Abstract

Pancreatic cancer carries a terrible prognosis, as the fourth most common cause of cancer death in the Western world. There is clearly a need for new therapies to treat this disease. One of the reasons no effective treatment has been developed in the past decade may in part be explained by the diverse influences exerted by the tumour microenvironment. The tumour stroma cross-talk in pancreatic cancer can influence chemotherapy delivery and response rate. Therefore, preclinical in vitro models which can bridge simple 2D in vitro cell based assays and complex in vivo models are required to understand the biology of pancreatic cancer. Here we discuss the evolution of 3D organotypic models, which recapitulate the morphological and functional features of pancreatic ductal adenocarcinoma (PDAC). Organotypic cultures are a valid high throughput preclinical in vitro model that maybe a useful tool to help establish new therapies for PDAC. A huge advantage of the organotypic model system is that any component of the model can be easily modulated in a short timeframe. This allows new therapies that can target the cancer, the stromal compartment or both to be tested in a model that mirrors the in vivo situation. A major challenge for the future is to expand the cellular composition of the organotypic model to further develop a system that mimics the PDAC environment more precisely. We discuss how this challenge is being met to increase our understanding of this terrible disease and develop novel therapies that can improve the prognosis for patients.

Key words: 3D organotypic model; Pancreatic cancer; Pancreatic stellate cell; Stroma; Preclinical models

Core tip: Pancreatic cancer carries a terrible prognosis, as the fourth most common cause of cancer death in the Western world. One of the reasons no effective treatment has been developed in the past decade may in part be explained by the influences exerted by the tumour microenvironment. The tumour stroma cross-talk in pancreatic cancer can influence chemotherapy delivery and response rate. Organotypic models of pancreatic cancer allow new therapies that can target the cancer, the stromal compartment or both to be tested in a model that mirrors the in vivo situation and can help improve patient prognosis.

Stacey J Coleman, Jennifer Watt, Prabhu Arumugam, Leonardo Solaini, Elisabeta Carapuca, Mohammed Ghallab, Richard P Grose, Hemant M Kocher

Stacey J Coleman, Jennifer Watt, Prabhu Arumugam, Leonardo Solaini, Elisabeta Carapuca, Mohammed Ghallab, Richard P Grose, Hemant M Kocher, Centre for Tumour Biology, Barts Cancer Institute - a CR-UK Centre of Excellence, Queen Mary University of London, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, United Kingdom

Jennifer Watt, Prabhu Arumugam, Leonardo Solaini, Hemant M Kocher, Barts and the London HPB Centre, The Royal London Hospital, Barts Health NHS Trust, London EC1M 6BQ, United Kingdom

Author contributions: Coleman SJ designed and wrote the paper; Watt J, Arumugam P, Solaini L, Carapuca E, Ghallab M and Grose RP contributed equally to the writing of the paper; Kocher HM made final amendments of the paper and approval of the version to be published.

Correspondence to: Hemant M Kocher, MS, MD, FRCS, Centre for Tumour Biology, Barts Cancer Institute - a CR-UK Centre of Excellence, Queen Mary University of London, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, United Kingdom. h.kocher@qmul.ac.uk

Telephone: +44-20-78823579 Fax: +44-20-78823884

Accepted: April 1, 2014

Published online: July 14, 2014

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: 3D organotypic model; Pancreatic cancer; Pancreatic stellate cell; Stroma; Preclinical models

Core tip: Pancreatic cancer carries a terrible prognosis, as the fourth most common cause of cancer death in the Western world. One of the reasons no effective treatment has been developed in the past decade may in part be explained by the influences exerted by the tumour microenvironment. The tumour stroma cross-talk in pancreatic cancer can influence chemotherapy delivery and response rate. Organotypic models of pancreatic cancer allow new therapies that can target the cancer, the stromal compartment or both to be tested in a model that mirrors the in vivo situation and can help improve patient prognosis.

Stacey J Coleman, Jennifer Watt, Prabhu Arumugam, Leonardo Solaini, Elisabeta Carapuca, Mohammed Ghallab, Richard P Grose, Hemant M Kocher

Stacey J Coleman, Jennifer Watt, Prabhu Arumugam, Leonardo Solaini, Elisabeta Carapuca, Mohammed Ghallab, Richard P Grose, Hemant M Kocher, Centre for Tumour Biology, Barts Cancer Institute - a CR-UK Centre of Excellence, Queen Mary University of London, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, United Kingdom

Jennifer Watt, Prabhu Arumugam, Leonardo Solaini, Hemant M Kocher, Barts and the London HPB Centre, The Royal London Hospital, Barts Health NHS Trust, London EC1M 6BQ, United Kingdom

Author contributions: Coleman SJ designed and wrote the paper; Watt J, Arumugam P, Solaini L, Carapuca E, Ghallab M and Grose RP contributed equally to the writing of the paper; Kocher HM made final amendments of the paper and approval of the version to be published.

Correspondence to: Hemant M Kocher, MS, MD, FRCS, Centre for Tumour Biology, Barts Cancer Institute - a CR-UK Centre of Excellence, Queen Mary University of London, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, United Kingdom. h.kocher@qmul.ac.uk

Telephone: +44-20-78823579 Fax: +44-20-78823884

Accepted: April 1, 2014

Published online: July 14, 2014

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: 3D organotypic model; Pancreatic cancer; Pancreatic stellate cell; Stroma; Preclinical models

Core tip: Pancreatic cancer carries a terrible prognosis, as the fourth most common cause of cancer death in the Western world. One of the reasons no effective treatment has been developed in the past decade may in part be explained by the influences exerted by the tumour microenvironment. The tumour stroma cross-talk in pancreatic cancer can influence chemotherapy delivery and response rate. Organotypic models of pancreatic cancer allow new therapies that can target the cancer, the stromal compartment or both to be tested in a model that mirrors the in vivo situation and can help improve patient prognosis.

