Pancreatology

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Pancreatology

Characterisation of Kinase Signaling Networks in Pancreatic Cancer by Phosphoproteomics

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Background: Despite extensive research, the key molecular defects that play a causal role in the development of pancreatic cancer (PC) and its progression to a highly metastatic state remain unknown. The central role of deregulated tyrosine kinase (TK) signaling in oncogenesis is widely documented, with over 30 receptor TKs implicated in human cancers. A detailed characterisation of TK signaling networks in PC may therefore identify novel disease phenotypes with associated therapeutic targets.

Methods: Mass spectrometry (MS)-based phosphoproteomics provides strategies for profiling the tyrosine phosphoproteome of cancer cells. A panel of 20 human PC cell lines and primary patient-derived xenografts were profiled for tyrosine-phosphorylated proteins by an immunoaffinity/LC-MSMS approach using an Orbitrap Velos. Spectra were quantified using the analysis tool MaxQuant and unsupervised hierarchal clustering undertaken to classify the cell lines and xenografts into subgroups based upon TK signalling.

Results and Conclusions: Phosphoproteomic profiling of these cells and xenografts identified 1300 tyrosine phosphorylation sites and 680 tyrosine phosphorylated proteins. Clustering revealed 3 major subgroups amongst PC cell lines with distinct tyrosine phosphorylation profiles. In particular, group 1 was characterised by the activation of the TKs HER3, EGFR, MET and RON and group 2 by the phosphorylation of tumour suppressor Syk, as well as a large network of hnRNP RNA binding proteins. Preliminary results indicate that subgroup 1 members have selective upregulation of ErbB signalling, and greater sensitivity to EGFR inhibition by Erlotinib. These lines also exhibit increased sensitivity to BEZ235, an inhibitor of PI-3 kinase, which couples HER3-mediated survival signals to AKT. Drug synergy assays are currently being performed to determine whether combinations of therapeutic agents improve response in those subgroups determined to show sensitivity to single agent therapy.

Collagen I – Increases the Expression of Transgelin and Lumican (Regulators of Cell Migration) by Activated Pancreatic Stellate Cells

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Activated pancreatic stellate cells (PSCs) play a central role in pancreatic fibrosis, a feature of chronic pancreatitis and pancreatic cancer. The fibrous matrix produced by PSCs can, in turn, regulate PSC function. Using microarrays, we have previously demonstrated that 146 genes were dysregulated (fold change>2, $p<0.001$, $q<0.25$, $n\geq3$) in PSCs cultured on collagen-I (mimics fibrotic pancreas) versus Matrigel™ (mimics normal pancreatic basement membrane). Interestingly, genes for transgelin and lumican (which code for proteins known to regulate cell migration) were upregulated in PSCs cultured on collagen-I compared to Matrigel.

Aim: To assess transgelin and lumican mRNA and protein expression in: i) PSCs cultured on matrigel versus collagen-I; ii) quiescent PSCs vs PSCs cultured on plastic.

Method: Transgelin and lumican expression was assessed by real-time PCR and western blotting in quiescent rat PSCs (24 hours after isolation or grown on Matrigel™ and activated PSCs (cultured on collagen-I for 72h or on plastic); $n=5$ preparations).

Results: Transgelin and lumican mRNA levels were upregulated by i) 45.5 and 24.7 fold ($^*p<0.05$) respectively in PSCs cultured on collagen-I vs Matrigel™. and ii) 20 and 4.2 fold ($^*p<0.05$) respectively in plastic-activated PSCs vs quiescent cells. Transgelin protein expresion was also increased (% of control [mean ± SE]: 295.7 ± 28.92, $^*p<0.05$) in activated PSCs compared to quiescent PSCs.

Conclusions: Transgelin and lumican are significantly increased in PSCs cultured on fibrous ECM and associated with PSC activation.

Implication: Characterisation of genes that may play a role in PSC transformation may allow the identification of specific therapeutic targets for the treatment of fibrosis.

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Silencing Heat Shock Proteins 27 and 47 Inhibit Platelet-Derived Growth Factor (PDGF)-Induced Pancreatic Stellate Cell Proliferation: Implication in Pancreatic Cancer Progression

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Heat shock proteins (HSPs) can regulate cell migration and proliferation and are known to be overexpressed in human pancreatic cancer (PC) tissue. The stromal reaction of PC is produced by activated pancreatic stellate cells (PSCs) which interact with PC cells to facilitate cancer progression. Growth factors (including PDGF) produced by tumour cells induce proliferation and migration of PSCs. We have also previously shown that PDGF induces the expression of several HSPs (27, 47, 70 and 90) in human cancer associated-PSCs (CA-hPSC). However, little is known about the role of HSPs in PSC function.

