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Discrimination of CD44 and Oct3/4 Expression in Pancreatic Adenocarcinoma from Benign Pancreatic Ducts in Small Biopsy Specimens

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Abstract

CD44 and Oct3/4 are important factors influencing cancer stem cell (CSC) development, but their clinical applications on pancreatic cancer are still unknown. Here, we tested the hypothesis that expression of CD44 and Oct3/4 correlates with the clinicopathological parameters of pancreatic ductal adenocarcinomas (PDACs). Firstly, data on the mRNA expression levels in PDACs and normal pancreatic tissues were collected from Gene Expression Omnibus (GEO) repository datasets. Immunohistochemical analyses of CD44 and Oct3/4 were next performed in tissue microarrays of 80 surgical specimens derived from a Chinese population, which included 9 normal pancreatic ducts and 71 PDACs, amongst which 12 were well differentiated, 47 moderately differentiated, and 12 poorly differentiated. From the GEO results, mRNA expression levels of both CD44 and Oct3/4 were higher in PDACs than in normal pancreatic tissues. In addition, immunostaining scores of these biomarkers were higher in most PDACs than in non-neoplastic pancreatic ducts. The intensity of CD44 and Oct3/4 staining in normal pancreatic tissues was weak and limited to small areas. Although CD44 and Oct3/4 overexpression in PDACs tended to be associated with advanced histologic grades of PDACs, the correlation of CD44 and Oct3/4 expression with the American Joint Committee on Cancer (AJCC) pathological stage was not statistically significant. In conclusion, CD44 and Oct3/4 overexpression may imply malignant transformation of pancreatic ducts and could help pathologists make a more accurate diagnosis and decision on clear surgical margins.

Key Words: pancreatic carcinoma, CD44, Oct3/4, cancer stem cell

Introduction

Pancreatic cancer is one of the most aggressive cancers and has a high mortality rate. In the United States, 5-year survival rates were only around 5% (27). Smoking, chronic pancreatitis, diabetes and obesity are considered risk factors for pancreatic neoplasms (2, 6). In addition, mutations in tumor suppressor

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genes, including *CDKN2A* (3), *STK11* (3), *BRCA2*[#] and *APC* (28), increase the risks of pancreatic tumors. However, no decisive evidence has shown which risk factor is the most important. A recent study has associated pancreatic cancer stem cells (CSCs) with chemoresistance, tumor metastasis and poor prognosis (5). CSCs and non-CSCs have different self-renewal capabilities and multilineage potentials. Therefore, combined use of several biomarkers, such as CD44, OCT3/4, CD24, CD133 and ESA, might help discriminate pancreatic CSCs from non-CSCs (14, 15, 20, 31). Of the above stated biomarkers, singly use of CD44 or OCT3/4 has been shown to be related to pathogenesis of some human cancers (10, 30).

CD44 is a transmembrane glycoprotein receptor that binds hyaluronic acid (HA). The CD44-HA complex effectively activates the Nanog and STAT3 pathways, which play important roles in cellular survival, differentiation, adhesion, and migration (1, 18, 34). CD44 are divided into standard (CD44s) and variant (CD44v) isoforms based on the positions of the RNA splice site (33). In recent studies, both CD44s and CD44v are associated with cancer-initiating cell development and tumor metastasis (18, 23, 34). Applying CD44 as a biomarker could effectively identify tumor stem cells in human cancers the breast, stomach, head and neck, pancreas and others (7, 34). Inhibition of CD44 expression could slow pancreatic cancer growth, invasion and recurrence rate (10, 33). Therefore, we tested the correlation of CD44 expression with American Joint Committee on Cancer (AJCC) pathologic stages and survival rate in PDAC.

Oct3/4, also known as OCT3, OCT4 or POU5F1, is a transcription factor, and Oct3/4 belongs to the *Pit-Oct-Unc* (*POU*) gene family (16). Oct3/4 maintains the pluripotency of embryonic stem cells and regulates the development of germ cells (11-13, 17). In addition, overexpression of Oct3/4 has been shown to enhance the 'stemness' of cancer stem cells and to induce the pathogenesis of several human cancers (4, 19, 24, 30). Suppression of Oct3/4 can slow cancer cell colony formation and improve survival rate (4, 24). Noguchi *et al.* (21) recently demonstrated that Oct3/4 may induce epigenetic modifications that suppress malignant transformation of pancreatic cancer stem cells *in vitro*. However, the clinicopathological role of Oct3/4 in pancreatic cancers is still unclear. Therefore, our aim was to clarify the relationship between Oct3/4 and clinicopathological parameters in PDACs.

