Increased expression of plakoglobin is associated with upregulated MAPK and PI3K/AKT signalling pathways in early resectable pancreatic ductal adenocarcinoma

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Abstract. Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancer types, and it is associated with a 5-year survival rate of <10% due to limited early detection methods and ineffective therapeutic options. Thus, an improved understanding of the mechanisms involved in the early stages of PDAC tumorigenesis is crucial in order to identify potential novel diagnostic and therapeutic targets. The most common signalling aberrations in PDAC occur in the Wnt/Notch signalling pathway, as well as within the epidermal growth factor receptor (EGFR) pathway and its associated ligands, EGF and transforming growth factor-β. In addition, the RAS family of oncogenes, which act downstream of EGFR, are found mutated in most pancreatic cancer samples. Plakoglobin, a component of the EGFR signalling pathway, serves an important role in normal cell adhesion; however, its role in PDAC is largely unknown. The present study used transcriptome sequencing and focussed proteome microarrays to identify dysregulated genes and proteins in PDAC. The presence of upregulated plakoglobin expression levels was identified as a distinguishing feature between the PDAC tumour environment and normal pancreatic tissue. Furthermore, plakoglobin was demonstrated to be associated with the differential upregulation of the PI3K/AKT and MAPK signalling pathways in the tumour microenvironment, which suggested that it may serve an important role in PDAC tumourigenesis.

Introduction

Despite the overall reduction in the number of cancer-related deaths following improved therapeutic options, the number of pancreatic ductal adenocarcinoma (PDAC)-related deaths continues to increase; it is associated with a median overall survival of <1 year and <10% survival at 5 years (1). Thus, an improved understanding of the mechanisms mediating PDAC tumourigenesis is required.

Plakoglobin, also known as γ-catenin, is a member of the catenin family and is an important component of desmosomes and adherens junctions in mammalian cells, where it facilitates cell-cell adhesion by linking desmosomal cadherins to the cytoskeleton (2,3). Plakoglobin can also be found localised in the nucleus, where it exerts nuclear functions, such as inhibiting the Wnt/β-catenin signalling pathway through regulating the transcription of specific components within the pathway (4,5). Nonetheless, the role that plakoglobin serves in tumorigenesis is disputed; whilst the majority of previous studies reported that plakoglobin suppressed tumour growth, others observed that plakoglobin exhibited pro-oncogenic features (5-9). For example, the overexpression of plakoglobin in SV40-transformed 3T3 cells suppressed tumour formation upon transplantation into syngeneic mice, and upon co-transfection of plakoglobin with N-cadherin, this tumour suppressive effect was considerably more effective than plakoglobin overexpression alone. Similarly, the overexpression of plakoglobin in a human renal carcinoma cell line that lacked the expression of cadherins or β-catenin also suppressed tumour formation in nude mice (6). In addition, plakoglobin reportedly affected several downstream signalling molecules involved in tumour suppressive pathways; plakoglobin reportedly regulated the expression and stability of the metastasis suppressor protein, Nm23, as well as the p53-regulated tumour suppressor, p14-3-3σ (4,7,10). On the other hand, a recent study demonstrated that high expression levels of plakoglobin promoted metastasis in invasive micropapillary carcinoma of the breast by promoting tumour cluster formation through activating PI3K/AKT/Bcl-2 signalling (9).
As a closely related homologue of β-catenin, a crucial transcriptional regulator of the canonical Wnt signalling pathway, plakoglobin shares structural and functional similarities with β-catenin (11). It has previously been demonstrated that plakoglobin competes with β-catenin, which leads to aberrations in Wnt signalling; both β-catenin and plakoglobin were observed to bind to the transcriptional co-activator, transcription factor 7-like 2 (also known as Tcfl4), although the latter bound with stronger affinity, and their co-expression led to the overall net inhibition of Wnt signalling (12,13). Plakoglobin was also discovered to activate T-cell factor/lymphocyte enhancer factor (TCF/LEF) transcriptional activity in the absence of β-catenin (11), and the decrease in plakoglobin expression levels correlated with poorer prognosis in malignancies such as non-small lung cancer (14).