Stacey J Coleman, Jennifer Watt, Prabhu Arumugam, Leonardo Solaini, Elisabeta Carapuca, Mohammed Ghallab, Richard P Grose, Hemant M Kocher
PANCREATIC CANCER

Pancreatic cancer has one of the highest mortality rates among malignancies, and is the fourth most common cause of cancer death in the Western world\[1,2\]. With an overall 5-year survival rate of 6% and median survival of less than six months, pancreatic ductal adenocarcinoma (PDAC) carries one of the bleakest prognoses in all of medicine. Surgery offers the only hope of a possible cure for patients; however even of those 10% of patients eligible for curative resection, only 21% will survive to five years\[3\]. This is due to the fact that, at diagnosis, distant metastases are common\[4\]. Clearly there is an urgent need for therapies for PDAC. One of the possible reasons that targeted therapies fail to improve the prognosis of patients with PDAC may, in part, be explained by the diverse influences exerted by the tumour microenvironment. Delineating the signalling networks within the tumour microenvironment, may help to explain the huge discrepancy between relative success and effectiveness of therapies in preclinical assay (predominately 2D cell based assays and xenograft mouse models) and their aberrant failure in human PDAC.

Many epithelial malignancies, including breast, prostate, skin and pancreatic cancers, often exhibit a significant stromal reaction around the tumour cells\[5-9\]. Once thought to be a bystander, it is becoming increasingly evident that the stroma not only functions as a mechanical barrier but also constitutes a dynamic compartment that can separate the tumour cells from the immune system. Quiescent PSCs are characterised by lipid droplets rich in vitamin A, resembling hepatic stellate cells (HSCs) first described by the 19th century\[26\]. They express desmin and glial fibrillary acidic protein (GFAP) marker which serve to distinguish them from pancreatic fibroblasts\[29\]. In acute and chronic inflammatory conditions, PSCs are activated. This is characterised by a loss of fat droplets, expression of α-smooth muscle actin (αSMA), and an increased synthesis and secretion of several ECM proteins such as fibronectin, laminin and collagen type I and III\[27-30\].

The isolation and immortalisation of PSCs from human and rat pancreas has provided an additional tool for studying PSC activation and can overcome the limitations of culturing primary stellate cells. While immortalised stellate cells have provided a valuable tool in the study of PSC function, it is important to validate findings using primary PSCs\[14\]. PSCs have been immortalised using either SV40 large T antigen or human telomerase in human PDAC. These studies have highlighted the importance of stroma-cancer cross-talk. Thus, just studying pancreatic cancer cells without any stromal representation does not reflect accurately the in vivo situation. Cells grown on 2D tissue culture plates or in Transwell™ inserts differ in their morphology, differentiation and cell-cell and cell-matrix interactions compared to cells in vivo\[24,25\]. There is a need for physiologically relevant in vitro model systems that allow us to investigate and interrogate cancer and stromal cell behaviour and their interactions. Thus, 3D organotypic models are an invaluable research tool\[20\].

MODELLING PDAC

In vitro (2D) studies of tumour stroma interactions in PDAC

Improved understanding of the mechanisms that mediate epithelial-stromal interactions in PDAC is now possible due to the isolation, and in vitro culture, of pancreatic stellate cells (PSC), the key cells driving the desmoplastic reaction\[21\]. In the healthy pancreas, PSCs make up 4%-7% of all pancreatic cell types and exist in a quiescent state\[27\]. Quiescent PSCs are characterised by lipid droplets rich in vitamin A, resembling hepatic stellate cells (HSCs) first described by the 19th century\[26\]. They express desmin and glial fibrillary acidic protein (GFAP) marker which serve to distinguish them from pancreatic fibroblasts\[29\]. In acute and chronic inflammatory conditions, PSCs are activated. This is characterised by a loss of fat droplets, expression of α-smooth muscle actin (αSMA), and an increased synthesis and secretion of several ECM proteins such as fibronectin, laminin and collagen type I and III\[27-30\].

The isolation and immortalisation of PSCs from human and rat pancreas has provided an additional tool for studying PSC activation and can overcome the limitations of culturing primary stellate cells. While immortalised stellate cells have provided a valuable tool in the study of PSC function, it is important to validate findings using primary PSCs\[14\]. PSCs have been immortalised using either SV40 large T antigen or human telomerase in human PSCs as we have previously successfully done in our laboratory\[24,25\]. Immortalised PSCs display an activated phenotype in 2D culture. Importantly PSC cell line is comparable to activated PSCs, which include expression of αSMA and ECM proteins. Importantly, expression profiling of primary and PSC cell lines have shown only a few differences, with differential differences expression of ECM proteins, cytokines and integrins\[27\]. In addition, both immortalised and primary PSCs respond to TGF-β or PDGF in a similar manner\[28\]. Thus primary and immortalised PSCs have facilitated for the dissection of
important cross-talk between PSCs and pancreatic cancer cells and are an important source to explore the tumour promoting aspects of tumour myofibroblasts in PDAC[16].

