The role of epithelial-mesenchymal transition in pancreatic cancer

Jen-Jung Pan[1], Muh-Hwa Yang[2,3]

[1]Division of Gastroenterology, Hepatology and Nutrition, Department of Internal Medicine, University of Texas Health Science Center at Houston, Texas, USA; [2]Institute of Clinical Medicine, National Yang Ming University, Taipei, Taiwan; [3]Division of Hematology-Oncology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan

ABSTRACT Pancreatic cancer is the fourth leading cause of cancer related death in the US. Despite the advances in medical and surgical treatment, the 5-year survival rate for such cancer is only approximately 5% when considering all stages of disease. The lethal nature of pancreatic cancer stems from its high metastatic potential to the lymphatic system and distant organs. Lack of effective chemotherapies, which is believed to be due to drug-resistance, also contributes to the high mortality of pancreatic cancer. Recent evidence suggests that epithelial-mesenchymal transition of pancreatic cancer cells contributes to the development of drug resistance and an increase in invasiveness. Future strategies that specifically target against epithelial-mesenchymal transition phenotype could potentially reduce tumoral drug resistance and invasiveness and hence prolong the survival of patients with pancreatic cancer.

KEY WORDS pancreatic cancer, epithelial-mesenchymal transition, cancer stem cell

J Gastrointest Oncol 2011; 2: 151-156. DOI: 10.3978/j.issn.2078-6891.2011.022

Introduction

Pancreatic cancer (PC) is the tenth cause of new cancer cases and the fourth leading cause of cancer related death in the US, with an estimated 43,140 new cases and 36,800 deaths in 2010 (1). Despite the advances in surgical and medical treatment, the 5-year survival rate for PC is only approximately 5% when considering all stages of disease (1). Without a specific diagnostic marker and being asymptomatic in early stage, PC is often diagnosed at an advanced/late stage when only palliative measures can be offered, which can only partially explain its observed poor prognosis (2). The 5-year survival rate of PC remains low at only 10-25% for those with locoregional disease due to local recurrence and/or distant metastasis after curative surgery (3). The lethal nature of PC therefore stems from its high metastatic potential to the lymphatic system and distant organs. In addition, lack of effective chemotherapies, which is believed to be due to drug-resistance, also contributes to the high mortality of patients diagnosed with PC (4). Recent evidence suggests that epithelial-mesenchymal transition (EMT) of PC cells contributes to the development of drug resistance (5).

EMT plays crucial roles in the formation of the body plan and in the differentiation of tissues and organs. During EMT, epithelial cells undergo profound phenotypic changes such as loss of cell-cell adhesion, loss of cell polarity, and acquisition of migratory and invasive properties (6). EMT not only occurs during embryonic development or as a physiological response to injury, but is also an important element in cancer progression through a variety of mechanisms. EMT endows cells with migratory and invasive properties, induces stem cell properties, prevents apoptosis and senescence, induces resistance to conventional chemotherapy, and contributes to immunosuppression (6).

To support the role of EMT in PC progression, several reports have shown the increased expression of EMT markers such as N-cadherin (7), transcription factors including Snail, Slug and Twist (8), fibronectin (9), and vimentin (9,10) in surgically resected PC specimens but not in the normal noncancerous pancreatic tissue. In

No potential conflict of interest.

Corresponding author: Muh-Hwa Yang, MD, PhD. Institute of Clinical Medicine,National Yang Ming University, No. 155, Sec. 2, Li-Nong Street, Peitou, Taipei 112, Taiwan. Tel: +886-228267000 ext 7911; Fax: +886-228235870; Email: mhyang2@vghtpe.gov.tw.

Submitted Apr 26, 2011. Accepted for publication May 16, 2011.
Available at www.thejgo.org

ISSN: 2078-6891

addition, the presence of EMT in PC is often associated with undifferentiated phenotype and overall poor survival compared to the tumors without EMT (9,10). As mentioned previously, EMT contributes to drug resistance in cancer cells probably through induction of the formation of cancer stem cells (CSCs) or stem-like cells (4,11). This concept is supported by the findings of the increased expression of stem cell markers in drug-resistant PC cells (12-14).