PDAC originates from both acinar and ductal cells and the well-described mechanism of tumorigenesis involves the early genetic alteration in the oncogene, K-RAS, whereby the expression of K-RAS is thought to induce the disease (15-17). However, studies in mice models have demonstrated that the expression of K-RAS in mature pancreatic acinar cells was insufficient to induce the PDAC precursor, pancreatic intraepithelial neoplasia (18), and that the accumulation of mutations in P16, TP53 and SMAD4 (15-17) are also required for the cancer to progress. Thus, nodal regulators of cellular responses have been proposed as potential strategies for inhibiting PDAC formation (18). It was noted in a previous study that compared with pancreatic acinar cells, ductal cell developed more rapidly into PDAC in the presence of pancreatic acinar cells, ductal cell developed more rapidly into PDAC in the presence of PDAC (18), and that the accumulation of mutations in P16, TP53 and SMAD4 (15-17) are also required for the cancer to progress. Thus, nodal regulators of cellular responses have been proposed as potential strategies for inhibiting PDAC formation (18).

RNA extraction. Total RNA was extracted from PDAC tumour tissue and non-malignant pancreatic tissue using an AllPrep DNA/RNA Mini kit (cat. no. 80201; Qiagen, Inc.) and DNase digestion was performed using a DNase kit (cat. no. AMPD1; Sigma-Aldrich; Merck KGaA), according to the manufacturers’ protocols. Total RNA was quantified using a NanoDrop ND-1000 UV spectrophotometer and a ratio of absorbance at 260 and 280 nm of >1.8 was observed in all samples.

Gene expression profiling using RNA sequencing. Total RNA extracted from tissue samples obtained from patients 7 and 8 (Table I) were treated with DNase to remove genomic DNA. The NEB Next rRNA Depletion kit (human/mouse/rat) (New England Biolabs Inc.; cat. no. E6310S) was subsequently used to remove the ribosomal RNA from the samples. The mRNA was converted into cDNA using the Ultra RNA Library Prep kit (New England Biolabs Inc.; cat. no. 7530L) and subsequently sequenced using the Illumina MiSeq system. The CLC Genomics Workbench version 11 software (www.qiagenbioinformatics.com/products/clc-genomics-workbench) was used for data normalization and differential gene analysis, with HGI9 serving as the reference genome.

Biomarker filtering analysis. The biomarker filtering tool available in the Ingenuity pathway analysis (IPA) software v01-08 (https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/) was used to identify novel potential biomarkers. Microsoft Excel files containing the differentially expressed genes (DEGs) obtained from RNA sequencing of tissue samples were uploaded onto the IPA platform and the biomarker filtering tool facilitated the identification of relevant and novel biomarkers based on the applied filters. The following filters were used: Species (Human); Tissues and cell lines (Pancreatic cancer cell lines); Node types (All nodes except complexes and groups); Diseases (Cancer); Biofluids (Blood); Molecules (All types); Biomarker (Not a known biomarker). Manual filtering was also conducted, including searching the NCBI database (https://www.ncbi.nlm.nih.gov/pubmed/) and pancreatic cancer database (http://pancreaticcancerdatabase.org/index.php) to further refine the biomarker analysis.

Validation by reverse transcription-quantitative PCR (RT-qPCR). The mRNA expression levels of potential biomarkers were validated using RT-2-qPCR primer assays (Qiagen, Inc.), according to the manufacturer’s protocol. The cycling condition was set as follows: 95°C for 10 min (1 cycle), 95°C for 15 secs (45 cycles) and 60°C for 1 min (45 cycles). At
Following incubation, the membranes were washed three times with P<0.05 were selected. Briefly, the membranes were incubated at room temperature in 2 ml block buffer and placed on a rocking platform shaker for 1 h. Subsequently, 2 ml diluted horseradish peroxidase conjugated-streptavidin was added to the membrane and incubated at room temperature for 30 min. Finally, the membrane was washed as previously described and 1 ml Chemi reagent mix was added to the membrane. Membranes were visualised using a Bio-Rad ChemiDoc imaging system (Bio-Rad Laboratories, Inc.).

