P11 is a potential prognostic marker in pancreatic cancer patients and contributes to cancer cell invasion

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Abstract

Background: Despite its rarity, pancreatic cancer (PC) remains the deadliest cancer type among all cancers with a 5-yr survival rate of only 4%. PC patients are often diagnosed late at which point the cancer has spread to other organs and sabotaged their function leading to patient death.

Rationale: The Waisman group has previously shown that the plasminogen receptor p11 is important for cancers to grow and spread to other organs. In fact, p11 is highly active on invasive cancer cells and acts as a "hub" to activate proteolytic enzymes which allow cancer cells to chew their way through neighboring fibrotic stroma, invade surrounding tissues, and metastasize to distant organs. However, the involvement of p11 in PC has not been addressed.

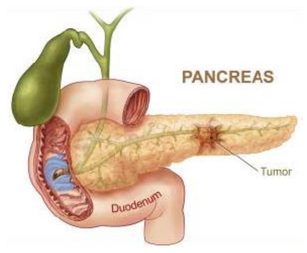
Methods: Here, the involvement of p11 in PC is being investigated using three different approaches:
1) culturing of human PC cell lines which were depleted of p11 (using RNAi technology) followed by in vitro evaluation of their ability to generate active enzymes (plasmin assays) and invade through an artificial matrix (invasion assays) compared to control cells.
2) utilizing a doxycycline-inducible PC mouse model that closely mimics the dynamics of PC in human patients.
3) examining p11 levels in tumors resected from PC patients admitted to the QEII hospital in Halifax, Nova Scotia, Canada.

Results: To examine whether p11 contributes to PC cell invasion, RNAi technology was used to deplete p11 protein levels. The results demonstrated that depletion of p11 in human PC cells hampers their ability to activate enzymes and invade by over 60%. Interestingly, p11 expression was found to be driven by oncogenic KRAS, a commonly mutated gene in PC (>95% of cases) and a well-established driver of oncogenesis. Immunohistochemical examination of pancreatic tumors isolated from the inducible mouse model revealed that p11 was highly expressed in tumorous ducts compared to normal ducts. This increase in expression was also observed in tumors isolated from two patients with invasive PC. We are currently in the process of examining a larger cohort of 96 PC samples from patients admitted to the QEII hospital from 2001 to 2011 in Halifax, Nova Scotia. Preliminary results show that p11 is variable across patients ranging from no expression to very high expression suggesting a potential prognostic role for p11 in PC.

Conclusion(s): In vitro results suggest that p11 contributes to the invasive properties of PC cells via enhancing the production of active proteases. This is further supported by the elevated p11 levels seen in mouse tumor samples as well as in patient samples with invasive PC. Currently, the full contribution of the KRas/p11 axis to PC is being studied using a larger cohort of patient samples (96 resected post-operative tumor samples). This may ultimately position p11 as a "druggable" target and a potential biomarker to predict outcome in newly diagnosed PC patients.

