Cousins Center for PNI, Semel Institute for Neuroscience and Human Behavior, UCLA AIDS Institute and Jonsson Comprehensive Cancer Center, University of California, Los Angeles, USA

Department of Pathology, The University of Melbourne, Parkville, Victoria 3010, Australia

Department of Cancer Anaesthesia and Pain Medicine, Peter MacCallum Cancer Centre, East Melbourne, Victoria 3002, Australia

Clinic of Surgery, Cantonal Hospital, Schaffhausen, Switzerland

Department of Mathematics and Statistics, The University of Melbourne, Parkville, Victoria 3010, Australia

Centre for Drug Candidate Optimisation, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria 3052, Australia

Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria 3052, Australia

1. Introduction

Pancreatic cancer cells intimately interact with a complex microenvironment that influences pancreatic cancer progression. The pancreas is innervated by fibers of the sympathetic nervous system (SNS) and pancreatic cancer cells have receptors for SNS neurotransmitters which suggests that pancreatic cancer may be sensitive to neural signaling. In vitro and non-orthotopic in vivo studies showed that neural signaling modulates tumour cell behavior. However the effect of SNS signaling on tumor progression within the pancreatic microenvironment has not previously been investigated. To address this, we used in vivo optical imaging to non-invasively track growth and dissemination of primary pancreatic cancer using an orthotopic mouse model that replicates the complex interaction between pancreatic tumor cells and their microenvironment. Stress-induced neural activation increased primary tumor growth and tumor cell dissemination to normal adjacent pancreas. These effects were associated with increased expression of invasion genes by tumor cells and pancreatic stromal cells. Pharmacological activation of β-adrenergic signaling induced similar effects to chronic stress, and pharmacological β-blockade reversed the effects of chronic stress on pancreatic cancer progression. These findings indicate that neural β-adrenergic signaling regulates pancreatic cancer progression and suggest β-blockade as a novel strategy to complement existing therapies for pancreatic cancer.

© 2014 Elsevier Inc. All rights reserved.
2. Methods

2.1. Orthotopic pancreatic cancer model

The human pancreatic ductal adenocarcinoma cell lines Panc-1, HPAF-II and Capan-1 were obtained from the American Type Culture Collection, and maintained at 37 °C, 5% CO₂. These cell lines were chosen because they have mutated TP53 and KRAS, which are common driver mutations in pancreatic cancer and because they range from well differentiated (Capan-1) to moderately and highly undifferentiated (HPAF-II and Panc-1, respectively) (Yachiida, 2012). Panc-1 cells were cultured in DMEM (Invitrogen) supplemented with 10% fetal bovine serum (Bovogen Biologicals) and 1% penicillin–streptomycin (Sigma–Aldrich). HPAF-II cells were cultured in RPMI (Invitrogen) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin. Capan-1 cells were cultured in DMEM supplemented with 20% fetal bovine serum and 1% penicillin–streptomycin. To model human pancreatic cancer, 4 × 10⁵ tumor cells in Matrigel (BD Bioscience) were injected into the tail of the pancreas of six week old female BALB/c-Foxn1nu nude athymic mice (The University of Adelaide, Australia) by laparotomy as previously described (Chai, 2013). Panc-1 was chosen for in vivo studies as tumors are poorly differentiated which is characteristic of patients who are diagnosed with advanced stage and grade (Chai, 2013; Hotz, 2003). To track tumor progression in vivo, cell lines were transduced with FUHLucW construct that expresses firefly luciferase under control of the ubiquitin C promoter (Morizono, 2005). Tumor progression was monitored longitudinally over 42 days by in vivo and ex vivo optical bioluminescent imaging using an IVIS Lumina II system (Perkin Elmer) as described previously (Chai, 2013; Sloan, 2010). The presence of tumor cell dissemination beyond the tumor margins and into adjacent normal pancreas and metastasis to distant organs was measured by ex vivo optical imaging using long exposure times (>60 s), and confirmed by hematoxylin and eosin staining. Findings were validated in 2–4 independent experiments. All procedures were conducted in accordance with protocols approved by Institutional Animal Care and Use Committee of Monash University.