Aim: To determine the effect of silencing HSPs (27, 47, 70 or 90) in CA-hPSCs on cell migration and proliferation.

Methods: CA-hPSC were isolated from resected pancreatic tissue from PC patients and treated (n=4) with siRNA targeting HSP27 (protein 1 or 2), HSP47, HSP70, HSP90 (>90% protein silencing achieved) or non-silencing siRNA (ns-siRNA). 48h post transfection CA-hPSCs were incubated ± PDGF (10ng/ml) for 48h. Cell proliferation assessed by the cell counting kit-8 and migration assessed using modified Boyden chambers with PDGF as a chemotactic agent.

Results: PDGF stimulated CA-hPSC proliferation and migration in ns-siRNA treated PSCs. Notably, silencing HSP27 (p1 and p2) or HSP47 (but not HSP 70 or 90) inhibited PDGF-induced proliferation (% of ns-siRNA without PDGF: ns-siRNA+PDGF 154.7±5.9#, HSP47 siRNA+PDGF 115.1±9.3*, HSP27(p1)-siRNA+PDGF 125.0±12.4*, HSP27(p2)-siRNA+PDGF 127.5±3.2*; #p<0.001 vs ns-siRNA; *p<0.01 vs ns-siRNA+PDGF). However, silencing HSP27 (p1 and p2) and HSP47 had no effect on basal or PDGF-induced PSC migration.

Conclusion: This is the first study to show that suppression of HSP27 or HSP47 inhibits PDGF-induced PSC proliferation.

Implication: Modulation of HSPs in PSCs may represent a novel approach to influence PSC function and PSC interactions with cancer cells.

Serum Apolipoprotein C-II is a Prognostic Marker for Long-Term Survival after Pancreatic Resection for Adenocarcinoma

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Objective: To examine the serum proteome for preoperative prognostic biomarkers to predict survival in pancreatic ductal adenocarcinoma (PDAC).

Methods: Forty training serum samples from preoperative patients were analysed using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI) and protein peaks were evaluated for a predictive model of survival using 6-fold cross-over logistic regression. This model was validated on 21 independent samples. Two predictive proteins were identified by tryptic peptide mass fingerprinting and sequencing. ELISA measures of these proteins were tested for prediction of survival on 57 independent Validation-2 samples, along with clinico-pathological measures using Kaplan-Meier and Cox proportional hazard analysis methods. The influences of these proteins on growth and invasion of cancer cell lines was tested in-vitro.

Results: The SELDI panel of 3,700, 8,222 and 11,522 *m/z* peaks predicted >12 months survival (ROC AUC: 0.79, 0.64–0.90; $P<0.039$). Addition of CA19-9 increased the ROC AUC to 0.95 (0.84–0.99; $P<0.0001$) although this increase was not significant ($P=0.11$). The *m/z* 8,222 and 11,522 proteins were identified as Serum Apolipoprotein (ApoC-II) and Amyloid A-1 (SAA-1). Validation-2 ELISA measures of ApoC-II were predictive of survival (Kaplan-Meier $P<0.009$) but were not for SAA-I. ApoC-II, CA19-9 and major vessel involvement predicted survival. Cox Proportional Hazard analysis confirmed their independent influence. *In vitro* pancreatic cancer cell line studies indicated that both ApoC-II and SAA-1 increased cell growth and invasion.

Conclusion: Serum levels of ApoC-II in combination with CA19-9 and major vessel invasion independently predict survival and could help decide the benefit of surgical resection.

Drug-Induced Acute Pancreatitis in a Cohort of 328 Patients – A Single-Centre Experience from Australia

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Background: Acute pancreatitis (AP) is associated with risk of morbidity and even mortality. Routine prescription drugs have been linked to the causation of AP.

Aim: To determine the incidence, presentation, course and outcome of drug-induced AP amongst patients admitted to a public hospital.

Methods: A retrospective analysis of patients presenting with AP to the Modbury hospital, South Australia from January 2006 to April 2011. Each admission was reviewed within the electronic database for patient details as well as to determine the aetiological factor. In patients with drug-induced AP, the WHO Probability Scale was used to evaluate causality relationship.

Results: 328 patients were treated for AP during the study period. Biliary and alcohol-induced AP accounted for 80% of cases. 11 patients (2 male and 9 female patients; median age: 59 years) were diagnosed with drug-induced AP. These included 5 cases of codeine-, 2 cases of azathioprine-, and 1 case each of chlorthiazide-, valproic acid-, oestradiol- and rosuvastatin-induced AP. 9 patients had a mild disease while 2 patients had severe AP with a median hospital stay of 4 days. Withdrawal of the drug resulted in cessation of the attacks in all patients over a median follow-up of 24 months.

