Stromal components and micro-RNAs as biomarkers in pancreatic cancer

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå Universitet för avläggande av filosofie/medicine doktorsexamen framläggs till offentligt förvar i hörsal B, målpunkt A, 9 trappor, Norrlands Universitetssjukhus Fredagen den 16 december, kl. 13:00. Avhandlingen kommer att försvaras på engelska.

Fakultetsopponent: Docent Marco Del Chiaro, Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska Institutet, Stockholm, Sweden.
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Abstract

Background Pancreatic ductal adenocarcinoma (PDAC) patients have the poorest 5-year survival rates of all cancer forms. It is difficult to diagnose at early disease stages, tumour relapse after surgery is common, and current chemotherapies are ineffective. Carbohydrate antigen 19-9 (CA 19-9), the only clinically implemented PDAC biomarker, is insufficient for diagnostic and screening purposes.

PDAC tumours are characterised by a voluminous stroma that is rich in extracellular matrix (ECM) molecules such as collagen, hyaluronan (HA) and matricellular proteins. These stromal components have been suggested to promote PDAC cell migration, proliferation, evasion of apoptosis and chemotherapy resistance. Those events are mediated via interactions with adhesion receptors, such as integrins and CD44 receptors expressed on cancer cell surfaces.

Micro-RNAs (miRNA) post-transcriptionally regulate gene expression in health and disease. At the time of PDAC diagnosis, miRNA levels are altered both in plasma and tumour tissue. Before PDAC diagnosis, tissue miRNA levels are altered in precursor lesions, raising the possibility that plasma miRNAs might aid in early detection.

In this thesis, it is hypothesised that stromal components and miRNAs can serve as tissue or blood based biomarkers in PDAC. The aims are: (1) to characterise the expression of stromal components and their receptors in normal and cancerous tissue; (2) to find potential stroma-associated tissue and blood-based biomarkers for diagnosis and prognosis estimates; (3) to determine the cellular effects of type IV collagen (Col IV) in PDAC; (4) to determine if plasma miRNAs that are altered in manifest PDAC can be used to diagnose PDAC earlier.

Methods The expression patterns of Col IV, Col IV-binding integrin subunits (α1, α2, β1), Endostatin, Osteopontin (OPN) and Tenascin C (TNC) were analysed in frozen PDAC and normal pancreatic tissue. A tissue microarray (TMA) was constructed using formalin-fixed, paraffin-embedded primary tumours and lymph node metastases. The TMA was used to study the expression levels and associations with survival of the standard CD44 receptor (CD44s), its variant isoform 6 (CD44v6), HA, OPN and Col IV. Circulating levels of HA, Col IV, Endostatin, OPN and TNC were measured in PDAC patients and healthy individuals, and compared with conventional tumour markers (Ca 19-9, CEA, Ca 125 and TPS). The functional roles of Col IV were studied in PDAC cell lines by: (1) growth on different matrices (2) blocking Col IV binding integrin subunits, (3) blocking the Col IV domains 7s, CB3 and NC1, and (4) by down regulation of PDAC cell synthesis of Col IV using siRNA transfection. Plasma miRNAs alterations were screened for in samples from patients with manifest disease, using real-time quantitative PCR (RT-qPCR). To find early miRNA alterations, levels of those miRNAs that were altered at diagnosis were measured in prediagnostic plasma samples.

Results High tissue expression of both the standard CD44 receptor (CD44s) and its variant isoform CD44v6 as well as low expression of stromal OPN were associated with poor survival. In addition, high CD44s and low OPN predicted poor survival independent of established prognostic factors.

Circulating Col IV, Endostatin, OPN, TNC and HA were increased in preoperative samples from PDAC patients. Preoperatively, higher levels of serum HA and plasma Endostatin were associated with shorter survival. Postoperatively, higher levels of Col IV, Endostatin and OPN were associated with shorter survival. On the contrary, only one of the conventional tumour markers was associated with survival (Ca 125).

Col IV stimulated PDAC cell proliferation and migration and inhibited apoptosis in vitro, dependent on the collagenous domain (CR3) of Col IV and the Col IV binding integrin subunit β1. Reduced endogenous Col IV synthesis inhibited these effects, suggesting that PDAC cells synthesise Col IV to stimulate tumour-promoting events via a newly discovered autocrine loop.

15 miRNAs were altered in early stage PDAC patients and the combination of these markers outperformed CA 19-9 in discriminating patients from healthy individuals. However, none of the miRNAs were altered in prediagnostic samples, suggesting that plasma miRNA alterations appear late in the disease course.

Conclusions Upregulated stromal components in PDAC tumours are detectable in blood samples and are potential diagnostic and prognostic biomarkers in PDAC. High circulating levels of Col IV, Endostatin, OPN and HA predict poor survival, as well as high expression of CD44s and CD44v6 and low expression of OPN in tumour tissue. PDAC cells synthesise Col IV, which forms BM-like structures close to cancer cells and promote tumour progression in vitro via an autocrine loop. Several plasma-miRNAs are altered in PDAC, but are not useful for early discovery.

Keywords
Pancreatic cancer, stroma, basement membrane, tumour markers, type IV collagen, endostatin, osteopontin, tenascin c, hyaluronan, CD44, micro-RNA

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