2.2. Chronic stress

Mice were randomly assigned to home cage control conditions (control) or 2 h per day restraint (stress) for 28 days commencing 7 days before tumor cell injection. Mice were restrained in a confined space that prevented them from moving freely but did not press on them (Thaker, 2006). This paradigm has been shown to induce chronic stress as shown by neuroendocrine activation (Thaker, 2006; Manni, 2008), weight loss (Smagin, 1999) (Supplementary Fig. 1), and anxiety-like behaviors (Hermann, 1994) but does not cause pain or wounding (Sheridan, 2004).

2.3. Pharmacological studies

For β-adrenergic antagonist studies 10 mg/kg/day (R/S)-propranolol (treatment) or water vehicle (placebo) was delivered to mice subcutaneously by osmotic minipump (Model 1004, Alzet). Propranolol was delivered for the duration of the experiment commencing seven days prior to tumor cell injection, with pumps replaced 24 days after implantation. Drug plasma concentration was assessed 20 days after pump implantation by UPLC-MS using a Micromass Quattro Premier coupled to an Acquity UPLC (Waters). For β-adrenergic agonist studies, 5 mg/kg/day (S)-isoproterenol (treatment) or 1 mM HCl vehicle (placebo) was delivered subcutaneously to mice via osmotic minipump (Model 1002, Alzet). Isoproterenol was delivered for 28 days, commencing seven days prior to tumor cell injection, with pumps replaced 14 days after implantation. Mice were maintained in their home cage for the duration of experiments that included isoproterenol treatment.

2.4. Invasion assay

2.5 × 10⁶ pancreatic cancer cells in serum free culture medium were seeded into the top well of a transwell chamber with 8.0 μm pores (BD Falcon) that was coated with 15 μg/mL Matrigel. Cells were allowed to migrate towards medium containing 20% serum for 22 h and then stained with DAPI. Cells that had migrated to the underside of the membrane were counted.

2.5. Proliferation assay

The influence of isoproterenol on proliferation was assessed using the CellTiter 96® Aqueous One Proliferation Assay (Promega). 8 × 10³ cells were seeded into a 96-well plate and assayed over 120 h, according to manufacturer’s instructions.

2.6. Gene expression studies

RNA was isolated from cell lines or primary pancreatic tumors using RNeasy Mini Kit (Qagen). Transcript levels were quantified by RT-PCR using iScript One-Step RT-PCR kit (Biorad) and species-specific Taqman probes (Applied Biosystems) to identify
2.7. cAMP assay

5 × 10^5 cells were seeded into a 96-well transparent plate and cultured overnight. Cells were washed with PBS and incubated in stimulation buffer (phenol-free DMEM, 0.1% BSA, 1 mM IBMX) at 37 °C for 60 min. Agonists were added for 30 min before cells were lysed with ice-cold 100% ethanol and rehydrated with lysis buffer (0.01% BSA, 5 mM HEPES, 0.3% Tween20). Cell lysates were incubated with AlphaScreen™ beads diluted in lysis buffer, followed by incubation with donor beads, and fluorescence signal was measured with a Fusion plate reader (Perkin Elmer). CAMP accumulation was expressed as a fraction of maximal stimulation induced by 10 μM forskolin.

2.8. Immunostaining

Cells were grown on slides, fixed in −20 °C acetone then incubated with antibodies against β2-adrenoceptor (H-20 rabbit polyclonal diluted 1:150, Santa Cruz Biotechnologies) for 16 h at 4 °C, followed by incubation with fluorescent Alexa-conjugated secondary antibodies (Invitrogen) and DAPI nuclear stain (Sigma). Immunostaining was imaged using an inverted microscope with fluorescence filters (Olympus). De-identified archival patient samples were obtained from Bern University Hospital in accordance with protocols approved by the Institutional Human Research Ethics Committee. Samples were dewaxed and incubated with antibodies as above and visualized by reaction with diaminobenzidine peroxidase (Vector) with hematoxylin counterstain.