In this study, we tested the hypothesis that higher expression of CD44 and Oct3/4 correlates with more advanced tumor progression and metastasis, and poor survival rate. To our knowledge, this is the first article to address the relationship of the expression levels of these two biomarkers with various clinicopathological parameters of PDAC in a Chinese population.

Materials and Methods

Study Ethics and GEO Database Interrogation of CD44 and Oct3/4 Gene Expression in PDACs

The institutional review board of Tri-Service General Hospital in Taipei, Taiwan, ROC, approved the protocol of this study (TSGHIRB No: 1-101-05-099). The methodology for analyses of functional genomic data was as previously described (8). Briefly, 52 sheets of de-linked data (GDS4102/212014_x_at/CD44) on CD44 mRNA expression in 36 PDAC and 16 normal pancreatic tissue samples were obtained from NCBI (http://www.ncbi.nlm.nih.gov/geo/tools/profileGraph.cgi?ID=GDS4102:212014_x_at). In addition, another database (GDS4103/208286_x_at/POU5F1P4) provided 52 sheets of data on Oct3/4 gene expression in 36 PDAC and 16 normal pancreatic tissue samples from NCBI (http://www.ncbi.nlm.nih.gov/geo/tools/profileGraph.cgi?ID=GDS4102:208286_x_at).

Tissue Array Construction

Small paraffin-embedded tumor tissue biopsy samples were obtained for construction of a tissue microarray slide. The tissue microarray consisted of 9 samples of normal pancreatic tissue and 71 samples of well- (n = 12), moderately- (n = 47), and poorly-differentiated (n = 12) PDAC tissues. All tumors were pathologically-staged according to the 2009 TNM system and were assigned a Fuhrman nuclear grade. The normal pancreatic tissues were taken at least 2 cm from the neoplasm. None of the specimens were from cases that had ever received radiation or chemotherapy before the surgery. Pathological diagnoses in these cases were reviewed by at least two experienced pathologists. To construct the tissue microarray, a core of 2-mm diameter was taken from a selected area of each paraffin-embedded tumor tissue, and the tissue microarray slides were constructed. The tissue microarray slide showed uniform staining as in the original paraffin-embedded specimens.

Immunohistochemistry

Tissue microarray sections were de-waxed in xylene, rehydrated in alcohol, immersed in 3% hydrogen peroxide for 5 min to suppress endogenous peroxidase activity, heated at 100°C for 30 min in 0.01 M sodium citrate buffer, pH 6.0, to retrieve antigens, rinsed 3 times in phosphate buffered saline (PBS), each for 5 min, incubated for 1 h at room temperature with a polyclonal mouse anti-human CD44 antibody (1:100, Thermo Scientific, Waltham, CA, USA), or a monoclonal rabbit anti-human Oct3/4 antibody (1:50, Spring

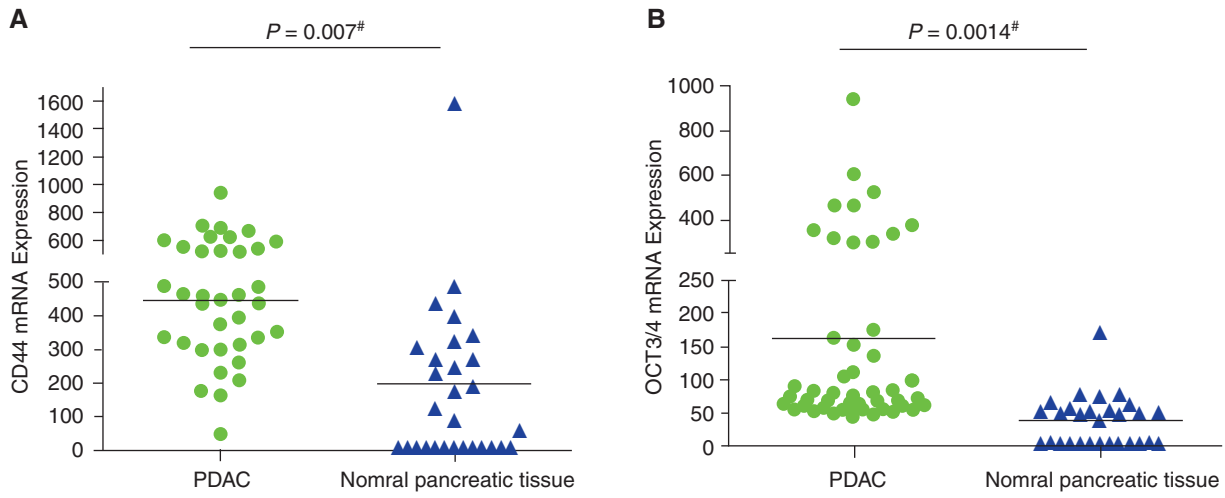

Fig. 1. GEO-derived CD44 (A) and Oct3/4 (B) mRNA expression in pancreatic ductal adenocarcinoma and normal pancreatic tissues. An adjusted *P* value was calculated for each comparison (n = 81).

