

Bindhu K Madhavan

Dr.sc hum

Combined evaluation of a panel of protein and miRNA serum-exosome biomarkers allows for highly sensitivity and specific diagnosis for pancreatic cancer

Promotionsfach: Chirurgie

Doktorvater: Prof. Dr. med. Markus W. Büchler

Pancreatic adenocarcinoma (PaCa) ranks fourth in cancer-related mortality. Chemotherapy, and radiation-resistance, early spread, and late diagnosis prohibiting resection account for non-satisfactory therapeutic progress. Serum markers like CA19-9 lack specificity and require additional diagnostic tools. Recently, two non-invasive diagnostic tools have come into focus. First, serum microRNA (miRNA) was repeatedly demonstrated for its applicability for differential diagnosis of cancer, where PaCa patients' serum miRNA may be able to differentiate between benign and malignant tumors or inflammation. Second, tumor-derived exosomes are easily detected in body fluids. Their protein and miRNA profiles may well help as diagnostic tools.

To study the protein or miRNA markers in exosomes, exosomes were isolated from patient's serum, (131 PaCa, 22 non-malignant pancreatic tumors, 25 chronic pancreatitis and 12 non-pancreatic malignancies patients) 20 healthy donor serum and 10 pancreatic cancer cell line culture supernatant by a method of differential centrifugation. The exosomes were analyzed for protein markers by flow-cytometry and for miRNA by qRT-PCR.

An initial screening for PaCa-initiating cell (PaCIC) marker expression on culture-derived exosomes revealed high recovery of CD44v6, c-Met, Tspan8, EpCAM, and CD104, which were used as PaCa S-Exo markers. CD9, CD63, and CD151, consistently recovered in healthy donor S-Exo served as controls. Percent staining and staining intensity for PaCIC markers of PaCa patients S-Exo differed significantly from that of healthy donor, non-malignant pancreatic tumors and CP. Nonetheless, CP patients S-Exo showed increased reactivity, which was reduced excluding anti-c-Met, while 92% of PaCa S-Exo remained positive. PaCa patients S-Exo reactivity was largely independent of tumor grading and staging, reactivity being already seen at early (Tis and T1) tumor stages and not being affected by metastasis.

To select for PaCa related miRNA, microarray analysis was performed with pools of serum exosomes and exosome-depleted serum from healthy donors and PaCa patients. PaCa culture supernatant exosomes served as control and interestingly, PaCa culture supernatant exosomes showed comparable results indicating relevant miRNA recovery in pooled serum to PaCa patient's S-Exo samples.

PaCa patients' serum exosome miRNA differed strikingly from that of healthy donors; such distinctions were not observed with miRNA from PaCa versus healthy donors' exosome-depleted serum. Surprisingly, miRNA with highest recovery in PaCa patients' serum exosomes also were highly enriched in PaCa culture supernatant exosomes. Based on these results, serum exosomes were analysed for highly expressed miR-1246, miR-4644, miR-3976, and miR-4306, not recovered in healthy donors' exosomes. These abundantly recovered miRNAs (miR-1246, miR-4644, miR-3976, and miR-4306) in PaCa S-Exo and Cult-Exo, were selected for evaluating diagnostic validity.

Concomitant PaCIC and miRNA evaluation of PaCa versus healthy, non-malignant pancreatic tumors, and CP revealed improved sensitivity without loss of specificity. Thus PaCa S-Exo may offer a breakthrough in PaCa diagnosis.