The bidirectional interaction between PSCs and pancreatic cancer cells has been studied using co-culture or well-established 2D in vitro assays such as wound assays or Transwell™ inserts to study migration[19]. Co-culturing of PSCs and pancreatic cancer cells showed that PSCs can increase the proliferation and migration of pancreatic cancer cells, while inhibiting apoptosis by the release of several cytokines and growth factors. Similarly, culturing PSCs in the conditioned medium of pancreatic cancer cells increases the proliferation, matrix synthesis and motility of PSCs, most likely via FGF-2, PDGF and TGF-β[22,44].

The desmoplasia in PDAC is believed to have a detrimental effect on the successful response to chemotherapy and radiotherapy[40,41]. In vitro experiments have shown that PSCs can increase the stem cell characteristic of pancreatic cancer cells, a possible mechanism of resistance to therapy[42]. Furthermore, in areas of the tumour that are hypoxic as a result of hypovascularity and profuse stroma provides a micro-environment in which pancreatic cancer cells thrive[43]. In vitro studies have shown that, co-culturing PSCs and pancreatic cancer cells under hypoxic conditions, PSCs are able to influence PCC invasion more strongly than in normoxic conditions[44]. Thus, pharmacological targeting of PSCs is an attractive option in treating PDAC.

**Role of the stroma in PDAC-in vivo studies**

Animal models, such as xenografts, orthotopic grafts or genetically engineered mice (GEM), have validated many in vitro findings. Early subcutaneous mouse models, in which PSCs and pancreatic cancer cells were injected into the flanks of immunocompromised mice, demonstrated that, in the presence of PSCs, pancreatic cancer cell proliferation increased and tumours formed more rapidly than when pancreatic cancer cells were injected alone[23]. Apte and colleagues showed that injection of pancreatic cancer cells (MiaPaCa-2 and AsPC-1 cell lines), together with primary human PSCs into the mouse pancreas was able to stimulate fibrosis, tumour growth and metastasis[45]. More recently, sex mismatch studies (injection of male PSCs and female pancreatic cancer cells into the pancreas of female mice), have shown that Y chromosome positive PSCs are able to migrate through blood vessels, together with cancer cells, localising to distant sites, such as the liver and diaphragm, where they are able to facilitate seeding, survival and growth of pancreatic cancer cells[40].

The development of genetically engineered mouse (GEM) models of PDAC has provided the most physiologically relevant model that closely mimics the situation in human cancer. Most of the GEM models of PDAC are based on the conditional, pancreas-specific, expression of the Kras oncogene (KRASG12D), present in 90% of human PDAC cases[40], this is facilitated by expressing Cre recombinase under the control of the embryonic pancreas lineage determining transcription factor Pdx-1 or Ptf1/p48 (“KC” mice). KC mice develop pancreatic tumours ranging from precursor pancreatic intra-ductal neoplasms (PanINs) to fully invasive and metastatic disease[46-48], albeit with a long latency period of up to a year. These KC mice have been crossed with mice harbouring several additional mutations, to investigate their contribution to the rapid progression to PDAC. GEM models of PDAC have been developed with activating mutations in TGFβ receptor and/or inactivation of tumoral suppressors such as p53 (“KPC” mice), INK4A/ARF and Smad4, which are the most common PDAC drivers[49]. There are several excellent reviews on the various GEM models that have been developed for studying the development of PDAC[50-52]. The generation of complex allele combinations together with the latency period involved in the development of tumour makes these models inherently expensive. Further criticism against GEM models of PDAC has focused on the multi-focality of their development of PDAC[47,48], involving of whole pancreas with tumours, histological variants commonly observed, presence of tumours in other organs as well as genetic homogeneity; features missing in the human PDAC[53]. Thus, 3D organotypic models may be an attractive option as a preclinical tool, bridging the gap between traditional 2D cell culture assays and the complex GEM models.

**Organotypic models used in other cancers**

The idea of recapitulating the physiomorphic 3D envi-

---

**Figure 1** Human pancreatic ductal adenocarcinoma has a dense desmoplastic stromal component. A: HE of human pancreatic cancer shows an area of invasive tumour; B: Stromal and epithelial components of the tumour are highlighted from figure A (scale bar 100 μm).
Coleman SJ et al. Pancreatic cancer organotypics as preclinical models

![diagram](image_url)

**Figure 2** Submerged organotypic culture models used to investigate pancreatic ductal adenocarcinoma. A: Cancer cells were embedded in the extracellular matrix mixture before it was allowed to polymerise. These cancer cells were then fed with culture media placed on top of the gel. B: Representative configuration of cells within the gel after 7 d of culture. The cancer cells forming duct-like structures within the gel. This model mimics the behaviour of invading cancer cells; C, D: Show the same model with both pancreatic stellate cells and cancer cells embedded within the extracellular matrix gel. Using this model the interaction between stellate cells and invading cancer cells can be examined; E: Stellate cells embedded in the gel prior to polymerisation, with cancer cells seeded on top of the gel 24 h later. In this model cancer cell invasion can be analysed in the presence of pancreatic stellate cells (F). Representative HE of these organotypic models are reviewed in Froeling et al[56].

Pancreatic cancer organotypics

Pancreatic cancer cell lines and normal pancreatic ductal epithelial cells (HPDE) previously have been cultured on type I glycosaminoglycan scaffolds and in collagen type I or Matrigel. Given only epithelial cells were in these models, the effect of the stroma on tumour cell behaviour was absent[57-59]. However, these studies were able to show that pancreatic cancer cells embedded into Matrigel formed spheroids with a distinct morphology and loss of apico-basal polarity as compared to culturing in 2D[57].