Bioscience, Pleasanton, CA, USA), each diluted in PBS, washed 3 times in PBS, each for 5 min, incubated with biotin-labeled secondary immunoglobulin (1:100, DAKO, Glostrup, Denmark) for 1 h at room temperature, washed 3 times, and incubated with AEC in the presence of substrate chromogen (DAKO, Glostrup, Denmark) at room temperature to detect peroxidase activities.

To assess the levels of CD44 immunostaining, the intensity of cytoplasmic and membrane immunostaining was scored on a scale of 0 (no staining) to 3 (strongest intensity), and the percentage of stained cells was estimated at each intensity. The percentage of cells (from 0 to 100) was multiplied by the corresponding immunostaining intensity (from 0 to 3) to obtain an immunostaining score in the range of 0 to 300. Similarly, to evaluate Oct3/4 staining, only the percentage of tumor cells with nuclear staining was recorded.

Protein-Protein Interaction Network and Signaling Pathways Analysis

To reveal protein-protein interactions between CD44 and Oct3/4, the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database version 10.0 (http://string-db.org) was used (29). Possible role of CD44-Oct3/4 interactions in CSC development was identified based on this information.

Statistical Analysis

Combined strategies were used to analyze the two Gene Expression Omnibus (GEO) repository datasets (GDS4102/212014_x_at/ CD44 and GDS4103/208286_x_at/POU5F1P4). The estimates of CD44 and Oct3/4 gene expression in PDAC and normal pancreatic tissues were evaluated using a one-tailed test. The immunostaining scores of CD44 and Oct3/4 were compared between PDAC samples and normal pancreatic ductal epithelium samples. Between-group differences were assessed using the Student *t*-test. A *P*-value of less than 0.05 was considered to be statistically significant. SigmaState software (Jandel Scientific, San Rafael, CA, USA) was used to perform the Pearson Product Moment analysis of the relationships of the expression of these two biomarkers with the clinicopathological parameters.

Results

CD44 and Oct3/4 mRNA Expression in PDACs and Normal Pancreatic Tissue

Using the gene expression data interrogated from the GEO database as described in the Materials and Methods section, PDACs showed higher levels of CD44 ($P = 0.007$) and Oct3/4 mRNA expression ($P = 0.014$) compared with normal pancreatic tissues (Fig. 1).

Clinicopathological Characteristics

In our study, 40 males and 31 females with various grades and AJCC stages of PDACs were included. The 71 PDAC cases were classified as AJCC stage IA (n = 5), IB (n = 7), IIA (n = 15), IIB (n = 19), III (n = 12), and IV (n = 13); other clinicopathologic characteristics, including the TNM stage distribution, are listed in Table 1.

CD44 Protein Expression in Normal Pancreatic Tissues and PDAC Tissues of Various histologic Grades and AJCC Pathologic Stages

Table 1. Clinicopathologic characteristics of 71 patients with PDACs

Variable	n
Gender	
Male	40
Female	31
Histopathological differentiation	
Well differentiated	12
Moderately differentiated	47
Poorly differentiated	12
TNM category	
T1	8
T2	19
T3	26
T4	18
N0	33
N1	38
M0	57
M1	14
AJCC stage	
IA	5
IB	7
IIA	15
IIB	19
III	12
IV	13

Table 2. Immunostaining patterns of CD44 and clinicopatholigcal parameters of PDACs

	Average intensity	Average % staining	Average score	Correlation[#]
Normal pancreatic ducts	0.14	13	5.37	
Tumor grading				
Grade I	1.45	25	43.63	Positive correlation (P = 0.048)
Grade II	1.91	37.27	82.27	
Grade III	1.73	43.33	98	
TNM stage				
T stage				
T1	2	23.33	70	No correlation (P = 0.880)
T2	1.8	41	86.33	
T3	1.69	34.38	79.06	
T4	1.89	42.22	87.78	
N stage				
N0	1.79	33.95	73.42	No correlation (P = 0.489)
N1	1.79	40.42	90.21	
M stage				
M0	1.69	34.69	74.06	No correlation (P = 0.213)
M1	2.09	45.91	108.18	
AJCC stage				
Stage IA	2	23.33	70	No correlation (P=0.376)
Stage IB	2	45	103.75	
Stage IIA	1.67	27.5	59.17	
Stage IIB	1.5	32.86	66.79	
Stage III	1.8	47	91	
Stage IV	2.09	45.91	108.18	