In this concise review, we will summarize the current knowledge regarding the mechanisms and implications of EMT in PC.

Molecular mechanisms of EMT

EMT is a process by which epithelial cells lose their polarity and are converted to a mesenchymal phenotype. EMT has been considered as the critical event inducing morphogenetic changes during embryonic development, organ fibrosis and tumor metastasis. Phenotypic changes of EMT include the downregulation of epithelial markers (e.g., E-cadherin, desmoplakin and plakoglobin) and upregulation of mesenchymal markers (e.g., vimentin, fibronectin and α-smooth muscle actin) (6,15,16). A variety of transcriptional factors, including Snail, Slug, Twist, Zeb1, SIP1, and E47, were shown to induce EMT through repression of E-cadherin transcription (17-22). In addition to transcriptional repression, other mechanisms can also repress E-cadherin expression. A previous study reported that promoter hypermethylation was associated with E-cadherin repression and induction of EMT (23). Recent evidences highlight the role of chromatin modification in E-cadherin repression. Snail interacts with histone deacetylase 1 (HDAC1)-histone deacetylase 2 (HDAC2), AJUBA-protein arginine methyltransferase 5 (PRMT5), or polycomb repressive complex 2 (PRC2) to repress E-cadherin expression (24-26). We recently demonstrated that regulation of the polycomb repressive complex 1 (PRC1) protein Bmi1 by Twist1 is essential in Twist1-induced suppression of E-cadherin (27).

Hypoxia is an important microenvironmental factor for triggering metastasis during cancer progression. Recent studies showed that hypoxia-inducible factor 1 and 2 (HIF-1α and HIF-2α) induces the expression and coordinates the interplay of EMT regulators. HIF-1α regulates the expression of EMT regulators such as Snail, Zeb1, SIP1 either directly or indirectly (28,29). We previously demonstrated the direct regulation of Twist1 by HIF-1α, suggesting the critical role of hypoxia in the induction of EMT (30). HIF-2α has also been shown to regulate Twist1 expression (31). The results from these studies suggest the critical role of intratumoral hypoxia in the induction of EMT through either HIF-1α or HIF-2α or both.

Accumulating evidences suggest that cells can acquire stem-like properties during induction of EMT (32,33). This finding provides a crucial link between the acquisition of metastatic traits and tumor-initiating capability in cancer cells undergoing EMT. To support this theory, we previously demonstrated the direct regulation of the stemness gene Bmi1 by Twist1. Twist1 and Bmi1 act cooperatively to repress E-cadherin and p16INK4A, leading to the induction of EMT and stem-like properties of cancer cells. A recent report showed that Bmi1 is induced by another EMT regulator Zeb1 through regulation of the miR-200 family in pancreatic cancer cells (34). It indicates that the polycomb repressive protein Bmi1 may play a central role in the induction of EMT and stemness in pancreatic cancers.

Pancreatic CSCs

Based on the CSC theory, a tumor contains a heterogeneous population of mature cancer cells and a small number of CSCs. These CSCs, similar to their normal counterparts, have the ability to self-renewal and undergo multilineage differentiation (35). Most of the CSCs are identified by their specific cell surface markers. Pancreatic CSCs have been identified based on the expression of CD24, CD44, and epithelial-specific antigen (ESA). These cells represent only 0.5% to 1% of all PC cells but have at least 100-fold greater tumor-initiating potential than the majority of the tumor cells that are negative for these markers. More importantly, tumors derived from $CD24^+CD44^+ESA^+$ PC cells have been shown to be able to copy the phenotypic diversity characterized in the original tumor (36,37). Different populations of pancreatic CSCs have also been reported based on their expression of CD133 and CXCR4 (38) and aldehyde dehydrogenase (ALDH) (39). Little overlap existed between the $ALDH^+$ and $CD24^+CD44^+$ cell population despite the fact that they had a similar tumor formation capacity in vivo (39). It is conceivable that multiple phenotypically distinct cell populations are clonogenic in an individual tumor. Alternatively, it is possible that the phenotype of CSCs changes in response to cellular activation status, interactions with the external microenvironment, or disease stage. Another possibility is that these different CSC populations are interrelated by a retained hierarchical arrangement in which the expression of each specific marker is restricted to a specific cellular compartment, which is reminiscent of the structured relationship between long- and short-term stem cells and progenitors

