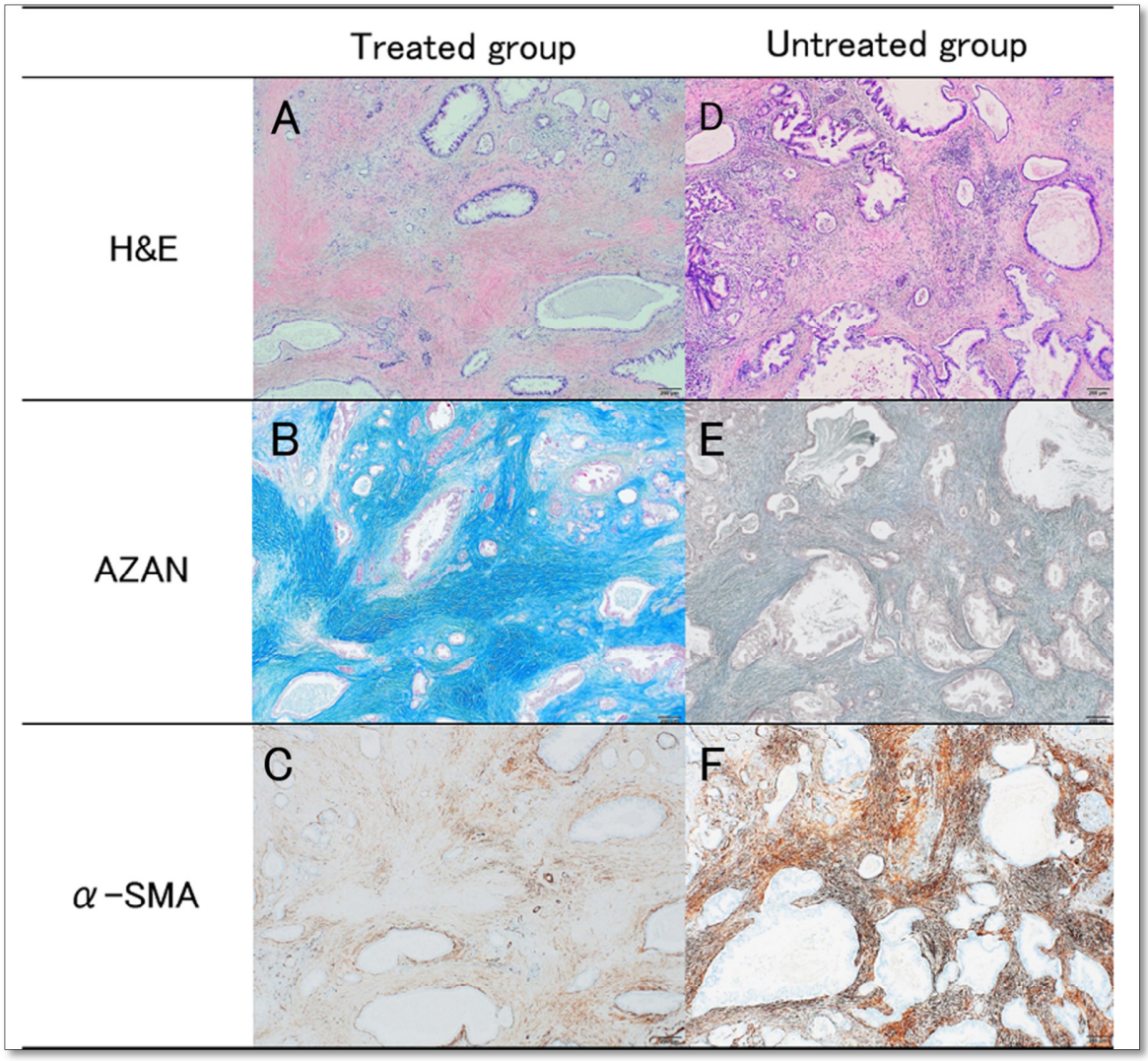


**Figure 1.** Pancreatic ductal adenocarcinoma is characterized by an invasive growth pattern with abundant fibrous stroma (A, left top, treated group; and D, right top, untreated group). The treated group shows aniline blue (AnB) positive collagen bundles in an intensively high density (B, left middle) and α-SMA positive fibroblasts in a low density (C, left bottom). On the other hand, the untreated group exhibits AnB positive collagen bundles in a low density (E, right middle) and α-SMA positive fibroblasts in a high density (F, right bottom).

**Table 1.** Image J analysis of aniline blue (AnB) positive area and α-SMA positive area in pancreatic ductal adenocarcinoma.

| Case No. | AnB positive area (pixels) | | α-SMA positive area (pixel) | |
|---|---|---|---|---|
| | Treated group | Untreated group | Treated group | Untreated group |
| **1** | 844,687 | 55,312 | 325,249 | 331,837 |
| **2** | 864,370 | 30,844 | 151,584 | 230,803 |
| **3** | 523,602 | 77,928 | 129,338 | 475,857 |
| **4** | 785,726 | 258,109 | 35,866 | 502,780 |
| **5** | 167,219 | 35,919 | 144,560 | 437,359 |
| **6** | 552,743 | 41,899 | 9,722 | 370,196 |
| **7** | 158,353 | 150,336 | 15,167 | 330,696 |
| **8** | 48,118 | 29,810 | 130,099 | 434,687 |
| **9** | 483,741 | 68,957 | 177,726 | 276,831 |
| **10** | 125,135 | 39,730 | 214,477 | 532,014 |
| **Average** | **455,369** | **78,884** | **133,379** | **392,306** |
| | **P-value<0.01** | | **P-value<0.01** | |

## DISCUSSION AND CONCLUSION

In the present study, we demonstrated the Nab-PTX neoadjuvant chemotherapy induced the unique fibrous stroma of pancreatic ductal adenocarcinoma, i.e., dense AnB positive fibrous stroma with limited numbers of CAFs. We think the dense collagenous fibrosis in the stroma contributes to tumor shrinkage in an anticancer effective manner. We have speculated that the pancreatic ductal adenocarcinomas in the untreated group continuously induced active CAFs in the stroma, and made use of the CAFs for the chemoresistance [5,6]. However, the treated group with Nab-PTX rapidly induced AnB positive collagen bundles in the stroma, and then decreased numbers of CAFs.

Our previous studies reported that the transcriptional factors associated epithelial-mesenchymal transition (EMT) contributed pancreatic cancer invasiveness/aggressiveness, and were thought to be related with CAFs [12,13]. The Nab-PTX neoadjuvant chemotherapy plays in roles of stromal collagenous fibrosis and inactivation of CAFs. However, details of molecular mechanisms of Nab-PTX are still unknown. We try to analyze the molecular mechanisms of collagenous fibrosis as the Nab-PTX anticancer effects in the near future.

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