[#]Correlation was analyzed by paired *t*-test and Pearson Product Moment method

Immunostaining results of CD44 expression in PDAC samples are shown in Fig. 2E-2H The average CD44 immunostaining scores in non-neoplastic pancreatic ducts and PDACs of grades I – III were 5.7, 43.64, 82.27, and 98, respectively (Table 2), indicating a significant correlation between CD44 expression level and histologic grade. Similarly, CD44 expression was higher in most specimens of PDAC than in those of non-neoplastic pancreatic ducts (Fig. 3A). Additionally, the CD44 immunostaining score was 70 in stage IA, 103.75 in stage IB, 59.17 in stage IIA, 66.29 in stage IIB, 91 in stage III, and 108.18 in stage IV PDACs (Table 2) Thus, there was no relationship between CD44 immunohistochemical staining and AJCC pathologic stages.

Oct3/4 Protein Expression in Normal Pancreatic Tissue and PDAC of Various Histologic Grades and AJCC Pathologic Stages

In non-neoplastic pancreatic ducts, only a small number of epithelial cells showed positive staining (Fig. 2I-2L) Oct3/4 immunostaining scores were significantly higher in most PDACs than in non-neoplastic pancreatic ducts (Fig. 3B). Similarly, the percentage of Oct3/4 stained tumor cells was positively correlated with histologic differentiation of PDACs ($P < 0.05$, Table 3). In addition, the percentage of cells expressing Oct3/4 in AJCC stages IA, IB, IIA, IIB, III, and IV PDAC was 23.33, 45, 27.5, 32.86, 47, and 45.91, respectively. The correlation of Oct3/4 expression with TNM and AJCC pathologic stages did not reach statistical significance.

Discussion

Cancer stem cells (CSCs) can regenerate tumor masses, promote distant metastasis, and cause therapeutic failure (26). Recent studies have shown the association of the Wnt, Shh, and Notch signaling pathways with the self-renewal activity of CSCs (9, 24, 26). Moreover, Ponnusamy *et al*. identified the product of pancreatic differentiation 2 (PD2) and Oct3/4 interaction as the main promoter of pancreatic CSC self renewal (25). Likewise, CD44-mediated regulation of tumor initiation, invasion, metastasis, and post-radiation recurrence were