in normal hematopoiesis (39).

EMT, Pancreatic CSCs, and drug resistance

Existing therapies for patients with cancer are largely against differentiated tumor cells, while sparing the relative quiescent CSCs (35). This paradigm can plausibly explain the commonly seen relapse after debulking chemotherapy due to the persistence of CSCs. The possible mechanisms underlying drug resistance in CSCs include the expression of energy-requiring transporters, the resistance to drug-induced apoptosis, and an active DNA-repair capacity (40). Du et al. (14) reported that chemoradiation-resistant PC cells acquired characteristics of CSCs and have high expression of anti-apoptotic protein bcl-2 and apoptosis inhibitory protein survivin. In another study, Hong et al. (41) reported that an ATP-binding cassette (ABC) transporter, ABCB1 (MDR1), was significantly augmented during the acquisition of drug resistance to gemcitabine. Pancreatic CSCs have been shown to be resistant to gemcitabine, the most commonly used chemotherapeutic agent for PC, in multiple studies (12,14,38,41,42). Treatment with gemcitabine can therefore enrich the CSC population likely through selection process that eventually leads to treatment failure (12,38,42). Emerging evidence suggests that Hedgehog pathway is important to CSC signaling (43). To support the critical role of pancreatic CSCs in the development of drug resistance, combined treatment with gemcitabine and cyclopamine, a small molecule smoothened antagonist, not only induced tumor regression but also decreased in CSC markers and Hedgehog signaling (42). In addition, ABC transporter inhibitor verapamil resensitized drug-resistant CSCs to gemcitabine in a dose-dependent manner (41).

Accumulating evidence suggests that EMT is important in cancer progression conceivably through commencing stem cell properties to cancer cells (4,6,11). Several studies have reported that pancreatic CSCs also possess mesenchymal features (12-14,39,44-46). During the EMT, mesenchymal cells are characterized by decreased expression of epithelial marker E-cadherin and increased expression of genes that encode members of the Snail family of transcriptional repressors (8,39). Rasheed et al. (39) reported that the expression of CDH1 that encodes for E-cadherin and of SNAI2 that encodes for Slug was decreased up to 5-fold and increased up to 51-fold, respectively, in $ALDH^+$ CSCs compared with unsorted tumor cells (39). Both Shah et al. (12) and Du et al. (14) reported that drug-resistant CSCs have decreased expression of E-cadherin and increased expression of vimentin, which are features of EMT. Transforming growth factor-β (TGF-β) is a regulator of many types of physiological and pathological EMT (11). When incubated in the presence of TGF-β, the side population (SP) cells, a CSC enriched fraction from PC cell line, changed their shape into mesenchymal-like appearance including spindle shaped assembly. This alteration was associated with significant reduction of E-cadherin expression level and induction of the expression of Snail and matrix metalloproteinase-2. When incubated in the absence of TGF-β, these cells restored epithelial-like appearance and the expression of E-cadherin. These results suggest that SP cells from PC possess superior potentials of phenotypic switch, i.e., EMT and mesenchymal-epithelial transition (MET) (44).