2.9. Statistical analyses

Student’s t test analyzed the effect of stress or isoproterenol on size and frequency of tumor cell dissemination and metastasis, and differences in gene expression levels. Data are presented as mean ± standard error. To determine the effect of stress on the longitudinal growth trajectory of tumors, and whether those effects were modified by pharmacological interventions that targeted β-adrenoceptors, we examined the stress × treatment interaction term in a 2 (control vs stress) × 2 (treatment vs placebo) experimental design in the context of mixed-effects linear model analysis (Demidenko, 2004). Data were analysed according to the model: \( y_{ij} = \alpha + \beta_1t + \beta_2d + \beta_3d \times t + \beta_4d \times t^2 + \epsilon_{ij} \) where: \( y \) is the tumor-specific luciferase activity for the ith mouse on the logarithmic scale, \( t \) is the time (days of followup), \( \alpha \) is the intercept parameter, \( \beta \) is the common growth rate parameter, \( d \) (j = 1, …, 3) are binary variables so that \( d_1 = 1 \) if the ith mouse belongs to the jth group and 0 otherwise; \( \beta_4d \times t^2 \) are independent mouse-specific random effects, which we assume to be normally distributed; and \( \epsilon \) is the error term. Estimated parameters are presented in Supplementary Table 1 where Model 1 (Fig. 3C) and Model 2 (Fig. 1C) have the same structure but Model 2 sets \( \beta_2 = \beta_3 = 0 \) for analysis of two (control and stress) groups. Parameter estimates were computed in R programming environment using the package nlme (Pinheiro and Bates, 2000).

3. Results

3.1. Chronic stress increases pancreatic cancer progression

To assess the effects of chronic stress on cancer progression within the pancreatic microenvironment, we used bioluminescence imaging to monitor primary tumor growth and metastatic dissemination in an orthotopic mouse model of human pancreatic cancer. To investigate the effect of chronic stress in the context of crossstalk between cancer cells and pancreatic stromal cells, luciferase-tagged Panc-1 cells were injected into the pancreas by laparotomy (Chai, 2013). Chronic stress was induced by subjecting mice to repeated daily restraint (Fig. 1A), which up regulates adrenergic stress response pathways as indicated by weight loss and increased tissue catecholamine levels (Supplementary Fig. 1) (Thaker, 2006). Longitudinal analysis found that stress increased the rate of pancreatic tumor growth by 10.92% ± 3.07 per day compared to mice maintained in their home cage (p < .001) (Fig. 1B). The effect of stress on primary tumor bioluminescence was apparent by day 21 after tumor cell injection and resulted in >10-fold increased tumor-specific bioluminescence at day 42 (p < .001) (Fig. 1B and C).

Pancreatic cancer morbidity and mortality is induced by primary tumor growth, tumor cell invasion of adjacent normal pancreas which may seed recurrence after resection, as well as metastatic dissemination to distant organs. To investigate the effect of stress on each of these contributors to disease progression, we evaluated primary tumor mass, tumor cell dissemination into adjacent normal pancreas and frequency of distant metastasis. Chronic stress increased primary tumor mass by 5-fold (7.5 mg ± 5 vs. 41 mg ± 13; p = 0.03) (Fig. 1D). To investigate the effect of stress on tumor cell dissemination beyond resection margins and into surrounding normal pancreas, the primary tumor was identified in the pancreatic tail adjacent to the spleen (Fig. 2A, left panel) and resected with 2 mm margins. R0 resection margins were confirmed by bioluminescence imaging (Fig. 2A, middle panel). The remaining pancreas was imaged ex vivo to identify disseminated luciferase-positive tumor cells (Fig. 2A, middle panel). Stress increased bioluminescence in adjacent normal pancreas by >10-fold (p = 0.004) (Fig. 2B). Local invasion of tumor cells in the pancreatic microenvironment was confirmed by histology (Fig. 2A, right panel). In addition to effects within the pancreas, stress induced metastatic dissemination of tumor cells to distant organs in 50% of mice compared to control where no distant metastasis was detected (Figs. 2C and 1B). Bioluminescence imaging and histological analyses confirmed the presence of liver metastasis (Fig. 2D), which also occurs in patients with pancreatic cancer (Michalski, 2008). These findings indicate that chronic stress promotes pancreatic cancer progression by accelerating primary tumor growth and dissemination of tumor cells within the pancreas and by inducing metastatic colonization of distant organs.