INTRODUCTION

I. Pancreatic cancer



5-year survival rate

Standard regimen

Diagnosis → Generalized SOC

Proposed regimen

Diagnosis → Biomarker discovery → Personalized SOC

II. The 3 steps of Cancer progression

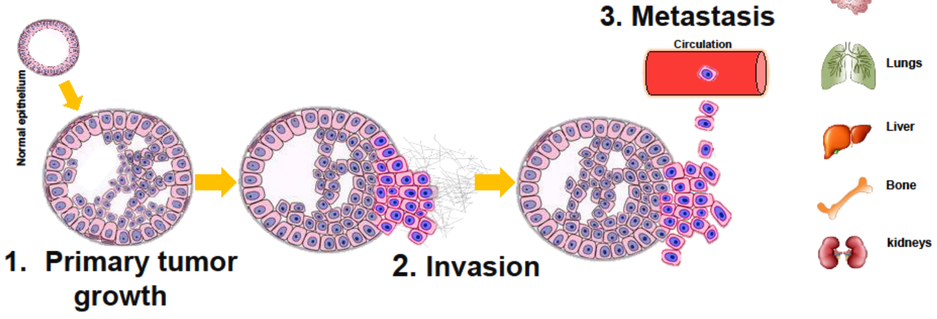
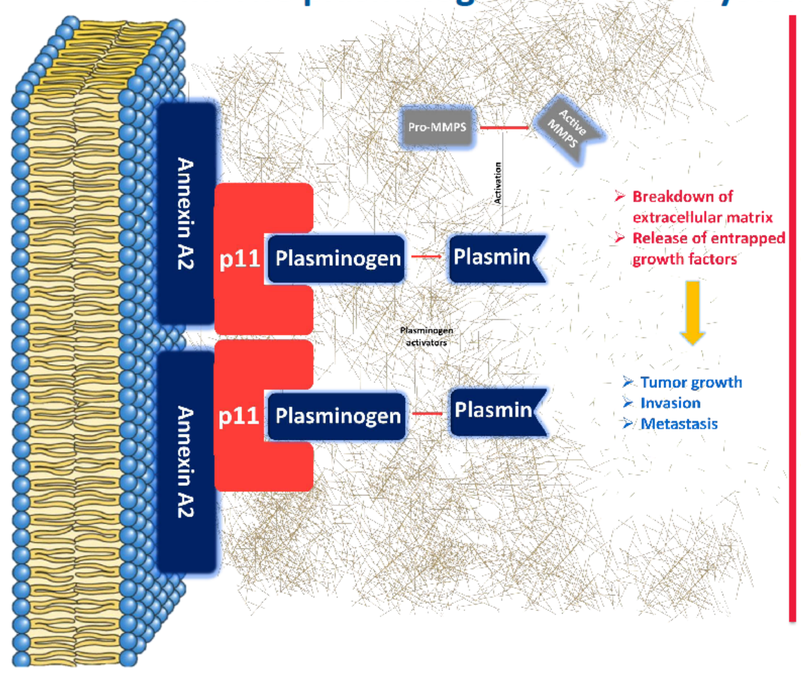


Figure 1. The three fundamental stages of tumor development. **(1)** Uncontrolled yet confined primary tumor growth. **(2)** infiltrating invasion characterized by epithelial to mesenchymal transition (EMT) and degradation/remodeling of the extracellular matrix (ECM). **(3)** Metastasis; tumor cell dissemination to vital organs.

III. The plasminogen activation system



Plasminogen binding to receptors is a **definitive step** in invasion.

Plasmin is a **major hub** in regulating cancer cell invasion and metastasis.

Figure 2. Contribution of plasminogen receptor-mediated ECM remodeling to cancer progression. The plasminogen receptor p11 binds the inactive protease plasminogen and mediates its activation into the active plasmin. The latter is a multifunctional protease that 1)cleaves extracellular matrix components, 2) releases trapped growth factors within the matrix, 3) activates pro-uPA into active uPA (urokinase plasminogen activator) (not shown), 4) activates other proteases such as pro-MMPs (matrix metalloproteinases) into active MMPs and 5) acts as a signal-transducing ligand. Active plasmin and MMPs break down impeding obstacles in the ECM, and mediate tumor cell growth, invasion, and metastasis.

RATIONALE

P11 mRNA is overexpressed in human pancreatic tumors

GSEA database:

Gene Set: GRUETZMANN_PANCREATIC_CANCER_UP

- Total number of patients: 41
- 364 genes up-regulated in pancreatic ductal adenocarcinoma (PDAC) identified in a meta analysis across four independent studies.