Reversal of EMT phenotype has been shown to restore drug sensitivity (5,46). Arumugam et al. (5) reported an inverse correlation between E-cadherin and Zeb-1, a transcriptional suppressor of E-cadherin, correlated closely with resistance to gemcitabine, 5-fluorouracil, and cisplatin. Silencing Zeb-1 in the mesenchymal PC lines not only increased the expression of E-cadherin but also restored drug sensitivity. They suggested that Zeb-1 and other regulators of EMT may maintain drug resistance in human PC cells (5). In another study, Li et al. (46) reported that the expression of several microRNAs (miRNA) including miR-200 were significantly down-regulated in gemcitabine-resistant PC cells. Emerging evidence has demonstrated the critical role of miRNA in various biological and pathological processes including EMT. These cells showed EMT characteristics such as elongated fibroblastoid morphology, lower expression of E-cadherin, and higher expression of vimentin and Zeb-1. By restoring the expression of miR-200, the expression of Zeb-1, Slug, and vimentin was down-regulated in the drug-resistant cells. These cells also showed reversal of EMT phenotype leading to epithelial morphology and had increased sensitivity to gemcitabine (46).

In summary, the current available treatment for cancer may select for drug resistant CSCs. Pancreatic CSCs could acquire drug resistance through EMT. Strategies target CSCs and/or EMT could potentially overcome the drug resistance problem during chemotherapy.

EMT and PC progression

As mentioned previously (9,10), the presence of EMT in PC is often associated with undifferentiated phenotype and overall poor survival compared to the tumors without EMT. EMT may not only induce drug resistance in CSCs but also increase tumorigenicity both in vitro and in vivo, migratory ability and invasiveness of PC cells (4,12-14,39,44,45).

MUC1, a transmembrane mucin glycoprotein, has been shown to be associated with the most invasive forms of PC (47). Roy et al. (47) reported that overexpression of MUC1 in PC cells triggered the molecular process of EMT, which translated to increased invasiveness and metastasis. $MUC1^+$ cells gained mesenchymal markers such as Slug, Snail and vimentin and lost E-cadherin expression. Furthermore, genes associated with metastasis and angiogenesis such as vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP)-2, 3, and 9 were significantly increased in $MUC1^+$ cells (47). MMPs have been implicated in facilitating the invasion and metastasis of PC (48). Bone morphogenetic proteins (BMPs) was reported to be able to induce EMT in PC cells, which resulted in an increase in invasiveness of the cells, in part through increased expression and activity of MMP-2 (49). In another study, overexpression of Slug significantly increased invasion and metastasis of PC cells through upregulation and activation of MMP-9 (50).

EMT is a dynamic process and is triggered by stimuli coming from extracellular matrix microenvironment and many secreted soluble factors. Among the many signaling pathways involved in this process, Wnt, TGF-β, Hedgehog, Notch, and nuclear factor-κB (NF-κB) signaling pathways are critical for EMT induction (51). Gordon et al (52) reported that loss of type III TGF-β receptor expression increased motility and invasiveness associated with EMT during PC progression. Wang et al. (45) reported that Notch-2 and its ligand, Jagged-1, were highly upregulated in gemcitabine-resistant PC cells. The finding is consistent with the role of the Notch signaling pathway in the acquisition of EMT phenotype. Down-regulation of Notch signaling pathway not only decreased invasive behavior of the drug-resistant cells but also led to partial reversal of the EMT phenotype, resulting in the MET, which was associated with decreased expression of vimentin, Zeb-1, Slug, Snail, and NF-κB (45). Their findings therefore provide a direct evidence of the association between EMT and PC invasiveness. In a recent study, Haque et al. (53) reported that Cyr61/CCN1 signaling is critical for EMT and promotes pancreatic carcinogenesis. Cyr61 (cysteine-rich 61) is a member of the CCN family of growth factors that includes CTGF, NOV, WISP-1, WISP-2 and WISP-3. Cyr61 is known to link cell surface and extracellular matrix and plays important roles on cell adhesion, proliferation, migration, differentiation, and angiogenesis during normal developmental and pathological processes (54). Cyr61 expression was detected in the early PC precursor lesions and its expression intensified with disease progression. Upon Cyr61 silencing, the aggressive behaviors of PC were reduced by obliterating interlinking events such as reversing EMT, blocking the expression of stem-cell-like traits and inhibiting migration. In contrast, addition of Cyr61 augmented EMT and stemness features in relatively less aggressive PC cells (53).