3.2. Beta-blockade slows pancreatic cancer progression

Chronic stress was found to accelerate progression of other solid tumor types and hematological malignancies through β-adrenergic signaling pathways (Sloan, 2010; Thaker, 2006; Lamkin, 2012; Ben-Eliyahu, 1999; Madden et al., 2011; Schuller, 2010). To investigate the role of β-adrenergic signaling in pancreatic tumor growth and dissemination, mice were treated with the nonselective β-blocker propranolol to block signaling through β-adrenoceptors. LC–MS analysis confirmed systemic exposure of propranolol was successfully maintained throughout the treatment period, with an average propranolol plasma concentration 41 ng/ml (range: 27–76 ng/ml). Consistent with previous findings...
Chronic stress increased pancreatic tumor progression. (A) Mice were exposed to chronic stress (daily restraint) vs home cage control conditions for 2 h per day for 28 days commencing 7 days prior to tumor cell injection. Where described, mice were treated with β-blockers for the duration of the experiment. (B) Primary tumor size was measured over time by non-invasive bioluminescence imaging. Luciferase activity: \( \times 10^6 \) p/s. Inset shows increased resolution over days 0–21, luciferase activity: \( \times 10^8 \) p/s. (C) Representative images of tumor-specific bioluminescence in mice exposed to control vs stress conditions. Luminescence scale: p/s/cm²/sr. (D) Primary tumor mass at 42 days after tumor cell injection. Data shown are representative of three replicate experiments.

**Fig. 1.** Chronic stress increased pancreatic tumor progression. (A) Mice were exposed to chronic stress (daily restraint) vs home cage control conditions for 2 h per day for 28 days commencing 7 days prior to tumor cell injection. Where described, mice were treated with β-blockers for the duration of the experiment. (B) Primary tumor size was measured over time by non-invasive bioluminescence imaging. Luciferase activity: \( \times 10^6 \) p/s. Inset shows increased resolution over days 0–21, luciferase activity: \( \times 10^8 \) p/s. (C) Representative images of tumor-specific bioluminescence in mice exposed to control vs stress conditions. Luminescence scale: p/s/cm²/sr. (D) Primary tumor mass at 42 days after tumor cell injection. Data shown are representative of three replicate experiments.

3.3. Beta-adrenergic signaling regulates pancreatic cancer cell invasion

To investigate if β-adrenergic signaling is sufficient to increase pancreatic cancer progression, mice were treated with β-adrenoceptor agonist isoproterenol vs vehicle control. Isoproterenol increased the rate of primary tumor growth (Fig. 4A and B), resulting in 1.9-fold increase in primary tumor mass at 42 days after tumor cell injection \((p = 0.04)\) (Fig. 4C). Isoproterenol treatment increased tumor cell dissemination into the adjacent pancreas by 3.9-fold \((p = 0.004)\) (Fig. 4D), and induced metastatic dissemination to distant organs in 50% of mice (Fig. 4E). These findings indicate that pharmacological β-adrenoceptor activation is sufficient to accelerate pancreatic cancer progression and show that β-adrenergic signaling is critical for the effects of chronic stress on primary tumor growth and tumor cell dissemination to the surrounding pancreatic microenvironment.
**Supplementary Fig. 3B**, suggesting that pancreatic cancer cells are responsive to β-adrenoceptor signaling. However, despite functional receptor coupling to downstream signaling pathways, isoproterenol treatment did not modulate proliferation of cultured tumor cells ([Supplementary Fig. 4]). This indicates that β-adrenoceptor signaling is insufficient to increase pancreatic cancer cell proliferation and suggests that the effects of stress on primary tumor growth in vivo (Fig. 1C) may require additional factors (e.g. β-adrenergic regulated stromal-derived growth factors) ([Schuller and Al-Wadei, 2010; Zhang, 2010; Schuller, 2007; Askari et al., 2005; Chan et al., 2008]).