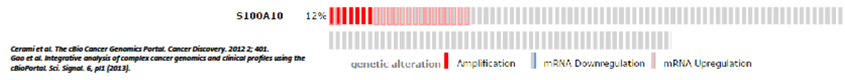
NM_002928	6004	RGS16	regulator of G-protein signaling 16
NM_002966	6281	S100A10	S100 calcium binding protein A10
NM_002997	6382	SDC1	syndecan 1
NM_003009	6415	SEPW1	selenoprotein W, 1

Grutzmann R. et al. (2005). Oncogene. 24(32):5079-88

TCGA database (cBioPortal):

Gene Set: PANCREATIC ADENOCARCINOMA (TCGA, Provisional)

- Total number of patients: 179
- P11 (S100A10) upregulated/amplified in 12% of cases.



Cerami et al. The cBio Cancer Genomics Portal. Cancer Discovery. 2012 2; 401.
Gao et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci. Signal. 6, pl1 (2013).

Expression Atlas database:

Gene Set: Transcription profiling by array of normal pancreas, primary pancreatic ductal adenocarcinoma (PDAC), normal liver, and pancreatic liver metastases using the Affymetrix HG-U133A array.

Total number of patients: 12

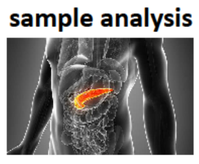
- S100A10 is upregulated in primary and liver metastasis of pancreatic ductal adenocarcinoma.

Gene name:S100A10	Gene ID:ENSG00000197747	
Gene expression analysis	Fold change	*P*-value
Liver Metastasis vs. normal liver	2.12	0.029
Primary tumor vs. normal pancreas	2.18	0.005

Barry S. et al. (2013). Clin Exp Metastasis. 30(3):251-264)

METHODS

1. **Human PC sample analysis**



2. **Animal models**

3. **Cell culture studies**

RESULTS

P11 is upregulated in tumors resected from NS pancreatic cancer patient/s.

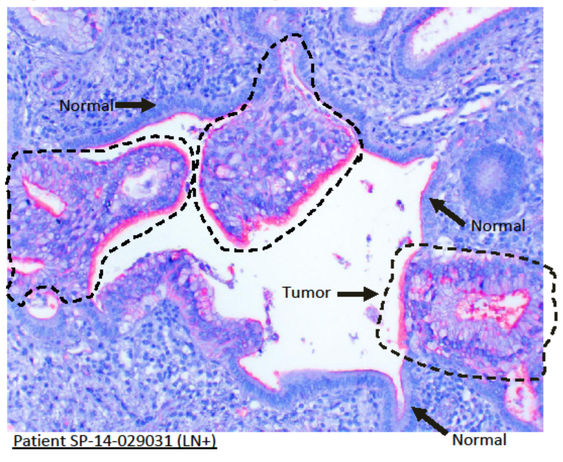


Figure 3. Immunohistochemical staining of p11 in fresh post-operative human pancreatic tumors. Immunohistochemistry staining of p11 in a fresh post-operative pancreatic tumor (Patient SP-14-029031) using the Vulcan Red chromogenic substrate.

P11 is overexpressed in tumorous pancreatic tissue compared to normal tissue or pancreatitis tissue