The introduction of stromal cells in PDAC 3D organotypic cultures was first demonstrated by our laboratory[26]. Depending on the hypothesis being explored, the flexible 3D models of PDAC can be set up distinctly. Pancreatic cancer cells can be embedded into the ECM gel consisting of collagen and Matrigel to simulate cells that have already invaded into the stroma. However, in order to understand the influence of PSCs on the behaviour of invaded pancreatic cancer cells these cells can be embedded in an ECM gel together with cancer cells (Figure 2).

Submerged ECM gels (when pancreatic cancer cells are grown on top of the gel and PSCs are embedded) are designed to model the early events in tumour progression. When pancreatic cancer cells are cultured on top of this model, they form luminal structures that resemble ducts (Figure 3). Using this model, we have shown that PSCs induce Ezrin translocation from the apical to the basal compartment of the cells is an early event in pancreatic cancer cell invasion[60,61]. This phenomenon has been validated across a range of human gastro-intestinal tumours[62,63]. Finally, in order to study the invasion of pancreatic cancer cells in the 3D model, the submerged...
culture system can be raised upon a grid (‘air-liquid’ model) and fed from underneath, creating a gradient that stimulates pancreatic cancer cells to invade while at the same time recapitulates cancer-stellate cell interaction in vivo (Figure 4).

Using the air liquid 3D model we have shown that the presence of PSCs leads to a significant increase, and altered sub-cellular distribution, of β-catenin in pancreatic cancer cells. Treating these 3D co cultures with All Trans Retinoic Acid (ATRA, which renders PSC quiescent) dampens Wnt-β catenin signalling resulting in reduced pancreatic cancer invasion [16]. Importantly, these results were confirmed in vivo, whereby treating KPC mice with ATRA led to disruption lead to disruption of the activated stroma and increase in apoptosis of tumour cells. These sets of observations validate the use of the organotypic model as a tool to assess new therapies in PDAC.

3D organotypic models provide a perfect intermediate between 2D cultures and GEM. Use of distinct cell types in these co-culture allows assessment of changes in signalling cascades and molecular targets resulting from cancer-stroma cross-talk in the absence of noise from other stromal elements present in vivo. Thus the relative contribution of each cell type in the complex microenvironment can be assessed. Using this approach Kadaba et al [14] isolated cancer cells from organotypic models of various organ including pancreas, skin and oesophagus after the cancer cells were exposed in 3D to their respective stromal cells (Figure 5). They demonstrated that cancer cell stromal interactions significantly alter proliferation, cell cycle, cell movement, cell signalling and inflammatory response in addition to changing stiffness in the ECM gel. Importantly, changes in stiffness of ECM gels was particularly prominent as the proportion of PSC in the ECM gel increased, a finding highly pertinent to drug delivery and perfusion in PDAC [41]. This study also highlighted the possible need for multidrug targeting or use of pleiotropic agents in PDAC therapy.

Despite the importance of multiple pathways in PDAC, the proto-oncogene Src has been heralded as a potential single molecular therapeutic target [82]. The conundrum of promise of Src inhibitors in combination with chemotherapy in vitro and the in vivo reduction of metastasis in KPC mice by 50%, was explored in organotypic cultures [82]. Using fluorescence lifetime imaging microscopy (FLIM) to measure fluorescence resonance...
energy transfer (FRET) an ECFP-YFP Src reporter, in PDAC cells in organotypic cultures Anderson and colleagues investigated the influence of tumour microenvironment on Dasatinib delivery in PDAC\cite{83}. In organotypic PDAC models with cancer cells expressing the Src biosensor cultured on top of an ECM gel with embedded primary human fibroblasts, they were able to show quantitatively that the microenvironment contribution to poor drug delivery to tumour cells is dependent on distance of cells from the invasive edge. This was validated in subcutaneous in vivo models due to the limitations of microscopy techniques precluding orthotopic or GEM models. This study demonstrated the adaptability of the organotypic model as powerful tool to address hypotheses at the molecular level in a complex microenvironment.

**Future applications and challenges**

3D organotypic models that mimic the morphological and functional features of their in vivo parental tissues have potential for bridging the gap between cell-based discovery research and animal models\cite{84,85}. A huge advantage of the organotypic system is that any component of the model can readily be modulated in a short time-frame. For example, the matrix composition can be altered to reflect the in vivo situation. The increase in ECM stiffness exerts elevated force on transformed cells increasing cellular response and resulting in increased tumour growth, survival and motility\cite{14,86}.

The relative paucity of primary stellate cells to conduct all the experiments in sufficient replicates lead us to generate a mini organotypic culture system (Figure 6) which give comparable results to the conventional “air liquid” co culture model\cite{84}. Additional cell types can be titrated in such as stellate cells\cite{84} or endothelial cells (Di Maggio, unpublished observations). For example, to assess the role of stroma on angiogenesis, in oesophageal cancer endothelial cells on a 2D monolayer have been cultured with fibroblast and cancer cells embedded in a collagen gel layered on top\cite{87}. Elsewhere investigation of the role of macrophages in malignant growth of human squamous cell carcinoma has been investigated in organotypic cultures\cite{88}. Immune response and inflammation play an important role in the desmoplastic reaction and inflammation is thought to activate pancreatic stellate cells\cite{13,89}.

Therapeutic agents such as chemotherapy (Gemen-
zitdis and Carapuca and Ghallab, unpublished observations), small molecules or RNAi (Arumugam and Watt, unpublished observations) can be tested in these organotypic cultures. The best dosage and regimen can then be taken in small animals thus reducing animal usage.