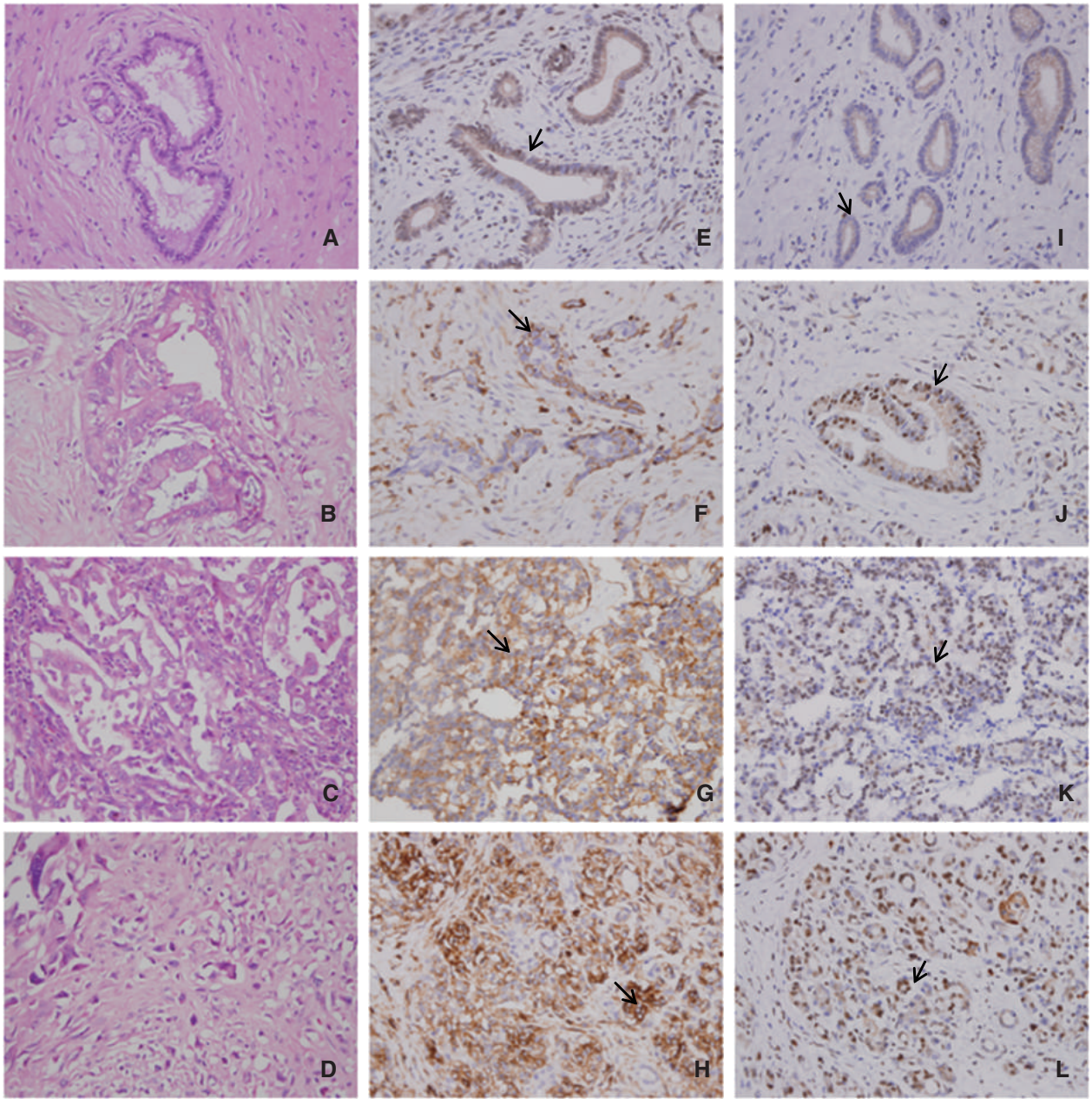

Fig. 2. Hematoxylin and eosin staining of normal pancreatic duct (A), and well-differentiated (B), moderately-differentiated (C) and poorly-differentiated (D) pancreatic ductal adenocarcinomas. Immunohistochemical analysis of CD44 (E–H) and Oct3/4 (I–L) in normal pancreatic duct (E and I), well-differentiated (F and J), moderately-differentiated (G and K), and poorly-differentiated (H and L) pancreatic ductal adenocarcinomas. The arrows indicate the stained tumor cells. Magnification, x 400.

shown to contribute to CSC development and epithelial-mesenchymal transition (18, 34). Therefore, the biomarkers CD44 and Oct3/4 are commonly used for not only the detection of pancreatic CSCs, but also the prediction of prognosis and therapeutic resistance (5, 15, 20, 32).

From the protein-protein interactions (PPI) database (STRING) and a previous study, we found that, by serving as the hub protein of a set of protein-protein interactions, CD44 is predicted to regulate transcriptional signals, including the phosphorylation of STAT3 and the activation of Nanog in the cytoplasm (Fig. 4) (18). In addition, the active-form of Nanog can be translocated into the nucleus to activate SOX2 and POU5F1 in the signaling pathway (19). The combination of Nanog and SOX2 can effectively initiate the development self-renewal ability of CSCs (19). Additionally, Oct3/4, also called POU5F1, is a transcriptional factor that can prolong cell survival and stimulate tumor overgrowth (17). Likewise, co-activation of CD44 with ERBB2 and

Table 3. Immunostaining patterns of Oct3/4 and clinicopatholigcal parameters of PDACs

	Average score	Correlation[#]
Normal pancreatic ducts	3.35	
Tumor grading		
Grade I	30.83	Positive correlation ($P = 0.043$)
Grade II	34.04	
Grade III	50.41	
TNM stage		
T stage		
T1	31.43	No correlation ($P = 0.171$)
T2	42.37	
T3	27.69	
T4	53.33	
N stage		
N0	33.95	No correlation ($P = 0.118$)
N1	40.42	
M stage		
M0	34.69	No correlation ($P = 0.197$)
M1	45.91	
AJCC stage		
Stage IA	23.33	No correlation ($P = 0.329$)
Stage IB	45	
Stage IIA	27.5	
Stage IIB	32.86	
Stage III	47	
Stage IV	45.91	