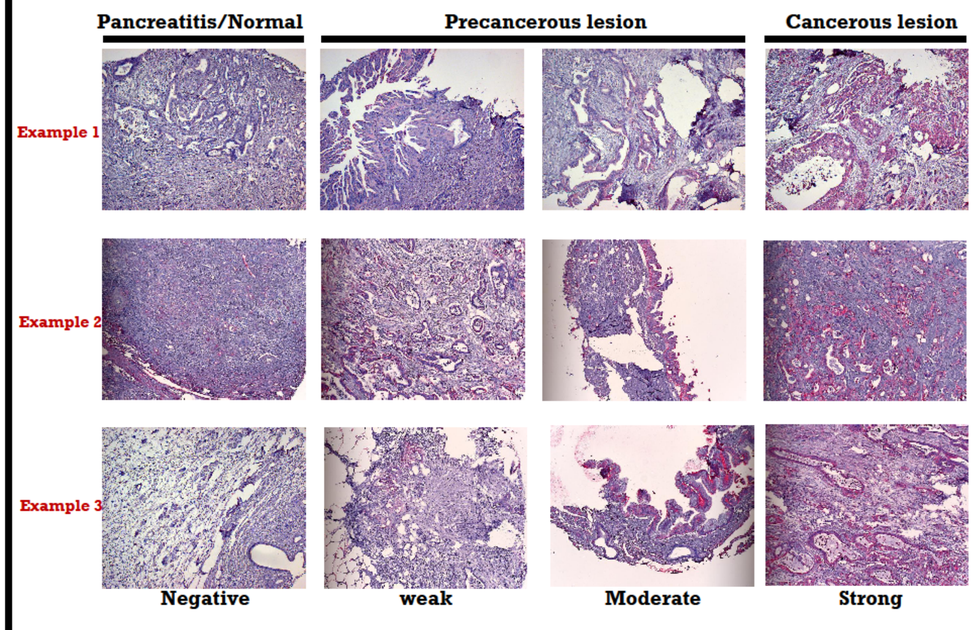


Figure 4. Immunohistochemical staining of p11 in three representative examples from frozen post-operative human pancreatic tumors. Immunohistochemistry staining of tissue microarrays of using the Vulcan Red chromogenic substrate. The expression of p11 in these tumors ranges from no staining (negative) to very intense staining (strong).

p11 depletion reduces *in vitro* invasiveness and plasmin generation of Panc-1 cells

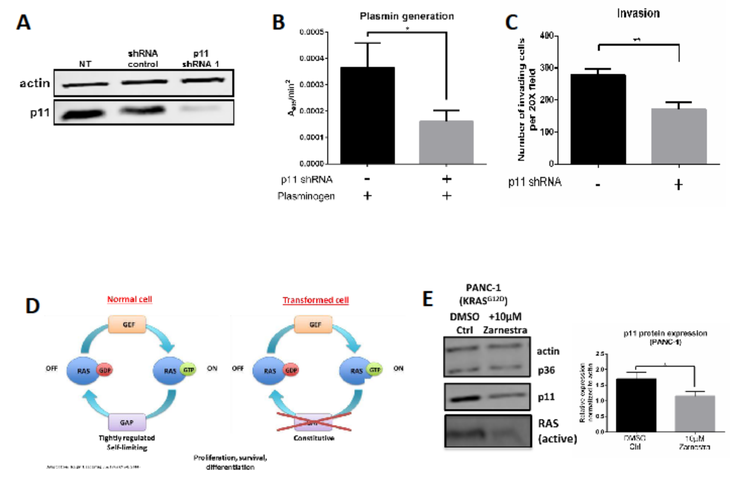


Figure 6. P11 depletion in Panc-1 cells reduces plasmin generation and *in vitro* invasiveness. (A) Western blot analysis of non-transfected (NT), shRNA control, and p11 shRNA (p11-depleted) Panc-1 cells. (B) Plasmin generation assay of control and p11-depleted Panc-1 cells; cells were incubated with 0.5μM plasminogen and plasmin-generating activity was measured using a chromogenic plasmin substrate (S2251). (C) Invasion assay of control and p11-depleted Panc-1; we used our well-established Boyden chamber assay to measure the ability of cells to invade through a Matrigel barrier (substitute for ECM) in response to a chemoattractant (20% FBS). (D) Schematic representation of wild-type and oncogenic KRAS in normal and transformed cells respectively. (E) Western blot analysis and quantification of p11 in Panc-1 cells that were treated with 10μM of the farnesyltransferase inhibitor Zarnestra for 72 hours.* $P < 0.05$, ** $P < 0.005$.

