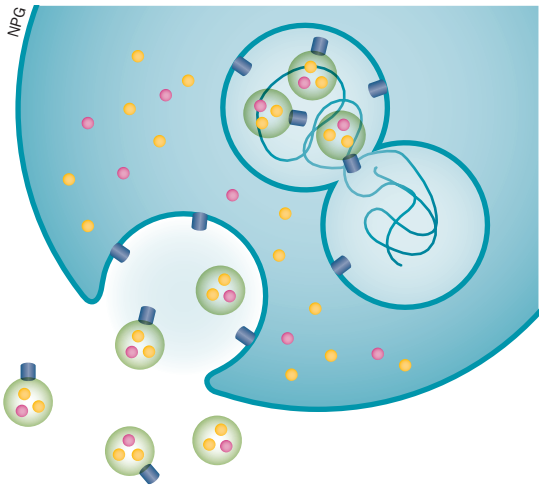
Nature Reviews Gastroenterology & Hepatology **12**, 427 (2015); published online 14 July 2015; doi:10.1038/nrgastro.2015.118

BIOMARKERS

The challenge to find biomarkers for the early detection of pancreatic cancer



Promising advances are currently being made in the search for reliable biomarkers that can facilitate the early detection of pancreatic cancer. In a study published in *Nature*, evidence was presented that glypican-1 (GPC1) expression on pancreatic-cancer-derived exosomes can be used to detect early pancreatic cancer and gain insights into disease progress and tumour burden.

Exosomes are formed when intracellular multivesicular endosomes bulge inwards to create small, membranous vesicles. These endosomes fuse with the plasma membrane, releasing exosomes into the extracellular environment and circulation where they can be detected using blood tests. Exosomes contain proteins and nucleic acids, and their composition varies widely depending on the type and condition of the cell they originate from, making them an ideal, easily accessible reservoir of cancer biomarkers to assess disease processes.

"We had an interest in understanding the biology of exosomes and their functions in cancer," explains corresponding author Raghu Kalluri. His research group analysed the protein contents of exosomes originating from pancreatic cancer cell lines and control cells using chromatography-mass spectrometry and flow cytometry. When they compared pancreatic cancer exosomes with those from non-tumorigenic cell lines, the cancer exosomes showed elevated levels of GPC1, a protein that has previously been reported as overexpressed in these cancers.

In blood samples from human patients with pancreatic ductal adenocarcinoma (PDAC, $n = 190$), substantially higher levels of circulating GPC1$^+$ exosomes were detected than in healthy donors ($n = 100$). In addition, the investigators assessed the tumour material for oncogenic *KRAS* mutations, a common feature in PDAC. In the same patients ($n = 15$) who harboured these variants in their tumour genome, GPC1$^+$ exosomes also contained identical *KRAS* mutations. However, the presence of oncogenic DNA in cancer exosomes was only evaluated in a subset of the whole patient population.

Interestingly, levels of circulating GPC1$^+$ exosomes were higher in patients with histologically confirmed pancreatic cancer precursor lesions than in patients with benign pancreatic disease or healthy individuals. The efficiency of GPC1$^+$ exosomes in distinguishing benign from neoplastic lesions and healthy individuals exceeded that of carbohydrate antigen 19-9 (CA19-9), a widely used tumour marker in PDAC. Tumour burden could also be assessed: higher levels of GPC1$^+$ exosomes were found in patients with PDAC and distant metastatic disease than in patients with metastases in lymph nodes or no metastases. In most patients exosome levels decreased after surgical resection of the tumour. A reduction in circulating GPC1$^+$ exosome levels was also correlated with increased survival in these patients.

When tested in a genetically engineered mouse model of PDAC that specifically recapitulates features of human disease progression, the investigators could show that Gpc1$^+$ exosome levels increase proportionally with time and tumour burden, as the disease progresses from early neoplastic stages to malignant disease. Reassuringly, an increase in Gpc1$^+$ exosome levels is evident before the mice develop pancreatic masses. By contrast, healthy mice retain a low level of Gpc1$^+$ exosomes during all time points, similarly to a mouse model of acute pancreatitis, which argues for cancer specificity of the test.

Researchers estimate that pancreatic cancer requires 15–20 years to evolve from early lesions to PDAC; even though lesions have to progress to certain stages to become detectable, a sufficient time window for cancer prevention might be achievable if suitable biomarkers are found. Currently, ~80% of patients are diagnosed when PDAC has already progressed to metastatic disease.

"Patients with very early stage disease are most likely to benefit from an early detection test," says Michael Goggins (Johns Hopkins University), who was not involved in the research. He acknowledges the study's potential strengths—high sensitivity of the test, the methods used to identify exosomes, and large study populations with resectable pancreatic cancer—but also the limitations.

"Specifically, the authors did not evaluate the test's performance in a sufficiently sized group of patients with very early stage disease (stage 1 pancreatic cancer or carcinoma-*in-situ*)." However, these limitations also reflect the dilemma the researchers had set out to solve. "It is not surprising they had few patients with stage 1 pancreatic cancer because it is very difficult to identify patients with stage 1 cancers," says Goggins. Although the findings from experiments in mouse models look promising, more meticulous work will be needed to confirm the efficiency of biomarkers in the early detection of cancerous lesions in humans. "We hope to test many more patients to validate our findings and to diagnose and track patients with pancreatic cancer," concludes Kalluri.

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Original article Melo, S. A. *et al*. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature* doi:10.1038/nature14581