Examples from other related fields include testing Metkinase inhibitor or COX-2 inhibitor in skin cancer models, tyrosine kinase inhibitors for breast cancers and Eps8 and HAX1 or β6 integrin RNAi in cancer cells prior to their incorporation into organotypic cultures to assess the effects on cell invasion.

Finally, many PDAC patients present very late with their disease when metastasis have already occurred. Thus, treating PDAC cells immediately after seeding in a 3D environment does not reflect the true clinical setting as tumours are well established at the time of patient treatment. We currently are investigating the effect of treating organotypic models once they are established and invasion of PDAC and/or stromal cells has begun. It is likely this would give a better understanding of the treatment regimen that is required when novel therapies emerge into a preclinical setting.

**CONCLUSION**

Organotypic culture models are valuable tools for studying the mechanisms of pancreatic cancer, providing an easily manipulated system in which specific questions can be addressed, thus facilitating the translation of basic science to the clinic. Allowing manipulation of cell types, matrix composition, and exogenous therapies, these physiologically relevant model systems are reproducible, experimentally flexible and offer targeted high-throughput platforms. Although the organotypic model provides a physiologically relevant means to study the tumour stroma interactions and the use of new therapies to target the cross talk, it remains a simplified representation of the complex *in vivo* situation and it still remains critical to test new therapies in orthotopic or transgenic models of the disease. However, the use of the organotypic model as a preclinical tool is becoming increasingly important and our group, as well as others, are modulating the 3D cultures to recapture other important aspects of the tumour microenvironment that can influence cancer cell behaviour. Thus, 3D organotypic models have potential for bridging the gap between cell based discovery and complex animal models. By providing an environment in which cell behaviour and novel treatment options can be investigated in an easily reproducible and controlled manner, these models more precisely mimic pancreatic cancer, thus providing a major contribution to preclinical drug and therapeutic discovery.
REFERENCES

1 Tassi E, Henke RT, Bowden ET, Swift MR, Kodack DP, Kuo AH, Maitra A, Wellstein A. Expression of a fibroblast growth factor-binding protein during the development of adenocarcinoma of the pancreas and colon. Cancer Res 2006; 66: 1191-1198 [PMID: 16424058 DOI: 10.1158/0008-5472-CAN-05-2926]

2 Harirhan D, Saied A, Kocher HM. Analysis of mortality rates for pancreatic cancer across the world. HPB (Oxford) 2008; 10: 58-62 [PMID: 18695761 DOI: 10.1016/j.hpb.2007.01.002]

3 Iqbal M, Lovegrove RE, Tilney HS, Abraham AT, Bhattacharya S, Tekkes FP, Kocher HM. A comparison of pancreaticoduodenectomy with extended pancreaticoduodenectomy: a meta-analysis of 1909 patients. Eur J Surg Oncol 2009; 35: 79-86 [PMID: 18356005 DOI: 10.1016/j.ejso.2008.01.002]

4 Obester PE, Olive KP. Pancreatic cancer: why is it so hard to treat? Ther Adv Gastroenterol 2013; 6: 321-337 [PMID: 23841611 DOI: 10.1177/1756283X13478680]

5 Moinfar F, Man YG, Brathbauer GL, Ratschek M, Tavassoli F.A. Genetic abnormalities in mammary ductal intraepithelial neoplasia-flat type ("clinging ductal carcinoma in situ"): a simulator of normal mammary epithelium. Cancer 2000; 88: 2072-2081 [PMID: 10813719 DOI: 10.1002/(SICI)1097-0142(20000501)88:4<2072::AID-CAN2>3.0.CO;2-D]

6 Shekhar MP, Werdell J, Santner SJ, Pauley RJ, Tait L. Breast stroma plays a dominant regulatory role in breast epithelial growth and differentiation: implications for tumor development and progression. Cancer Res 2001; 61: 1320-1326 [PMID: 11245428]

7 Olumfi AF, Grossfeld GD, Hayward SW, Carroll PR, Tsai Y. The pathogenesis of cancer metastasis: the ‘seed and soil’ hypothesis revisited. Nat Rev Cancer 2003; 3: 435-458 [PMID: 12778135 DOI: 10.1038/nrc1098]

8 Mueller MM, Fusenig NE. Friends or foes - bipolar effects of the tumour stroma in cancer. Nat Rev Cancer 2004; 4: 839-849 [PMID: 15516957 DOI: 10.1038/nrc1477]

9 Mahadevan D, Von Hoff DD. Tumor-stroma interactions in pancreatic ductal adenocarcinoma. Mol Cancer Ther 2007; 6: 1186-1197 [PMID: 17460631 DOI: 10.1186/1535-7567-MCT-06-066]

10 Ene-Obong A, Clear AJ, Watt J, Wang J, Fatah R, Riches JC, Marshall JF, Chinnaleong J, Chelala C, Gribben JG, Ramsay AG, Kocher HM. Activated pancreatic stellate cells sequester CD8+ T cells to reduce their infiltration of the juxtatumoral compartment of pancreatic ductal adenocarcinoma. Gastroenterology 2013; 145: 1121-1132 [PMID: 23891972 DOI: 10.1053/j.gastro.2013.07.025]

11 Kadaba R, Birke H, Wang J, Hooper S, Andl CD, Di Maggio F, Soylu E, Ghallab M, Bor D, Foeuling FE, Bhattacharya S, Rustgi AK, Sahai E, Chelala C, Sasieni P, Kocher HM. Imbalance of desmoplastic stromal cell numbers drives aggressive cancer processes. J Pathol 2013; 230: 107-117 [PMID: 23359139 DOI: 10.1002/path.4172]