Taken together, PC with EMT features has more aggressive behaviors and is associated with poor patient survival. Multiple proteins and signaling pathways are involved in this process. Reversal of EMT phenotype could potentially reduce PC invasiveness and hence prevent metastasis.

Conclusion

Accumulating evidences suggest that EMT plays important roles in PC progression through several plausible mechanisms. PC cells may acquire stemness properties and become drug resistant during undergoing EMT. PC with EMT features is more aggressive and is associated with poor patient survival. Future strategies that specifically target against EMT phenotype could potentially reduce tumoral drug resistance and invasiveness and hence prolong the survival of patients with PC.

References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010;60:277-300.
2. Hidalgo M. Pancreatic cancer. N Eng J Med 2010;362:1605-17.
3. Yeo TP, Hruban RH, Leach SD, Wilentz RE, Sohn TA, Kern SE, et al. Pancreatic cancer. Curr Probl Cancer 2002;26:176-275.
4. Sarkar FH, Li Y, Wang Z, Kong D. Pancreatic cancer stem cells and EMT in drug resistance and metastasis. Minerva Chir 2009;64:489-500.
5. Arumugam T, Ramachandran V, Fournier KF, Wang H, Marquis L, Abbruzzese JL, et al. Epithelial to mesenchymal transition contributes to drug resistance in pancreatic cancer. Cancer Res 2009;69:5820-8.
6. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell 2009;139:871-90.
7. Nakajima S, Doi R, Toyoda E, Tsuji S, Wada M, Koizumi M, et al. N-cadherin expression and epithelial-mesenchymal transition in pancreatic carcinoma. Clin Cancer Res 2004;10:4125-33.
8. Hotz B, Arndt M, Dullat S, Bhargava S, Buhr HJ, Hotz HG. Epithelial to mesenchymal transition: expression of the regulators snail, slug, and twist in pancreatic cancer. Clin Cancer Res 2007;13:4769-76.
9. Javle MM, Gibbs JF, Iwata KK, Pak Y, Rutledge P, Yu J, et al. Epithelial-mesenchymal transition (EMT) and activated extracellular signal-regulated kinase (p-Erk) in surgically resected pancreatic cancer. Ann Surg Oncol 2007;14:3527-33.
10. Masugi Y, Yamazaki K, Hibi T, Aiura K, Kitagawa Y, Sakamoto M. Solitary cell infiltration is a novel indicator of poor prognosis and

epithelial-mesenchymal transition in pancreatic cancer. Hum Pathol 2010;41:1061-8.