### Table I. Characteristics of patients enrolled in the present study.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Alcohol use</th>
<th>Diabetes</th>
<th>Differentiation</th>
<th>TNM</th>
<th>Stage</th>
<th>Alive 12/12</th>
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<td>Yes</td>
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<td>T3N1M0</td>
<td>IIB</td>
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<tr>
<td>2</td>
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<td>IA</td>
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<td>No</td>
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<tr>
<td>4</td>
<td>Male</td>
<td>63.5</td>
<td>Yes</td>
<td>No</td>
<td>Mod diff</td>
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<td>IIB</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
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<td>54.3</td>
<td>Yes</td>
<td>No</td>
<td>Poor diff</td>
<td>T2N1M0</td>
<td>IIB</td>
<td>Yes</td>
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<td>6</td>
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<td>52.2</td>
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<tr>
<td>7</td>
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<td>57.3</td>
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<td>8</td>
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<td>43.4</td>
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<td>No</td>
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<td>IIA</td>
<td>Yes</td>
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<tr>
<td>9</td>
<td>Female</td>
<td>52.6</td>
<td>No</td>
<td>No</td>
<td>Mod diff</td>
<td>T2N1M0</td>
<td>IIA</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*aSamples used in RNA sequencing. *bSamples used for proteome profiling. diff, differentiated.

the end of the cycles the Cp values were obtained to be used for analysis. Kits were purchased targeting JUP (cat on pancreatic tissue. no. PPH07138F), COPG (cat. no. PPH07138F), TCIRG1 (cat. no. PPH09294A) and the reference gene MRPL19 (cat. no. PPH09294A), a housekeeping gene whose expression remains unchanged in PDAC carcinogenesis (25). A Roche LightCycler-480 was used with the cut off Cp set at 3s, according to the manufacturer's protocol. Experiments adhered to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments guidelines, including performing three independent experiments (26).

Expression levels were quantified by uploading the data into the Relative Expression Software Tool (REST) software (27).

**Protein extraction and quantification.** Total protein from 20 mg PDAC and non-cancerous pancreatic tissue was extracted using a lysis buffer [(0.5% igepal, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate, 150 mM sodium chloride and 50 mM Tris-HCl (pH 7.5) containing a protease inhibitor cocktail of aprotinin (0.5 µg/ml) and PMSF (1 mM)]. Tissues were homogenized using TissueRuptor and centrifuged at 2,000 x g for 10 min at 4°C. The supernatant was transferred into a new tube and the total protein was quantified using the Quick Start™ Bradford protein assay kit (cat. no. 5000006; Bio-Rad Laboratories, Inc.), according to the manufacturer's protocol.

**Focused proteomic profiling on pancreatic tissue using Human Oncology Proteome Profiler Array.** Protein expression profiling was conducted to determine the relative expression levels of 84 cancer-related proteins using the Proteome Profiler Human XL Oncology Array kit (cat. no. ARY026; R&D Systems Europe, Ltd.), according to the manufacturer's protocol. Quick Spots image analysis software vPCM.22.0.0.i (R&D Systems Europe, Ltd.) was used to measure the intensity of each spot and identify differentially expressed proteins (DEPs). The intensity of each protein was compared between the tumour and corresponding normal pancreatic tissues, and differentially expressed proteins (DEPs) with P<0.05 were selected. Briefly, the membranes were incubated at room temperature in 2 ml block buffer and placed on a rocking platform for 1 h. Subsequently, 500 µl Array Buffer 4 was added and the membranes were incubated overnight at 4°C. Following incubation, the membranes were washed three times with 1 ml wash buffer. Then, 30 ml detection antibody cocktail was added to 1.5 ml 1X Array buffer 4/6 and incubated at room temperature with the membrane on a rocking platform shaker for 1 h. Subsequently, 2 ml diluted horseradish peroxidase conjugated-streptavidin was added to the membrane and incubated at room temperature for 30 min. Finally, the membrane was washed as previously described and 1 ml Chemi reagent mix was added to the membrane. Membranes were visualised using a Bio-Rad ChemiDoc imaging system (Bio-Rad Laboratories, Inc.).

**Statistical and bioinformatics analysis.** Statistical analysis on three biological replicates was performed using Microsoft Office Excel (2016) and data are presented as the mean ± SEM. Differences between groups were analysed using Student's t-test. P<0.05 was considered to indicate a statistically significant difference. The Reactome database (28) and PANTHER version 6 software (29) were used to identify dysregulated pathways (P<0.05). For statistical analysis of the real-time PCR data, the Pair Wise Fixed Reallocation Randomisation Test was performed by the REST software.