12 Neesse A, Michl P, Frese KK, Feig C, Cook N, Jacobetz MA, Lolkema MP, Buchholz M, Olive KP, Gress TM, Tuveson DA. Stromal biology and therapy in pancreatic cancer. Gut 2011; 60: 861-868 [PMID: 20966025 DOI: 10.1136/gut.2010.226092]

13 Foeuling FE, Feig C, Chelala C, Dobson R, Mein CE, Tuveson DA, Clevers H, Hart IR, Kocher HM. Retinoic acid-induced pancreatic stellate cell quiescence reduces paracrine Wnt-β-catenin signaling to slow tumor progression. Gastroenterology 2011; 141: 1486-1497, 1486-1497 [PMID: 21704588 DOI: 10.1053/j.gastro.2011.06.047]

14 Li D, Abbuzzese J.L. New strategies in pancreatic cancer: emerging epidemiologic and therapeutic concepts. Clin Cancer Res 2010; 16: 4313-4318 [PMID: 20647474 DOI: 10.1158/1078-0432.CCR-09-1942]

15 Ozaifa F, Friess H, Tempia-Caliera A, Kleeff J, Büchler MW. Growth factors and their receptors in pancreatic cancer. Tumor Carcinog Mutagen 2001; 21: 27-44 [PMID: 11135319]

16 Atepe MV, Wilson JS. Stellate cell activation in alcoholic pancreatitis. Pancreas 2003; 27: 316-320 [PMID: 14576494 DOI: 10.1097/01000667-200311000-00008]

17 Atepe MV, Haber PS, Darby SJ, Rodgers SC, McCaughan GW, Korsten MA, Pirola RC, Wilson JS. Pancreatic stellate cells are activated by proinflammatory cytokines: implications for pancreatic fibrogenesis. Gut 1999; 44: 534-541 [PMID: 10075961 DOI: 10.1136/gut.44.4.534]

18 Bachem MG, Schüneemann M, Ramadani M, Siech M, Beger H, Buck A, Zhou S, Schmid-Kotsas A, Adler G. Pancreatic carcinoma cells induce fibrosis by stimulating proliferation and matrix synthesis of stellate cells. Gastroenterology 2005; 128: 907-921 [PMID: 15825074 DOI: 10.1053/j.gastro.2004.12.036]

19 Korec M. Growth factors and pancreatic cancer. Int J Pancreatol 1991; 9: 87-91 [PMID: 1744452]

20 Foeuling FE, Mirza TA, Feakins RM, Seedhar A, Elia G, Hart IR, Kocher HM. Organotypic culture model of pancreatic cancer demonstrates that stromal cells modulate E-cadherin, beta-catenin, and Ezrin expression in tumor cells. Am J Pathol 2009; 175: 636-648 [PMID: 19688876 DOI: 10.2353/ajpath.2009.090131]

21 Debnath J, Brugge JS. Modelling glandular epithelial cancers in three-dimensional cultures. Nat Rev Cancer 2005; 5: 675-688 [PMID: 16148884 DOI: 10.1038/nrc1695]

22 Foeuling FE, Marshall JF, Kocher HM. Pancreatic cancer organotypic cultures. J Biotechnol 2010; 148: 16-23 [PMID: 20833848 DOI: 10.1016/j.jbiotec.2010.01.008]

23 Atepe MV, Haber PS, Applegate TL, Norton ID, McCaughan GW, Korsten MA, Pirola RC, Wilson JS. Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture. Gut 1998; 43: 128-133 [PMID: 9771417 DOI: 10.1136/gut.43.1.128]

24 Wake K. “Stemzellen” in the liver: perisinusoidal cells with special reference to storage of vitamin A. Am J Anat 1971; 132: 429-462 [PMID: 4942297 DOI: 10.1002/ajaj.1010320404]

25 Omary MB, Luagea L, Lowe AW, Pandol SJ. The pancreatic stellate cell: a star on the rise in pancreatic diseases. J Clin Invest 2007; 117: 50-59 [PMID: 17200706 DOI: 10.1172/JCI30082]

26 Fujita H, Ohuchida K, Mizumoto K, Nakata K, Yu J, Kayashima T, Cui L, Manabe T, Ohutsuka T, Tanaka M. alpha-Smooth Muscle Actin Expressing Stromal Cells Promotes an Aggressive Tumor Biology in Pancreatic Ductal Adenocarcinoma. Pancreas 2010; Epub ahead of print [PMID: 20467342 DOI: 10.1097/MPA.0b013e3181bd6f67]

27 Bachem MG, Schneider E, Gross H, Weidenbach H, Schmid RM, Menke A, Siech M, Beger H, Grünert A, Adler G. Iden-
tification, culture, and characterization of pancreatic stellate cells in rats and humans. Gastroenterology 1998; 115: 421-432 [PMID: 9679048 DOI: 10.1016/S0016-5085(98)70209-4]

Hwang RF, Moore T, Arumugam T, Ramachandran V, Amos KD, Rivera A, Ji B, Evans DB, Logsdon CD. Cancer-associated stromal fibroblasts promote pancreatic tumor progression. Cancer Res 2008; 68: 918-926 [PMID: 18245495 DOI: 10.1158/0008-5472.CAN-07-5714]

Jesnowski R, Fürst D, Ringel J, Chen Y, Schrödel A, Kleeff J, Kolb A, Schareck WD, Lörh M. Immortalization of pancreatic stellate cells as an in vitro model of pancreatic fibrosis: deactivation is induced by matrilin and N-acetylcysteine. Lab Invest 2005; 85: 1276-1291 [PMID: 16127427 DOI: 10.1038/labinvest.3700029]