Conclusions: Routine prescription drugs, as an aetiological factor, accounted for 3.3% of cases of AP. The disease appeared to be more common in middle-aged women. It is likely that the overall incidence of this entity is under-reported owing to the stringent criteria needed to conclusively determine a causal relationship.

Mechanisms Underlying feG in the Prevention of Injury in a Rodent Model of Acute Pancreatitis-Associated Lung Injury

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The salivary tripeptide feG is a novel anti-inflammatory derivative that we have shown to prevent injury in animal models of acute pancreatitis (AP). Acute lung injury (ALI) is a common complication of AP and contributes to the majority of associated deaths. We previously demonstrated prophylactic and therapeutic efficacy of feG as an agent to resolve ALI in a clinically relevant "two-hit" AP-LPS lung injury model. However, the specific mechanisms underlying feG's actions have not been fully identified.

Aim: To determine the effect of feG on inflammatory mediator expression in the lung.

Methods: Total RNA from rat lungs of "two-hit" AP-LPS ALI with or without prophylactic feG treatment was isolated (Trizol) before reverse transcription to single-stranded cDNA (Applied Biosystems high capacity kit). Quantitative gene expression from cDNA was performed using Applied Biosystems TaqMan Express Plates. Amplification data, gene functions and expression relationships were analysed using RQ Manager 1.2, IPA 9.0 and GNCPro.

Results: Twenty-one genes of interest decreased in expression in feG treated rats. Relationship analysis confirmed the important integrated roles in inflammatory cell trafficking via adhesion, infiltration and activation of these genes. However, functional correlation between co-expressed genes indicates that this may be an indirect function of feG in some cases. Of particular importance were phospholipase A2, vascular cell adhesion molecule-1, and integrin alpha-L and beta-2.

Conclusion: In the context of ALI following AP-LPS, feG appears to downregulate various genes that stimulate inflammatory mediator release and inflammatory cell chemotaxis, activation and migration during injury.

Can Serum Pancreatic Enzyme Levels Predict Severity of Acute Paediatric Pancreatitis?

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Background: Paediatric pancreatitis is poorly understood and recognised. Adult predictive systems for severe acute pancreatitis (AP) require information not readily collected/available, especially when the diagnosis of pancreatitis is unsuspected.

Table 1. Summary of statistically significant early laboratory parameters in children with mild and severe pancreatitis. *Values recorded within the first 24 hours of admission; §peak lipase/amylase values recorded; ‡Values recorded within the same day as peak lipase result. Values are recorded as median (range) or mean (SD). Adm, admission (Coffey and Ooi)

Laboratory Parameter	Timing	Mild	Severe	*P*-value
Lipase (U/L)	0 to 24-h (Adm)*	274 (177–740)	922 (400–2148)	0.0018
	Peak§	300 (181–893)	1040 (466–1980)	0.0007
Amylase (U/L)	0 to 24-h (Adm)*	312 (198–672)	726 (240–1430)	0.0469
	Lipase-Peak‡	332 (249–672)	746 (277–1300)	0.0278
	Peak§	322 (221–756)	811 (305–1439)	0.0240
White Cell Count (x 10^9/L)	0 to 24-h (Adm)*	10.4 (0.7)	13.9 (1.5)	0.0162
	Lipase-Peak‡	10.5 (0.6)	13.9 (1.5)	0.0176
Neutrophils (x 10^9/L)	0 to 24-h (Adm)*	7.6 (0.6)	10.9 (1.6)	0.0311
	Lipase-Peak‡	7.3 (0.6)	11.1 (1.6)	0.0090
Haemoglobin (g/L)	0 to 24-h (Adm)*	126.1 (3.5)	139.5 (4.1)	0.0221
	Lipase-Peak‡	123.8 (2.8)	139.3 (3.8)	0.0016
Haemotocrit	0 to 24-h (Adm)*	0.37 (0.34–0.40)	0.39 (0.37–0.43)	0.0256
	Lipase-Peak‡	0.36 (0.34–0.40)	0.39 (0.38–0.43)	0.0055
Phosphate (mmol/L)	0 to 24-h (Adm)*	1.6 (1.4–1.7)	1.3 (1.1–1.5)	0.0389

Aims: We compared laboratory results of mild and severe paediatric AP and recurrent acute pancreatitis (RAP) to identify potential predictors for severe AP.