11. Singh A, Settleman J. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene 2010;29:4741-51.
12. Shah AN, Summy JM, Zhang J, Park SI, Parikh NU, Gallick GE. Development and characterization of gemcitabine-resistant pancreatic tumor cells. Ann Surg Oncol 2007;14:3629-37.
13. Dembinski JL, krauss S. Characterization and functional analysis of a slow cycling stem cell-like subpopulation in pancreas adenocarcinoma. Clin Exp Metastasis 2009;26:611-23.
14. Du Z, Qin R, Wei C, Wang M, Shi C, Tian R, et al. Pancreatic cancer cells resistant to chemoradiotherapy rich in "stem-cell-like" tumor cells. Dig Dis Sci 2011;56:741-50.
15. Thompson EW, Newgreen DF, Tarin D. Carcinoma invasion and metastasis: a role for epithelial-mesenchymal transition? Cancer Res 2005;65:5991-5; discussion 5995.
16. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 2006;7:131-42.
17. Cano A, Pérez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, et al. The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. Nat Cell Biol 2000;2:76-83.
18. Hajra KM, Chen DY, Fearon ER. The SLUG zinc-finger protein represses E-cadherin in breast cancer. Cancer Res 2002;62:1613-8.
19. Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 2004;117:927-39.
20. Grooteclaes ML, Frisch SM. Evidence for a function of CtBP in epithelial gene regulation and anoikis. Oncogene 2000;19:3823-8.
21. Comijn J, Berx G, Vermassen P, Verschueren K, van Grunsven L, Bruyneel E, et al. The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion. Mol Cell 2001;7:1267-78.
22. Perez-Moreno MA, Locascio A, Rodrigo I, Dhondt G, Portillo F, Nieto MA, et al. A new role for E12/E47 in the repression of E-cadherin expression and epithelial-mesenchymal transitions. J Biol Chem 2001;276:27424-31.
23. Tamura G, Yin J, Wang S, Fleisher AS, Zou T, Abraham JM, et al. E-Cadherin gene promoter hypermethylation in primary human gastric carcinomas. J Natl Cancer Inst 2000;92:569-73.
24. Peinado H, Ballestar E, Esteller M, Cano A. Snail mediates E-cadherin repression by the recruitment of the Sin3A/histone deacetylase 1 (HDAC1)/HDAC2 complex. Mol Cell Biol 2004;24:306-19.
25. Hou Z, Peng H, Ayyanathan K, Yan KP, Langer EM, Longmore GD, et al. The LIM protein AJUBA recruits protein arginine methyltransferase 5 to mediate SNAIL-dependent transcriptional repression. Mol Cell Biol 2008;28:3198-207.
26. Herranz N, Pasini D, Díaz VM, Francí C, Gutierrez A, Dave N, et al. Polycomb complex 2 is required for E-cadherin repression by the Snail1 transcription factor. Mol Cell Biol 2008;28:4772-81.
27. Yang MH, Hsu DS, Wang HW, Wang HJ, Lan HY, Yang WH, et al. Bmi1 is essential in Twist1-induced epithelial-mesenchymal transition. Nat Cell Biol 2010;12:982-92.
28. Krishnamachary B, Zagzag D, Nagasawa H, Rainey K, Okuyama H, Baek JH, et al. Hypoxia-inducible factor-1-dependent repression of E-cadherin in von Hippel-Lindau tumor suppressor-null renal carcinoma mediated by TCF3, ZFHX1A, and ZFHX1B. Cancer Res 2006;66:2725-31.
29. Evans AJ, Russell RC, Roche O, Burry TN, Fish JE, Chow VW, et al. VHL promotes E2 box-dependent E-cadherin transcription by HIF-mediated regulation of SIP1 and Snail. Mol Cell Biol 2007;27:157-69.
30. Yang MH, Wu MZ, Chiou SH, Chen PM, Chang SY, Liu CJ, et al. Direct regulation of TWIST by HIF-1alpha promotes metastasis. Nat Cell Biol 2008;10:295-305.
31. Gort EH, van Haaften G, Verlaan I, Groot AJ, Plasterk RH, Shvarts A, et al. The TWIST1 oncogene is a direct target of hypoxia-inducible factor-2alpha. Oncogene 2008;27:1501-10.
32. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008;133:704-15.
33. Morel AP, Lièvre M, Thomas C, Hinkal G, Ansieau S, Puisieux A. Generation of breast cancer stem cells through epithelial-mesenchymal transition. PLoS ONE 2008;3:e2888.
34. Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, et al. The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nat Cell Biol 2009;11:1487-95.
35. TReya T, Morrison SJ, Clarke MF, Weissman IL. Stem cell, cancer, and cancer stem cells. Nature 2001;414;105-11.
36. Lee CJ, Dosch J, Simeone DM. Pancreatic cancer stem cells. J Clin Oncol 2008;26:2806-12.
37. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, et al. Identification of pancreatic cancer stem cells. Cancer Res 2007;67:1030-7.
38. Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M, et al. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell 2007;1:313-23.
39. Rasheed ZA, Yang J, Wang Q, Kowalski J, Freed I, Murter C, et al. Prognostic significance of tumorigenic cell with mesenchymal features in pancreatic adenocarcinoma. J Natl Cancer Inst 2010;102:340-51.
40. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer 2005;5:275-84.
41. Hong SP, Wen J, Bang S, Park S, Song SY. CD44-positive cells are responsible for gemcitabine resistance in pancreatic cancer cells. Int J Cancer 2009;125:2323-31.
42. Jimeno A, Feldmann G, Suarez-Gauthier A, Rasheed Z, Solomon A, Zou GM, et al. A direct pancreatic cancer xenograft model as a platform for cancer stem cell therapeutic development. Mol Cancer Ther 2009;8:310-4.
43. Peacock CD, Wang Q, Gesell GS, Corcoran-Schwartz IM, Jones E, Kim J, et al. Hedgehog signaling maintains a tumor stem cell compartment in multiple myeloma. Proc Natl Acad Sci USA 2007;104;4048-53.