Masamune A, Satoh M, Kikutaka Y, Suzuki N, Shimosegawa T. Establishment and characterization of a rat pancreatic stellate cell line by spontaneous immortalization. World J Gastroenterol 2003; 9: 2751-2758 [PMID: 14669327]

Mathison A, Liebl A, Bharucha J, Mukhopadhhyay D, Lomberk G, Shah V, Urrutia R. Pancreatic stellate cell model cells for transcriptional studies of desmoplakia-associated genes. Pancreatology 2010; 10: 505-516 [PMID: 20847583 DOI: 10.1159/0003020540]

Satoh M, Masamune A, Sakai Y, Kikutaka Y, Hamada H, Shimosegawa T. Establishment and characterization of a simian virus 40-immortalized rat pancreatic stellate cell line. Tohoku J Exp Med 2002; 198: 55-69 [PMID: 12498351 DOI: 10.1620/tjem.198.55]

Sparmann G, Hohenadl C, Tornøe J, Jaster R, Fitzner B, Liebl A, Bharucha J, Mukhopadhyay D, Logsdon CD, Siveke JT. Genetically engineered mouse models of pancreatic cancer. Cell 2005; 121: 852-855 [PMID: 16034487 DOI: 10.1101/gad.3004208.412]

Erkan M, Adler G, Apte MV, Bachem MG, Buchholz M, De Angelis M, Dowsett M, Logsdon CD, Siveke JT. The use of targeted mouse models in cancer research. Gastroenterology 2010; 35: 1079-1092 [PMID: 20795355 DOI: 10.1016/j.gastro.2012.06.051]

Xu Z, Vonlaufen A, Phillips PA, Fiala-Beer E, Zhang X, Yang L, Biankin AV, Goldstein D, Prolla RC, Wilson JS, Apte MV. Role of pancreatic stellate cells in pancreatic cancer metastasis. Am J Pathol 2010; 177: 2585-2596 [PMID: 20934792 DOI: 10.2353/apath.2010.008899]

Almeguer A, Shibata D, Forrester K, Martin J, Arheim N, Peruchó M. Most human carcinomas of the exocrine pancreas contain mutant k-ras genes. Cell 1988; 53: 549-554 [PMID: 2453289 DOI: 10.1016/0092-8674(88)90571-5]

Grippo P, Tuveson DA. Deploying mouse models of pancreatic cancer for chemoprevention studies. Cancer Prev Res (Phila) 2010; 3: 1392-1397 [PMID: 21045161 DOI: 10.1158/1940-6207.CAPR-10-0258]

Olive KP, Tuveson DA. The use of targeted mouse models for preclinical testing of novel cancer therapeutics. Clin Cancer Res 2006; 12: 5277-5287 [PMID: 17008660 DOI: 10.1158/1078-0432.CCR-06-0436]

Hruban RH, Iacobuzio-Donahue C, Wilentz RE, Goggins M, Kern SE. Molecular pathology of pancreatic cancer. Cancer 2001; 7: 251-258 [PMID: 11561601]

Herreros-Villanueva M, Hijonosa E, Cosme A, Bujanda L. Mouse models of pancreatic cancer. World J Gastroenterol 2012; 18: 1286-1294 [PMID: 22493452 DOI: 10.3748/wjg.v18.i12.1286]

Hidalgo M, Von Hoff DD. Translational therapeutic opportunities in ductal adenocarcinoma of the pancreas. Clin Cancer Res 2012; 18: 4249-4256 [PMID: 22896691 DOI: 10.1158/1078-0432.CCR-12-1327]

Pérez-Mancera PA, Guerra C, Barbaric M, Tuveson DA. What we have learned about pancreatic cancer from mouse models. Gastroenterology 2012; 142: 1079-1092 [PMID: 22406637 DOI: 10.1053/j.gastro.2012.03.002]

Mazur PK, Siveke JT. Genetically engineered mouse models of pancreatic cancer: unravelling tumour biology and progressing translational oncology. Gut 2012; 61: 1488-1500 [PMID: 21875467 DOI: 10.1136/gutjnl-2011-300756]

Eguchi D, Ikenaga N, Ohuchida K, Kozono S, Cui L, Fujiwara K, Fujino M, Ohtsuka T, Mizumoto K, Tanaka M. Hypoxia enhances the interaction between pancreatic stellate cells and cancer cells via increased secretion of connective tissue growth factor. J Surg Res 2013; 181: 225-233 [PMID: 22795535 DOI: 10.1016/j.jss.2012.06.051]

WJG | www.wjgnet.com 8479

July 14, 2014 | Volume 20 | Issue 26 |
Engineering tissues and organs. A new perspective in epithelial biology. 