Methods: A retrospective review (January 2000–July 2011) was performed in Sydney Children's Hospital Randwick to identify patients with AP and RAP, and their laboratory parameters were recorded. AP was defined as abdominal pain not due to other causes *plus* elevated lipase and/or amylase (>2x upper limit normal [ULN]) and/or imaging evidence of AP. AP was defined as severe if associated with: pseudocyst, necrosis, haemorrhage, abscess, death, pancreatic surgery, ICU admission, or organ dysfunction from AP ($PaO_2 < 60$, O_2 requirement or systolic blood pressure < 90 mmHg).

Results: There were 65 (87.8%) patients with AP (median age = 11.7 years [IQR = 6.0–13.8, range = 0.3–17.9]; 64.4% male) and 9 (12.2%) with RAP (median age = 9.3 years [IQR = 8.2–12.1, range = 2.7–16.8]; 44.4% male) identified. 52 (66.7%) and 26 (33.3%) were mild and severe episodes of pancreatitis respectively. Statistically significant differences in laboratory parameters between mild and severe pancreatitis within the first 24 hours of admission and at the time of peak serum lipase were noted (Table 1).

Conclusion: Both peak serum lipase and amylase, and serum lipase and amylase within the first 24 hours of admission were significantly higher in severe than mild episodes of AP and RAP in children. This observation may suggest their potential role in predicting the severity of paediatric AP.

Principal Components Analysis May Provide New Insights into the Association of Nutrient and Other Substance Intakes with Chronic Pancreatitis

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Background: Alcohol is the main aetiological factor for chronic pancreatitis in Western countries. However not all heavy drinkers succumb, suggesting moderation by co-factors such as smoking, genetic mutations and nutrient intakes. Numerous studies have sought evidence for the impact of ingested substances other than alcohol (eg. anti-oxidants) on the pathogenesis of chronic pancreatitis. This study used a statistical pattern analysis technique to explore intake associations with acute exacerbations of chronic pancreatitis.

Methods: Incident cases of acute pancreatitis were consecutively recruited as part of a prospective cohort study. Underlying chronic pancreatitis was defined by composite of clinical, morphological and biochemical criteria. Data on cofactors were collected, including smoking, alcohol intake, and diet in the 24 hours prior to acute symptom onset. The latter was analysed by Foodworks 2007™ to yield intakes of carbohydrate, fat, protein, vitamin A, vitamin C and folate. Associations were sought by univariable and multivariable analysis. The latter also included Principal Components Analysis (PCA) to reveal unforeseen patterns of intake behaviour.

Results: There were no significant differences in usual alcohol intake between those with and without chronic pancreatitis. Preceding an acute exacerbation, after adjustment for sex, indigeneity and body mass index, chronic pancreatitis patients were more likely to substitute food-based intake for combinations of other substances eg. alcohol and tobacco, or tobacco and coffee. By contrast, in standard multiple logistic regression, only intakes of fat showed a slight inverse association and coffee a slight positive one.

Conclusion: Given the complexity of interactions between nutritional and other substance intakes, PCA may yield more meaningful and realistic associations with chronic pancreatitis. Greater insight into pathogenesis may result from further work involving longitudinal data dietary data collection over a longer timeframe, and analysis of whole-food groups rather than pre-calculated micro- and macro-nutrients.

A Novel Culture System for Pancreatic Cancer Reveals Tumour Heterogeneity and May Improve Mutation Detection in Low Cellularity Pancreatic Tumours

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Pancreatic cancer (PC) is the fourth leading cause of cancer death in Western societies with a 5-year survival rate of less than 5%. There are few therapeutic options for patients with PC, and new insights into the pathogenesis of this lethal disease are urgently needed.

Advances in "-omic" technologies have led to large scale efforts in characterizing the full range of aberrations in cancer, including the International Cancer Genome Consortium (ICGC). As part of the ICGC, we aim to deeply sequence tumour and normal gDNA, survey global gene expression and determine genome wide patterns of DNA methylation of ~ 400 patients with PC. Low epithelial cellularity of pancreatic tumours presents significant challenges for sequencing studies to detect mutations in an admixture of normal and malignant epithelium.

We have developed a method to enrich the epithelial content for low cellularity tumours using xenografting and flow cytometry with antibodies to deplete stromal elements. Moreover, we have generated primary cell lines from PC xenografts that retain key aberrations identified in the resected patient samples. Our preliminary SNP-chip data suggest that increasing the amount of usable material with this method may facilitate mutation detection.

Establishment of relevant preclinical models of PC, which will be extensively characterised by the ICGC, present a valuable resource for the evaluation of novel targetable molecular mechanisms in PC.

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