**Results**

**JUP is upregulated in early resectable PDAC tumours.** Differential gene expression analysis of the sequenced transcripts from patients 1 and 2 revealed that 4,911 genes were commonly upregulated and 5 were downregulated (Fig. 1A). Using the IPA biomarker module and aforementioned filter parameters, JUP encoding plakoglobin, COPG encoding coatamer protein complex, subunit gamma and TCIRG1 encoding T-cell immune regulator 1 were identified as potential biomarkers. Validation of these genes using RT-qPCR demonstrated that JUP expression levels were increased in all PDAC samples compared with the corresponding normal pancreatic tissue sample.[p(H1)=0.012](Fig. 1B). However, the expression levels of COPG in PDAC tissues were not significantly different compared with normal tissues, with a probability of the alternate hypothesis [p(H1)=0.703]. TCIRG1 expression levels were also unchanged between normal and PDAC tissue samples [p(H1)=0.597]. Although the expression levels of JUP were significantly increased in tumour tissues, it was observed to have a wide 95% confidence interval of 1.25-1,856.39.
Dysregulated pathways in PDAC identified by gene expression analysis. Bioinformatics analysis predicated that the insulin growth factor-1 (IGF-1) pathway and mitogen-activated protein kinase (MAPK) kinase signalling cascade were the most upregulated pathway in PDAC tumours (Tables II and SI). The other most upregulated and active signalling pathways were the integrin (P=9.65x10^-15), RAS (P=1.10x10^-3), platelet-derived growth factor (P=1.06x10^-6), epidermal growth factor receptor (EGF; P=7.72x10^-6), angiogenesis (P=5.58x10^-7), CCKR signaling map (P=1.16x10^-6), VEGF signaling pathway (P=4.11x10^-2), B cell activation (P=4.11x10^-2) and Notch (P=2.6x10^-6) signalling pathways (Tables II and SII-SX). Pathways found to be downregulated in PDAC included the proton/oligopeptide co-transportation system, Golgi-ER trafficking and MHC II antigen presentation pathways (Table III). Additional analysis using Reactome confirmed the dysregulation of these pathways.

Upregulated proteins and their signalling pathways. Protein expression profiling of PDAC and normal tissue identified nineteen significantly upregulated proteins and no downregulated proteins (Fig. 2A and Table SXI). The adherens junction protein, nectin-4, was observed to be the most significantly overexpressed (P=4.85x10^-6), followed by p27^Kip1 (P=5.966x10^-3), mucin-1 (MUC-1; P=8.420x10^-3) and gelsolin-like actin-capping protein (CapG; P=1.021x10^-2; Fig. 2B). Further analysis of these proteins demonstrated the enrichment of several pathways (Fig. S1 and Table IV).

Discussion

In the present study, using data produced by transcriptome sequencing and a focused proteome microarray, plakoglobin was found to be significantly upregulated in early stage PDAC tumours compared with normal pancreatic tissue. In addition, analysis of dysregulated pathways within the

Table II. Top upregulated pathways identified in tumours using the Reactome and PANTHER databases following RNA sequencing.

<table>
<thead>
<tr>
<th>Pathways</th>
<th>P-value</th>
<th>Number of genes involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin/IGF pathway-mitogen activated protein</td>
<td>2.19x10^-2</td>
<td>19</td>
</tr>
<tr>
<td>kinase/MAP kinase cascade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Integrin signaling pathway</td>
<td>9.65x10^-15</td>
<td>112</td>
</tr>
<tr>
<td>Ras pathway</td>
<td>1.10x10^-5</td>
<td>39</td>
</tr>
<tr>
<td>PDGF signaling pathway</td>
<td>1.06x10^-6</td>
<td>74</td>
</tr>
<tr>
<td>EGF receptor signaling pathway</td>
<td>7.72x10^-6</td>
<td>70</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>5.58x10^-7</td>
<td>88</td>
</tr>
<tr>
<td>CCKR signaling map</td>
<td>1.16x10^-6</td>
<td>120</td>
</tr>
<tr>
<td>VEGF signaling pathway</td>
<td>4.11x10^-2</td>
<td>35</td>
</tr>
<tr>
<td>B cell activation</td>
<td>4.11x10^-2</td>
<td>33</td>
</tr>
<tr>
<td>Notch signaling pathway</td>
<td>2.6x10^-6</td>
<td>38</td>
</tr>
</tbody>
</table>
PDAC tissue revealed that the IGF-1 and the EGF receptors (IGF1R and EGFR, respectively), as well as components of the K-RAS signalling pathway were also upregulated. Plakoglobin, also known as γ-catenin, is encoded by the junction plakoglobin (JUP) gene and belongs to the catenin protein family (8). It serves a critical role in cell adhesion as an important component of desmosomes and adherens junctions; through interacting as a complex with α- or β-catenin, plakoglobin associates with the cytoplasmic domains of E-cadherin or N-cadherin to facilitate the linkage between adherens junctions and the cytoskeleton (30,31). Recently, plakoglobin was identified as a transcriptional factor involved in the modulation of WNT signalling (8). It was also demonstrated to act as a global regulator of gene expression; it was found to interact