[#]Correlation was analyzed by paired *t*-test and Pearson Product Moment method

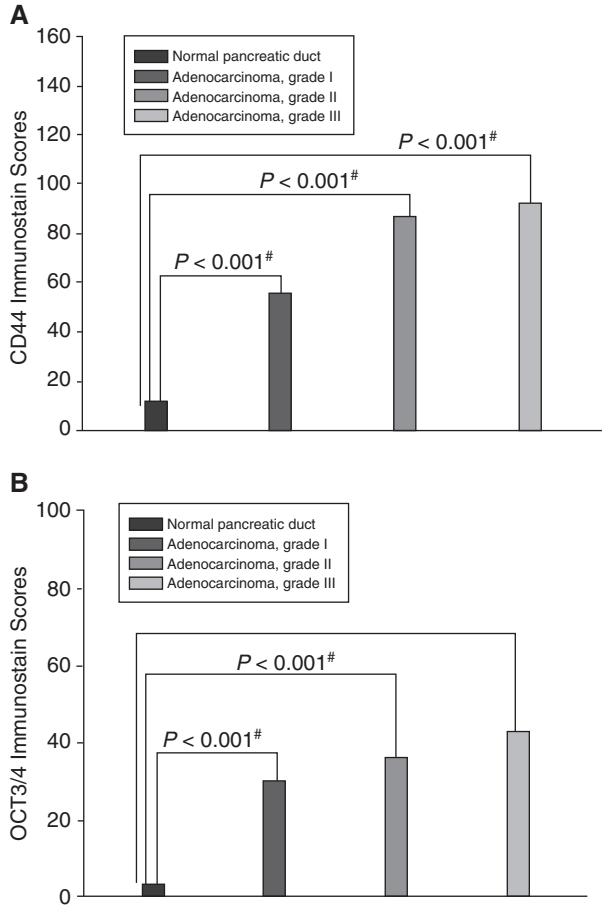

Fig. 3. CD44 (A) and Oct3/4 (B) immunostaining scores in non-neoplastic pancreatic tissues, and grades I – III pancreatic ductal adenocarcinomas.

SPP1 is involved in the regulation of cellular proliferation and chemo-resistance (22, 31). Interestingly, the regulation of pancreatic cancer invasion and epithelial-mesenchymal transition depends on the expression of membranous metalloproteinase MT1/MMP (34). The PPI network clearly shows a connection between CD44 and MMP9.

In our study, the expression of CD44 and Oct3/4 was found to be significantly higher in PDAC than in normal pancreatic tissue, implying that these two factors might be involved in the carcinogenesis of pancreatic cancers. Additionally, CD44 and Oct3/4 were also correlated with tumor histologic grades, but not with AJCC pathologic stages. The difference between our and previous findings might be attributed to the ethnicity of our patient population, which was Chinese (5, 22). However, due to the small number of cases studied here, more data are needed for a more definite conclusion.

Endoscopic retrograde cholangiopancreatography remains the most common diagnostic tool used to detect pancreatic tumors and in tissue sampling. Unfortunately, the most common pancreatic cancer diagnosis is well-to-moderately differentiated adenocarcinoma, which is difficult to distinguish from non-neoplastic pancreatic ducts. In this study, we demonstrated that the application of CD44 and Oct3/4 immunostaining might improve the accuracy of PDAC diagnosis and help define an adequate safety margin even in small biopsy specimens.

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References

1. Aruffo, A., Stamenkovic, I., Melnick, M., Underhill, C.B. and Seed, B. CD44 is the principal cell surface receptor for hyaluronate. *Cell* 61: 1303-1313, 1990.
2. Bosman, F.T., Carneiro, F., Hruban, R.H. and Theise, N.D. World Health Organization Classification of Tumours of the Digestive System. Lyon: IARC Press, 2010, pp. 281-313.
3. Campbell, P.J., Yachida, S., Mudie, L.J., Stephens, P.J., Pleas-

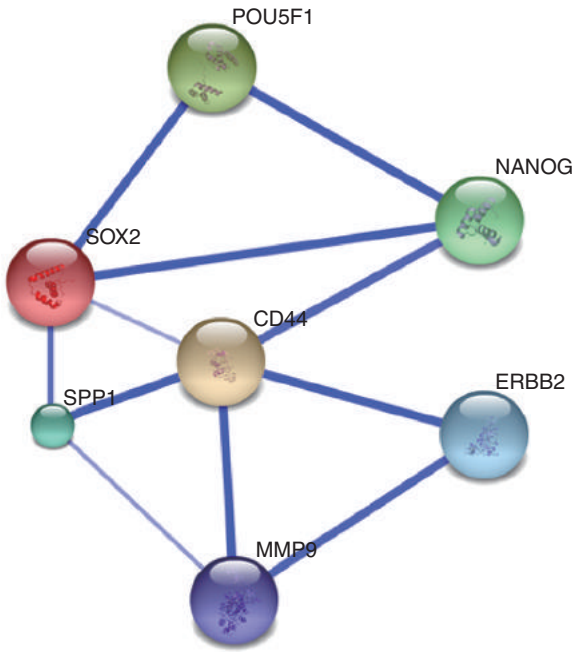

Fig. 4. Confidence view of the protein–protein interaction (PPI) network generated by the STRING database. CD44 is the controlling hub used in the search. POU5F1 is the same as Oct3/4 protein.