To investigate if β-adrenoceptor signaling directly to tumor cells is sufficient to modulate tumor cell invasion, cells were treated with isoproterenol and the effects on expression of invasion related genes and on basement membrane invasion were assayed. Isoproterenol induced modest expression of genes involved in tumor cell invasion including matrix metalloprotease 2 (MMP2) and MMP9 (2-fold to 4-fold increase, \( p < 0.01 \)), and this was associated with increased invasion through Matrigel (\( p < 0.01 \)) ([Fig. 5D]). Tumor cell invasion was blocked by propranolol, indicating a requirement for β-adrenoceptor signaling. However, in contrast to the modest effects observed in cultured tumor cells, chronic stress significantly up regulated expression of invasion genes in the pancreatic microenvironment. Species-specific qRT-PCR analyses found that stress preferentially up-regulated tumor cell MMP9 expression in primary pancreatic tumors (54-fold increase vs. control, \( p < 0.001 \)) and stromal cell MMP2 expression (>100-fold increase, \( p < 0.01 \)) in primary pancreatic tumors ([Fig. 5E]), consistent with patterns of MMP expression observed in pancreatic tumors from patients ([Maatta, 2000]). Collectively, these findings suggest that β-adrenoceptor signaling directly to pancreatic cancer cells may impact invasion, and emphasize the importance of the pancreatic microenvironment in regulating tumor cell proliferation and invasion.

**4. Discussion**

These studies found that chronic stress acts through β-adrenergic signaling pathways to increase pancreatic tumor growth and invasion. In the context of the pancreatic microenvironment, β-adrenergic signaling accelerated growth of primary pancreatic tumors and significantly enhanced tumor cell dissemination through adjacent normal pancreas and to distant organs. Even with potentially curative R0 resection, prognosis of patients with pancreatic cancer is exceptionally poor and autopsy results suggest that tumor recurrence approaches 100% ([Hishinuma, 2006; Sperti, 1997; Takahashi, 1995]). These findings raise the possibility that chronic stress may contribute to pancreatic tumor recurrence by facilitating dissemination of tumor cells into adjacent normal pancreas where they may seed recurrent tumor growth and metastasis even after resection of the primary tumor. Pharmacologic blockade of β-adrenergic signaling with propranolol stopped tumor cell invasion of adjacent pancreas, which suggests that β-blockers may complement existing chemotherapeutic strategies to slow or prevent pancreatic tumor growth and invasion, and improve survival of patients with pancreatic cancer.

Beta-adrenergic regulation of primary pancreatic tumor growth contrasts with neural regulation of other solid tumor types including breast cancer where β-adrenoceptor signaling did not in-
crease primary tumor size but selectively accelerated metastatic dissemination (Sloan, 2010; Madden et al., 2011; Perez Pinero, 2012). Pancreatic tumors frequently arise in the head of the pancreas where their growth may obstruct the bile duct and pancreatic duct, leading to jaundice, pruritus and liver metastasis (Bond-Smith, 2012). This suggests that β-adrenergic regulation of primary pancreatic tumor growth – in addition to its effects on tumor cell invasion and dissemination – may contribute to the morbidity and mortality associated with pancreatic cancer. The physiological mechanisms for these differential effects on primary tumor growth.

Fig. 4. Beta-adrenergic signaling is sufficient to accelerate pancreatic cancer progression. (A) Tumor progression was tracked using non-invasive bioluminescence imaging in mice treated with isoproterenol (iso) vs. placebo (control). Y-axis: fold change over day 0 luciferase activity. (B) Representative images of tumor-specific bioluminescence in mice treated with iso vs control. Luminescence scale: p/s/cm²/sr. (C) Primary tumor mass was determined on day 42 after tumor cell injection in mice treated with iso vs control. (D) The magnitude of tumor cell dissemination into pancreas adjacent to the primary tumor was quantified by ex vivo imaging after surgical resection of the primary tumor. Luciferase activity: x10⁸ p/s. (E) The frequency of metastasis-bearing mice was determined at 42 days after tumor cell injection.