[Atala A, Engineering tissues and organs. *Curr Opin Urol* 1999; 9: 517-526 [PMID: 10668571 DOI: 10.1097/00002437-199908000-00035]

[61] Pampaloni F, Reynaud EG, Stelzer EH. The third dimension bridges the gap between cell culture and live tissue. *Nat Rev Mol Cell Biol* 2007; 8: 839-845 [PMID: 17684528 DOI: 10.1038/nrm2236]

[62] Schmeichel KL, Bissell MJ. Modeling tissue-specific signaling and organ function in three dimensions. *J Cell Sci* 2003; 116: 2377-2388 [PMID: 12766184 DOI: 10.1242/jcs.005030]

[63] Kopan R, Fuchs E. A new look into an old problem: keratin as tools to investigate determination, morphogenesis, and differentiation in skin. *Genes Dev* 1998; 3: 1-15 [PMID: 2468556 DOI: 10.1101/gad.3.1.1]

[64] Fartsch M, Ponec M. Improved barrier structure formation in air-exposed human keratinocyte culture systems. *J Invest Dermatol* 1994; 102: 366-374 [PMID: 8120421 DOI: 10.1111/j.1523-1747.ep12571979]

[65] Wang F, Weaver VM, Petersen OW, Larabell CA, Dederh S, Briand P, Lupa R, Bissell MJ. Reciprocal interactions between beta-talin and epidermal growth factor receptor in three-dimensional basement membrane breast cultures: a different perspective in epithelial biology. *Proc Natl Acad Sci USA* 1998; 95: 14821-14826 [PMID: 9843973 DOI: 10.1073/pnas.95.25.14821]

[66] Hoffman MP, Kibbey MC, Letterio JJ, Kleinman HK. Role of laminin-1 and TGF-beta 3 in acinar differentiation of a human submandibular gland cell line (HSG). *J Cell Sci* 1996; 109 (Pt 8): 2013-2021 [PMID: 8856497]

[67] Sakamoto T, Hiirano K, Morishima Y, Masuyama K, Ishii Y, Nomura A, Uchida Y, Ohtsuka M, Sekizawa K. Maintenance of the differentiated type II cell characteristics by culture on an acellular human amnion membrane. *In Vitro Cell Dev Biol Anim* 2001; 37: 471-479 [PMID: 11669280 DOI: 10.1097/01.tdv.0000057-00471: MDTFT1-2.0.CO;2]

[68] Vukicevic S, Luyten FP, Kleinman HK, Reddi AH. Differentiation of canalicular cell processes in bone cells by basement membrane matrix components: regulation by discrete domains of laminin. *Cell 1990; 63: 437-445 [PMID: 2208292 DOI: 10.1016/0092-8674(90)00176-F]

[69] Sanderson IR, Ezzell RM, Kedinger M, Erlanger M, Xu ZX, Pringault E, Leon-Robine S, Louvard D, Walker WA. Human fetal enterocytes in vitro: modulation of the phenotype by extracellular matrix. *Proc Natl Acad Sci USA* 1996; 93: 7267-7272 [PMID: 8755542 DOI: 10.1073/pnas.93.15.7717]

[70] Mauchamp J, Mirrione A, Alquier C, André F. Filopodic-like structure and polarized monolayer: role of the extracellular matrix on thyroid cell organization in primary culture. *Biol Cell 1998; 90: 369-380 [PMID: 9835011]

[71] Boudreau N, Bissell MJ. Extracellular matrix signaling: integration of form and function in normal and malignant cells. *Curr Opin Cell Biol* 1998; 10: 640-646 [PMID: 9818175 DOI: 10.1016/S0955-0674(98)80340-9]

[72] Howlett AR, Bailey N, Dansky C, Petersen OW, Bissell MJ. Cellular growth and survival are mediated by beta 1 integrins in normal human breast epithelium but not in breast carcinoma. *J Cell Sci 1995; 108 (Pt 5): 1945-1957 [PMID: 7544739]

[73] Radisky D, Muschler J, Bissell MJ. Order and disorder: the role of extracellular matrix in epithelial cancer. *Cancer Invest* 2002; 20: 139-153 [PMID: 11852996 DOI: 10.1081/CNV-120003074]

[74] Zutter MM, Santoro SA, Staat WD, Tsung YL. Re-expression of the alpha 2 beta 1 integrin abrogates the malignant phenotype of a breast carcinoma cells. *Proc Natl Acad Sci USA* 1995; 92: 7411-7415 [PMID: 7638207 DOI: 10.1073/pnas.92.16.7411]

[75] Grzesiak JF, Bouvet M. Determination of the ligand-binding specificities of the alpha2beta1 and alpha1beta1 integrins in a novel 3-dimensional in vitro model of pancreatic cancer.
Coleman SJ et al. Pancreatic cancer organotypics as preclinical models

90 Coleman SJ, Chioni AM, Ghallab M, Anderson RK, Lemoine NR, Kocher HM, Grose RP. Nuclear translocation of FGFR1 and FGF2 in pancreatic stellate cells facilitates pancreatic cancer cell invasion. EMBO Mol Med 2014; 6: 467-481 [PMID: 24503018]

91 Nystrom ML, McCulloch D, Weinreb PH, Violette SM, Speight PM, Marshall JF, Hart IR, Thomas GJ. Cyclooxygenase-2 inhibition suppresses alphavbeta6 integrin-dependent oral squamous carcinoma invasion. Cancer Res 2006; 66: 10833-10842 [PMID: 17108119 DOI: 10.1158/0008-5472.

92 Chioni AM, Grose R. FGFR1 cleavage and nuclear translocation regulates breast cancer cell behavior. J Cell Biol 2012; 197: 801-817 [PMID: 22665522 DOI: 10.1083/jcb.201108077]

93 Ramsay AG, Keppler MD, Jazayeri M, Thomas GJ, Parsons M, Violette S, Weinreb P, Hart IR, Marshall JF. HSI-associated protein X-1 regulates carcinoma cell migration and invasion via clathrin-mediated endocytosis of integrin alphavbeta6. Cancer Res 2007; 67: 5275-5284 [PMID: 17545607 DOI: 10.1158/0008-5472.CAN-07-0318]

P- Reviewers: Apte MV, Liu QD S- Editor: Ma YJ
L- Editor: A E- Editor: Zhang DN