ance, E.D., Stebbings, L.A., Morsberger, L.A., Latimer, C., McLaren, S., Lin, M.L., McBride, D.J., Varela, I., Nik-Zainal, S.A., Leroy, C., Jia, M., Menzies, A., Butler, A.P., Teague, J.W., Griffin, C.A., Burton, J., Swerdlow, H., Quail, M.A., Stratton, M.R., Iacobuzio-Donahue, C. and Futreal, P.A. The patterns and dynamics of genomic instability in metastatic pancreatic cancer. *Nature* 467: 1109-1113, 2010.

4. Du, Z., Jia, D., Liu, S., Wang, F., Li, G., Zhang, Y., Cao, X., Ling, E.A. and Hao, A. Oct4 is expressed in human gliomas and promotes colony formation in glioma cells. *Glia* 57: 724-733, 2009.
5. Fitzgerald, T.L. and McCubrey, J.A. Pancreatic cancer stem cells: association with cell surface markers, prognosis, resistance, metastasis and treatment. *Adv. Biol. Regul*. 56: 45-50, 2014.
6. Gall, T.M., Tsakok, M., Wasan, H. and Jiao, L.R. Pancreatic cancer: current management and treatment strategies. *Postgrad. Med. J*. 91: 601-607, 2015.
7. Hu, Y. and Fu, L. Targeting cancer stem cells: a new therapy to cure cancer patients. *Am. J. Cancer Res*. 2: 340-356, 2012.
8. Hueng, D.Y., Lin, G.J., Huang, S.H., Liu, L.W., Ju, D.T., Chen, Y.W., Sytwu, H.K., Chang, C., Huang, S.M., Yeh, Y.S., Lee, H.M. and Ma, H.I. Inhibition of Nodal suppresses angiogenesis and growth of human gliomas. *J. Neurooncol*. 104: 21-31, 2011.
9. Ivanova, N., Dobrin, R., Lu, R., Kotenko, I., Levorse, J., DeCoste, C., Schafer, X., Lun, Y. and Lemischka, I.R. Dissecting self-renewal in stem cells with RNA interference. *Nature* 442: 533-538, 2006.
10. Jiang, W., Zhang, Y., Kane, K.T., Collins, M.A., Simeone, D.M., di Magliano, M.P. and Nguyen, K.T. CD44 regulates pancreatic cancer invasion through MT1-MMP. *Mol. Cancer Res*. 13: 9-15, 2015.
11. Jones, T.D., Ulbright, T.M., Eble, J.N., Baldridge, L.A. and Cheng, L. OCT4 staining in testicular tumors: a sensitive and specific marker for seminoma and embryonal carcinoma. *Am. J. Surg. Pathol*. 28: 935-940, 2004.
12. Jordan, C.T., Guzman, M.L. and Noble, M. Cancer stem cells. *N. Engl. J. Med*. 355: 1253-1261, 2006.
13. Kekuda, R., Prasad, P.D., Wu, X., Wang, H., Fei, Y.J., Leibach, F.H. and Ganapathy, V. Cloning and functional characterization of a potential-sensitive, polyspecific organic cation transporter (OCT3) most abundantly expressed in placenta. *J. Biol. Chem*. 273: 15971-15979, 1998.
14. Lee, C.J., Dosch, J. and Simeone, D.M. Pancreatic cancer stem cells. *J. Clin. Oncol*. 26: 2806-2812, 2008.
15. Li, C., Heidt, D.G., Dalerba, P., Burant, C.F., Zhang, L., Adsay, V., Wicha, M., Clarke, M.F. and Simeone, D.M. Identification of pancreatic cancer stem cells. *Cancer Res*. 67: 1030-1037, 2007.
16. Ling, G.Q., Chen, D.B., Wang, B.Q. and Zhang, L.S. Expression of the pluripotency markers Oct3/4, Nanog and Sox2 in human breast cancer cell lines. *Oncol. Lett*. 4: 1264-1268, 2012.
17. Looijenga, L.H., Stoop, H., de Leeuw, H.P., de Gouveia Brazao, C.A., Gillis, A.J., van Roozendaal, K.E., van Zoelen, E.J., Weber, R.F., Wolffenbuttel, K.P., van Dekken, H., Honecker, F., Bokemeyer, C., Perlman, E.J., Schneider, D.T., Kononen, J., Sauter, G. and Oosterhuis, J.W. POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. *Cancer Res*. 63: 2244-2250, 2003.