Fig. 5. Beta-adrenergic signaling induced pancreatic cancer cell invasion. (A) Immunostaining for β1-adrenoceptor (β1AR) and β2-adrenoceptor (β2AR, red) on Panc-1 pancreatic cancer cells (Upper and middle panel, blue: DAPI, inset: isotype control, scale bar: 5 μm) and archived human pancreatic cancer (Lower panel, scale bar: 100 μm). (B) Quantitative RT-PCR analyses of ADRB1 and ADRB2 expression on Panc-1 cells, normalized to RPL30 expression. (C) cAMP accumulation in Panc-1 cells presented as percent relative to maximal stimulation by 10 μM forskolin. (D) Matrigel invasion by Panc-1 cells treated with increasing concentration of isoproterenol (0, 0.01–10 μM), or with 10 μM propranolol ± 10 μM isoproterenol. (E) Quantitative RT-PCR analyses of tumor cell (T) or stromal cell (S) MMP2 and MMP9 expression in primary pancreatic tumors.
are unclear but may reflect how stress signals are delivered to the tumor microenvironment and which stromal cells are responsive to those signals. In the context of breast cancer, chronic stress
modulates the tumor microenvironment by recruiting M2 macrophages to primary tumors, which supports a switch to pro-meta-
static gene expression (Sloan, 2010; Madden et al., 2011; Perez
Pinero, 2012). Unlike breast, pancreas is densely innervated by
SNS fibers, and it is possible that direct neurotransmitter activation
of pancreatic stromal cell types such as pancreatic stellate cells
may impact primary tumor growth. Pancreatic stellate cells are
fibroblast-like cells specific to the pancreas that contribute to
inflammation and tumorigenesis (Vonlaufen, 2008; Mace, 2013).
Pancreatic stellate cells produce cytokines and growth factors
and induce a desmoplastic reaction that has been implicated in
chemoresistance (Apte, 2013). Pancreatic stellate cells are closely
related to hepatic stellate cells, which express β-adrenoceptors
and are sensitive to catecholaminergic neurotransmitter signaling
(Sigala, 2013). The effect of β-adrenoceptor signaling on pancreatic
stellate cells is yet to be investigated and may provide insight to
the effects of chronic stress on primary pancreatic cancer growth.
To fully understand the impact of chronic stress on pancreatic
cancer progression, it will be important to further investigate the
effects of β-adrenergic signaling to tumor cells versus stromal cells
in the pancreatic microenvironment. Matrix metalloproteases
facilitate tumor cell invasion and contribute to pancreatic cancer
progression. MMP2 and MMP9 are differentially regulated by pan-
creatic tumor cells and pancreatic stromal cells in patient samples
(Maatta, 2000). However the physiological factors that modulate
pancreatic cancer MMP expression are unknown. Our findings
identify chronic stress as a novel regulator of MMP expression,
which selectively up-regulated MMP9 in tumor cells and MMP2
in stromal cells in the pancreatic microenvironment. These find-
ings suggest beta-blockade as a pharmacological intervention to
limit expression of invasive genes and to prevent pancreatic cancer
cell dissemination (Fig. 3D).

Use of an orthotopic model of pancreatic cancer allowed the first investigation of the effects of chronic stress on tumor develop-
ment and progression in the pancreatic microenvironment. In con-
trast, interpretation of previous studies that investigated pancreatic tumor growth in flank was limited by the context of non-
physiological intercellular interactions (Lin, 2012; Al-Wadei,
2012; Schuller, 2011; Al-Wadei et al., 2009). To better understand
β-adrenergic regulation of pancreatic cancer onset it will be impor-
tant to investigate the effects of chronic stress in transgenic models
that spontaneously develop pancreatic cancer (Herreros-Villanueva,
2012). Modified study design will also be required to investigate β-
adrenergic regulation of pancreatic cancer metastasis. Both physi-
ologic and pharmacologic β-adrenergic activation induced meta-
tasis (Figs. 2C and 4E). However, β-blockade did not modulate this
effect in the short (six week) timeframe of these studies. This study
design was chosen to focus on events that occur early in tu-
mor development as studies of other tumor types suggest that this
time point is sensitive to β-adrenergic signaling (Sloan, 2010; Tha-
ker, 2006; Lamkin, 2012). However, this design limited investiga-
tion of metastatic dissemination from pancreas to distant organs
as few metastases arose during the six week timeframe of tumor
growth. In future studies, a modified design that includes surgical
resection of the primary tumor and longitudinal imaging follow up
of tumor recurrence and metastasis would better facilitate assess-
ment of β-adrenergic regulation of tumor recurrence and metaста-
sis (Chai, 2013).