<table>
<thead>
<tr>
<th>Pathways</th>
<th>Genes involved</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proton/oligopeptide co-transportation (Drug transportation)</td>
<td>SLC15A1, SLC15A2, SLC15A4</td>
<td>1.21x10^-7</td>
</tr>
<tr>
<td>Organelle trafficking</td>
<td>KIFC, GALNT1, KIF5A, MANIA1</td>
<td>1.12x10^-3</td>
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<tr>
<td>Membrane trafficking</td>
<td>SEC23B, KIF5C, GALNT1, KIF5A, DVL2, MANIA1</td>
<td>2.82x10^-3</td>
</tr>
<tr>
<td>MHC Class II antigen presentation (Immune activation)</td>
<td>SEC23B, KIF5C, KIF5A</td>
<td>3.87x10^-3</td>
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</tbody>
</table>
with p53 transcription regulatory elements to transcriptionally modulate the TCF/LEF family in an APC-dependent manner (11,32), and upon addition of exogenous plakoglobin to a mesothelioma cell line, plakoglobin accumulated in the nucleus and exhibited transcriptional activity (11). Overall, this suggests that plakoglobin may serve as a transcription factor.

The insulin/IGF-1R, which may signal through the rat sarcoma (RAS) signalling pathway, is important for transducing signals that promote the development of pancreatic cancer; the crosstalk between insulin/IGF and G protein-coupled receptor signaling was observed to depend on mTOR complex 1 in pancreatic cell lines (33). The findings from the present study suggested that the MAPK and PI3K/AKT signalling pathways may be upregulated in the early PDAC tumour microenvironment; however, the mechanism behind this dual signaling remains largely unknown. Alterations in single or groups of genes involved in one pathway often trigger tumorigenic cellular behaviour. It has been reported that ~95% of PDAC tumours harbour activating K-RAS mutations (34) and RAS is a common signaling component of both the MAPK and PI3K/AKT signaling pathways; however, although RAS isoforms are structurally similar, they demonstrate distinct roles in different subcellular environments to mediate different signalling pathways (35). A comprehensive analysis of active signaling pathways in pancreatic cancer demonstrated that 12 of these pathways were often associated with single or multiple gene alterations that arose from different signaling pathways (20). Notably, in the present study, N-RAS was the only isoform identified as being upregulated in PDAC through transcriptome sequencing (Table SIII); N-RAS mutations are frequently observed in melanoma (35).

Receptor tyrosine kinases such as the IGF-1R and EGFR, which were both found to be upregulated in PDAC in the present study, transduce signals via the insulin receptor substrate-1 (IRS) to either the MAPK or PI3K/AKT signaling pathway to control cell proliferation and survival (33). EGFR expression levels are upregulated in ~90% of pancreatic tumours, which contributes to chemoresistance (36-38). Thus, the combination of molecules found upregulated in the PDAC tumour microenvironment in the present study further suggested that the PI3K/AKT and MAPK signaling pathways may be upregulated in PDAC compared to within normal pancreatic tissue.