P11 is upregulated in tumors isolated from an inducible PC mouse model

Doxycycline-inducible pancreas-specific mouse model

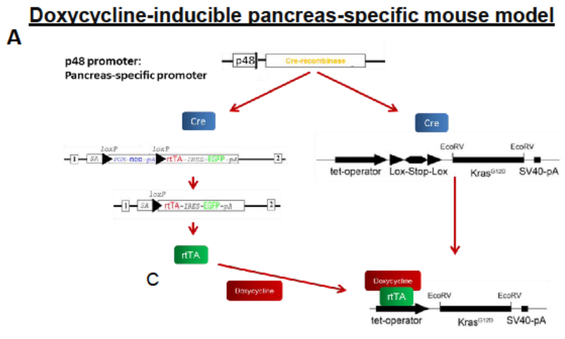


Figure 5. p11 expression and localization in tumors isolated from a spontaneous pancreas-specific mouse model. (A) Tet-On pancreatic cancer mouse model; the model utilizes the Cre-LoxP system which allows doxycycline-inducible pancreas-specific expression of K-Ras^{G12D}. The expression of the Cre recombinase is controlled by a p48 promoter allowing pancreas-specific expression of K-Ras^{G12D}. Cre also allows expression of a reverse tetracycline transactivator (rtTA) which binds to administered doxycycline and both then bind the Tet-operator (Tet-O) upstream of K-Ras^{G12D}. The G12D mutation in K-Ras is present in over 90% of human PDAC. Mice expressing K-Ras^{G12D} will develop pancreatic tumors which closely mimic human pancreatic cancer. (B) Immunohistochemical analysis of a normal pancreas vs. a pancreatic tumor.

CONCLUSIONS

1) P11 plays an important role in pancreatic cancer cell invasion.
2) P11 is upregulated and differentially localized in both human and mouse pancreatic tumors.
3) Oncogenic KRAS regulates p11 levels and increases plasmin activity.
4) P11 is a promising prognostic marker of human PC.

FUTURE DIRECTIONS

1) Finalize a p11 tissue microarray on a larger cohort of 96 PC patient samples.
2) Complete survival and clinical correlation analysis.
3) Cross iKRAS mice with p11 -/- mice and investigate the effect of p11 loss on tumor growth and/or metastasis.
4) Examine the effect of p11 depletion in KRAS^{G12D}-expressing on plasmin generation and invasiveness.
5) Decipher the molecular pathway by which oncogenic KRAS regulates p11 expression.

KRAS^{G12D} up-regulates p11 which is concomitant with increase in plasmin generation and plasmin-dependent invasion

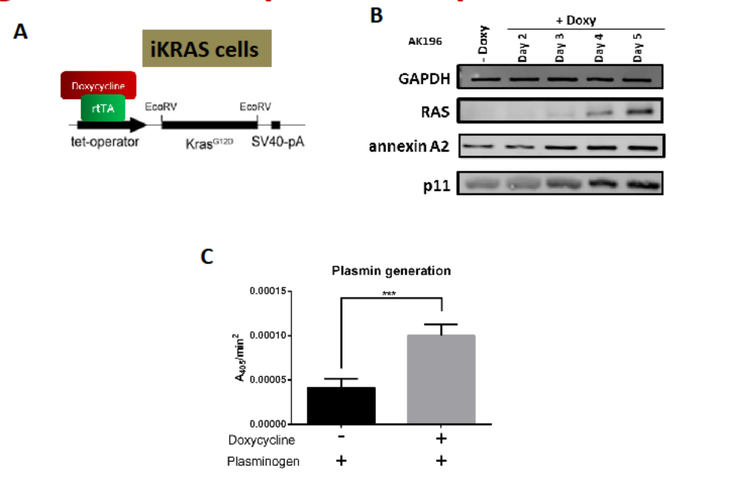


Figure 7. Inducible expression of KRAS^{G12D} upregulates p11 and annexin A2 and increases cancer cell invasiveness and their plasmin-generating capacity. (A) Genetic setup of AK196 (iKRAS) pancreatic cancer cells; rtTA is a reverse tetracycline transactivator and is required for doxycycline-inducible expression of KRAS. (B) western blot analysis of AK196 cells treated with 1μg/ml doxycycline for 5 days. (C) Plasmin generation assay of KRAS^{G12D}-expressing cells (+Doxy) and control cells (-Doxy).* $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$.

acknowledgements

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