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DIAGNOSIS

Fishing for exosomes

Pancreatic ductal adenocarcinoma (PDAC) is frequently asymptomatic until late-stage disease develops, meaning that most patients have unresectable primary tumours, metastases and a dismal prognosis when they are diagnosed. Thus, there is a real need to develop sensitive and affordable diagnostics to identify patients with PDAC early in order to treat them more effectively and reduce PDAC-associated mortality rates.

Exosomes are a type of extracellular vesicle that are secreted by all cells, including cancer cells. Exosomes contain various proteins, RNAs and DNA from the cell of origin, so they may be useful for diagnostics. However, a key issue has been how to determine which exosomes from the myriad in a blood sample are those derived from tumour cells.

Melo, Luecke, Kahlert *et al.* isolated exosomes from MDA-MB-231 human breast cancer cells, from transformed human fibroblast cell lines and from non-transformed control cell lines and carried out ultra-performance liquid chromatography–mass spectrometry to identify cancer-selective exosome markers. Of the 48 proteins that were only expressed by the cancer cell line-derived exosomes, only 1 was membrane-anchored: glypican 1 (GPC1), which is known to be overexpressed in breast cancer and PDAC. Circulating exosomes from nude mice with MDA-MB-231 xenografts also expressed GPC1, and the expression of GPC1 differentiated between circulating exosomes from the MDA-MB-231 tumours and those from non-tumour cells.

Next, the authors isolated circulating exosomes from 32 patients with breast cancer and 190 patients with PDAC, as well as from 100 healthy controls. Of the circulating exosomes from healthy controls, on average only 2.3% were GPC1⁺; 24 of the breast cancer samples had GPC1⁺ exosomes at a higher proportion than controls. Strikingly, however, all 190 PDAC samples had significantly higher levels of GPC1⁺ exosomes than controls. Furthermore, the authors showed that quantitative PCR of mRNA in circulating GPC1⁺ exosomes from 15 patients for whom the primary tumour material was available correctly identified the *KRAS* mutations present in these tumours. Therefore, circulating GPC1⁺ exosomes specifically indicate the presence of PDAC (and also of some breast cancers), and the material they contain can be used to molecularly characterize the tumour.

Focusing on PDAC, the authors assessed how early in PDAC development GPC1⁺ exosomes appear in the circulation. They isolated exosomes from blood samples derived from 5 patients with intraductal papillary mucinous neoplasm (IPMN; a PDAC precursor lesion) and from 26 patients with benign pancreatic disease (BPD) and found that the levels of circulating GPC1⁺ exosomes were higher in the patients with IPMN than in patients with BPD (who had equivalent levels to healthy controls). Using PKT mice — a genetically engineered mouse model that progressively develops PDAC — they also showed that the

levels of circulating GPC1⁺ exosomes increased with disease burden and that these high levels were detectable when pancreatic lesions were pre-neoplastic and before lesions were detected by histology or magnetic resonance imaging.

CA19-9 is the standard biomarker for PDAC (detected in patient sera), so the authors compared CA19-9 with circulating GPC1⁺ exosomes for their effectiveness as biomarkers. As well as in patients with PDAC, CA19-9 levels were significantly higher in sera from patients with BPD but did not identify patients with IPMN. Conversely, the presence of circulating GPC1⁺ exosomes had 100% specificity and sensitivity for all stages of PDAC; this was confirmed in a second, independent cohort. Furthermore, a longitudinal study of blood collections from 29 patients with PDAC showed that 28 of these patients exhibited reduced levels of circulating GPC1⁺ exosomes after surgery. If this decrease in circulating GPC1⁺ exosomes was greater than the median decrease for all of the samples, then these patients had a significantly higher overall and disease-specific survival.

It is clear that expensive imaging techniques and current biomarkers are insufficient for the early diagnosis of, or even screening for, PDAC. Interestingly, the authors found that an enzyme-linked immunosorbent assay (ELISA) for GPC1 was equivalent in sensitivity and specificity to CA19-9, indicating that a simple and cheap method for isolating circulating exosomes is needed in the clinic.

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ORIGINAL RESEARCH PAPER Melo, S. A. *et al.* Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature* **523**, 177–182 (2015)

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