The precise signalling role plakoglobin serves during pancreatic carcinogenesis is largely unclear; however, one study reported that plakoglobin promoted the phosphorylation of AKT and ERK in an EGFR-dependent manner, and that its overexpression promoted cell mobility and migration (39). The data from the present study demonstrated that both EGFR and insulin/IGF-1R were upregulated in the PDAC tumour microenvironment. In normal muscle tissue, plakoglobin bound to the insulin receptor and the p85 regulatory subunit of PI3K to promote the activation of the PI3K-AKT-FoxO pathway and muscle growth; in this context, plakoglobin was tightly regulated by the E3 ligase, Trim-32; however, it is also important to note that changes in plakoglobin expression alone were insufficient to influence the signal transduction and muscle growth (40). In invasive micropapillary carcinoma of the breast, plakoglobin was observed to be overexpressed in metastatic tissue, and it contributed to metastasis through activating the PI3K/AKT/Bcl-2 signalling pathway (9). This suggested a critical role for plakoglobin in PI3K/AKT signalling. It has also been reported that both the MAPK and PI3K/AKT signalling pathways exist in pancreatic cancer cell lines and that the inhibition of both pathways leads to cell cycle arrest and apoptosis (41). In addition, plakoglobin was demonstrated to have an important role in maintaining cell adhesion in keratinocytes through inhibiting p38 MAPK, hence, the loss of function of plakoglobin resulted in the activation of p38 MAPK in these cells (42). However, the activation of p38 is context-dependent; for example, insulin stimulated p38 in cultured mouse adipocyte cells but downregulated it in chick neuronal cells (43,44). Overall, the findings from the present study, considered alongside the existing research, suggest that plakoglobin may be an important context-dependent factor in carcinogenesis. A conceptual schematic diagram to illustrate the signalling networks upregulated in early PDAC as well as the hypothesised mechanism of action exerted by plakoglobin is presented in Fig. 3.

Plakoglobin may also influence cellular outcomes through its nuclear functions. A candidate nuclear molecule identified in the present study was MUC1, whose overexpression was reported in the proteomic study. A previous study observed that MUC1 was transported in complex with plakoglobin to the nucleolus in HER2-positive primary invasive ductal breast carcinomas following treatment with heregulin/neuregulin-1 (HRG) (39). This HRG-induced nucleolar translocation of the plakoglobin-MUC1 complex was dependent on MUC1, because a mutation in its cytoplasmic RKK motif prevented its nuclear transfer (45). Notably, both MUC1 and HER2 were upregulated in the proteomic study and the transcriptome sequence data demonstrated that HER1 was also one of the genes representative of EGFR signalling (Table SV). In addition, both the transcriptomic and proteomic data analyses revealed further proteins directly or indirectly associated with the insulin/IGF-1 and EGFR signalling pathways (Fig. 2); this included the adherens junction protein nectin-4, which has previously been identified as a tumour-associated
marker for breast cancer and as a potential serum biomarker for ovarian cancer (46,47). Other important proteins included CapG, a potential oncogene associated with tumour invasion and metastasis (48) and the cathepsins, which are proteinases involved in tumour invasion (49). Inflammatory stimuli transcriptionally driven by NF-κB, which is most likely derived from stromal cells, reportedly served an important role in the early development of cancer (21). In conclusion, the present study suggested that components of both the MAPK and PI3K/AKT signalling pathways may be dysregulated in the PDAC tumour microenvironment. These findings proposed an association between the overexpression of plakoglobin and the upregulation of the insulin/IGF and MAPK pathways in early resectable PDAC. The identification of plakoglobin as an important protein in PDAC may help to understand the mechanisms that mediate the initiation of tumorigenesis, with the prospect of modulating plakoglobin to prevent carcinogenesis providing a possible target for future therapeutic intervention; for example, targeting the negative regulator of plakoglobin, Trim-32, may be one approach. The potential involvement of N-RAS in early PDAC has not been reported before and requires further investigation.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

EN contributed to project concept and design, all experimental work, and data acquisition, analysis and interpretation. EN drafted the article. MN contributed to the design of the project, data analysis and interpretation, writing of the manuscript and obtained funding. MB and JD contributed in data acquisition. MS contributed to the project concept. GC contributed to the project concept and design, and data interpretation. All authors revised the work for intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Human Research Ethics Committee of The University of Witwatersrand approved the collection of biological samples and data from patients with pancreatic adenocarcinoma (approval no. M150778) at Chris Hani Baragwanath Academic Hospital in Johannesburg. All patients gave informed voluntary consent.
Patient consent for publication

All patients provided written consent for sample and data to be used for the current study.

Competing interests

The authors declare that they have no competing interests.

References