44. Kabashima A, Higuchi H, Takaishi H, Matsuzaki Y, Suzuki S, Izumiya M, et al. Side population of pancreatic cancer cells predominates in TGF-beta-mediated epithelial to mesenchymal transition and invasion. Int J Cancer 2009;124:2771-9.
45. Wang Z, Li Y, Kong D, Banerjee S, Ahmad A, Azmi AS, et al. Acquisition of epithelial-mesenchymal transition phenotype of gemcitabine-resistant pancreatic cancer cells is linked with activation of the notch signaling pathway. Cancer Res 2009;69:2400-7.
46. Li Y, VandenBoom TG 2nd, Kong D, Wang Z, Ali S, Philip PA, et al. Up-regulation of miR-200 and let-7 by natural agents leads to the reversal of epithelial-to-mesenchymal transition in gemcitabine-resistant pancreatic cancer cells. Cancer Res 2009;69:6704-12.
47. Roy LD, Sahraei M, Subramani DB, Besmer D, Nath S, Tinder TL, et al. MUC1 enhances invasiveness of pancreatic cancer cells by inducing epithelial to mesenchymal transition. Oncogene 2011;30;1449-59.
48. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002;2:161-74.
49. Gordon KJ, Kirkbride KC, How T, Blobe GC. Bone morphogenetic proteins induce pancreatic cancer cell invasiveness through a Smad1-dependent mechanism that involves matrix metalloproteinase-2. Carcinogenesis 2009;30:238-48.
50. Zhang K, Chen D, Jiao X, Zhang S, Liu X, Cao J, et al. Slug enhances invasion ability of pancreatic cancer cells through upregulation of matrix metalloproteinase-9 and actin cytoskeleton remodeling. Lab Invest 2011;91:426-38.
51. Wu Y, Zhou BP. New insights of epithelial-mesenchymal transition in cancer metastasis. Acta Biochim Biophys Sin (Shanghai) 2008;40:643-50.
52. Gordon KJ, Dong M, Chislock EM, Fields TA, Blobe GC. Loss of type III transforming growth factor beta receptor expression increases motility and invasiveness associated with epithelial to mesenchymal transition during pancreatic cancer progression. Carcinogenesis 2008;29:252-62.
53. Haque I, Mehta S, Majumder M, Dhar K, De A, McGregor D, et al. Cyr61/CCN1 signaling is critical for epithelial-mesenchymal transition and stemness and promotes pancreatic carcinogenesis. Mol Cancer 2011;10:8.
54. Perbal B. CCN proteins: multifunctional signaling regulators. Lancet 2004;363:62-4.