As primary tumor growth and tumor cell invasion of surround-
ing pancreas impact patient survival, the findings of the current
study have important implications for management of chronic stress
in patients with pancreatic cancer. Diagnosis of pancreatic
cancer is associated with high levels of distress (Carlson, 2004;
Zabora, 2001), and beta-blocker treatment may be one strategy
to reduce distress (Lindgren, 2013). Findings presented here sug-
gest that in addition to improving quality of life, therapeutic inter-
vention of β-adrenergic stress response pathways might also affect
cancer progression. To translate these findings it will be important
to prospectively investigate the effect of β-blockade on disease
progression in pancreatic cancer patients and to define the patient
populations that will optimally benefit from adjuvant beta-blocker
therapy.

Acknowledgments

The authors thank Mr Ming Chai for preparing the cell lines, Dr
Kouki Morizono for luciferase expression vectors, Ms Anna Winter
for human tissue staining, Dr Anna Cook and Dr Chris Langmead for
guidance with cAMP assays, Dr Francis Chiou for UPLC-MS analysis
and Dr Sarah Creed, Dr Jonathan Hiller and Dr Phoebe Phillips for
thoughtful discussion of this research. This work was supported by
the Australian National Health and Medical Research Council (1008865, 1049561), the Australian Research Council (LE1110100125), and the National Cancer Institute (CA160890). Dr Corina Kim-Fuchs is supported by a fellowship from the Swiss Cancer League and an HDR scholarship from Monash University. Ms Caroline Le is supported by an Australian Postgraduate Award and a PhD scholarship from the Co-operative Research Centre for Cancer Therapeutics. Dr Eliane Angst is supported by a grant from the Bern Cancer League. Dr Erica Sloan is supported by an Early Ca-
reer Fellowship from the National Breast Cancer Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in
the online version, at http://dx.doi.org/10.1016/j.bbi.2014.02.019.

References

Al-Wadei, H.A. et al., 2012. Colecxib and GABA cooperatively prevent the
progression of pancreatic cancer in vitro and in xenograft models of stress-
free and stress-exposed mice. PLoS ONE 7 (8), e43786.
pancreatic cancer xenografts by systemic increase in stress neurotransmitters
and suppression of the inhibitory neurotransmitter gamma-aminobutyric acid.
Carcinogenesis 30 (3), 506–511.
Apte, M.V. et al., 2013. A starring role for stellate cells in the pancreatic cancer
microenvironment. Gastroenterology 144 (6), 1210–1219.
Askari, M.D., Tsao, M.S., Schuller, H.M., 2005. The tobacco-specific carcinogen, 4-
(methyltriazolomino)-1-(3-pyridyl)-1-butanal stimulates proliferation of
immortalized human pancreatic duct epithelia through beta-adrenergic
Ben-Eliyahu, S. et al., 1999. Evidence that stress and surgical interventions promote
tumor development by suppressing natural killer cell activity. Int. J. Cancer
80 (6), 880–888.
Bilimoria, K.Y. et al., 2007. National failure to operate on early stage pancreatic
Carlson, L.E. et al., 2004. High levels of untreated distress and fatigue in cancer
Chai, M.G. et al., 2013. Bioluminescent orthotopic model of pancreatic cancer
growth. J. Vis. Exp. (76).
proliferation and IL-6 levels of human pancreatic duct epithelial cells and can be
inhibited by the dietary agent, sulforaphane. Int. J. Oncol. 33 (2), 415–419.
Conroy, T. et al., 2011. FOLIRINOX versus gemcitabine for metastatic pancreatic
Hoboken, New Jersey.
Farrow, B., Albo, D., Berger, D.H., 2008. The role of the tumor microenvironment in
(16), 4266–4276.

Please cite this article in press as: Kim-Fuchs, C., et al. Chronic stress accelerates pancreatic cancer growth and invasion: A critical role for beta-adrenergic
C. Kim-Fuchs et al. / Brain, Behavior, and Immunity xxx (2014) xxx–xxx


Sigala, B. et al., 2013. Sympathetic nervous system catecholamines and neuropeptide Y neurotransmitters are upregulated in human NAFLD and modulate the fibrogenic function of hepatic stellate cells. PLoS ONE 8 (9), e72928.