18. Marhaba, R. and Zoller, M. CD44 in cancer progression: adhesion, migration and growth regulation. *J. Mol. Histol*. 35: 211-231, 2004.
19. Matsuoka, J., Yashiro, M., Sakurai, K., Kubo, N., Tanaka, H., Muguruma, K., Sawada, T., Ohira, M. and Hirakawa, K. Role of the stemness factors sox2, oct3/4, and nanog in gastric carcinoma. *J. Surg. Res*. 174: 130-135, 2012.
20. Mimeault, M. and Batra, S.K. Molecular biomarkers of cancer stem/progenitor cells associated with progression, metastases, and treatment resistance of aggressive cancers. *Cancer Epidemiol. Biomarkers Prev*. 23: 234-254, 2014.
21. Noguchi, K., Eguchi, H., Konno, M., Kawamoto, K., Nishida, N., Koseki, J., Wada, H., Marubashi, S., Nagano, H., Doki, Y., Mori, M. and Ishii, H. Susceptibility of pancreatic cancer stem cells to reprogramming. *Cancer Sci*. 106: 1182-1187, 2015.
22. Ohara, Y., Oda, T., Sugano, M., Hashimoto, S., Enomoto, T., Yamada, K., Akashi, Y., Miyamoto, R., Kobayashi, A., Fukunaga, K., Morishita, Y. and Ohkohchi, N. Histological and prognostic importance of CD44+/CD24+/EpCAM+ expression in clinical pancreatic cancer. *Cancer Sci*. 104: 1127-1134, 2013.
23. Orian-Rousseau, V. CD44, a therapeutic target for metastasising tumours. *Eur. J. Cancer* 46: 1271-1277, 2010.
24. Ponnusamy, M.P. and Batra, S.K. Ovarian cancer: emerging concept on cancer stem cells. *J. Ovarian Res*. 1: 4, 2008.
25. Ponnusamy, M.P., Deb, S., Dey, P., Chakraborty, S., Rachagani, S., Senapati, S. and Batra, S.K. RNA polymerase II associated factor 1/PD2 maintains self-renewal by its interaction with Oct3/4 in mouse embryonic stem cells. *Stem Cells* 27: 3001-3011, 2009.
26. Reya, T., Morrison, S.J., Clarke, M.F. and Weissman, I.L. Stem cells, cancer, and cancer stem cells. *Nature* 414: 105-111, 2001.
27. Reynolds, R.B. and Folloder, J. Clinical management of pancreatic cancer. *J. Adv. Pract. Oncol*. 5: 356-364, 2015.
28. Roberts, N.J. and Klein, A.P. Genome-wide sequencing to identify the cause of hereditary cancer syndromes: with examples from familial pancreatic cancer. *Cancer Lett*. 340: 227-233, 2013.
29. Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., Simonovic, M., Roth, A., Santos, A., Tsafou, K.P., Kuhn, M., Bork, P., Jensen, L.J. and von Mering, C. STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res*. 43: D447-D452, 2015.
30. Tai, M.H., Chang, C.C., Kiupel, M., Webster, J.D., Olson, L.K. and Trosko, J.E. Oct4 expression in adult human stem cells: evidence in support of the stem cell theory of carcinogenesis. *Carcinogenesis* 26: 495-502, 2005.
31. Trosko, J.E. From adult stem cells to cancer stem cells: Oct-4 Gene, cell-cell communication, and hormones during tumor pro-

motion. *Ann. N. Y. Acad. Sci*. 1089: 36-58, 2006.
32. Vaz, A.P., Ponnusamy, M.P., Rachagani, S., Dey, P., Ganti, A.K. and Batra, S.K. Novel role of pancreatic differentiation 2 in facilitating self-renewal and drug resistance of pancreatic cancer stem cells. *Brit. J. Cancer* 111: 486-496, 2014.
33. Wood, N.J. Pancreatic cancer: pancreatic tumour formation and recurrence after radiotherapy are blocked by targeting CD44. *Nat. Rev. Gastroenterol. Hepatol*. 11: 73, 2014.
34. Zöller, M. CD44: can a cancer-initiating cell profit from an abundantly expressed molecule? *Nat. Rev. Cancer* 11: 254-267, 2011.