Pancreatic cancer specific nanoparticles aided glycovariant assays

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INTRODUCTION

Pancreatic cancer is among the most lethal malignancies. The cancer antigen 19.9 (CA19-9) also known as sialyl Lewis A (sLeA) antigen is the most used and best-validated serum tumor marker for PanCa, but there are several critical aspects for its clinical use. False negative results in subjects with Lewis (a-b-) genotype and false positive elevation in patients with benign diseases, such as pancreatitis compromise the utility of CA19.9.

Cancer-derived extracellular vesicles (EVs) are strongly glycosylated, being rich in specific glyco-conjugates such as integrins, tetraspanins and mucins reflecting the cell of origin and altered glycosylation of circulatory biomarkers is a well-known phenomenon.

Two aims were set for this study. First, the optimization and clinical evaluation of CA125 ($CA125^{WGA}$) glycovariant (GV) assay previously identified from cell line glycoprofiling. Second, the profiling with passively coated purified EVs compared to soluble protein (SP) enriched fractions from of PanCa, benign and healthy serum pools in collaboration with FastEV company (FastEV.fi).

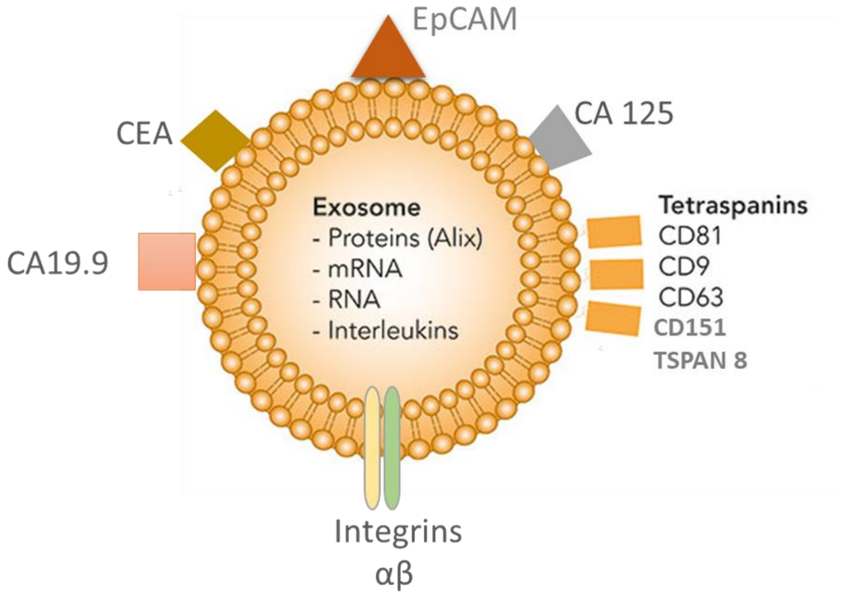
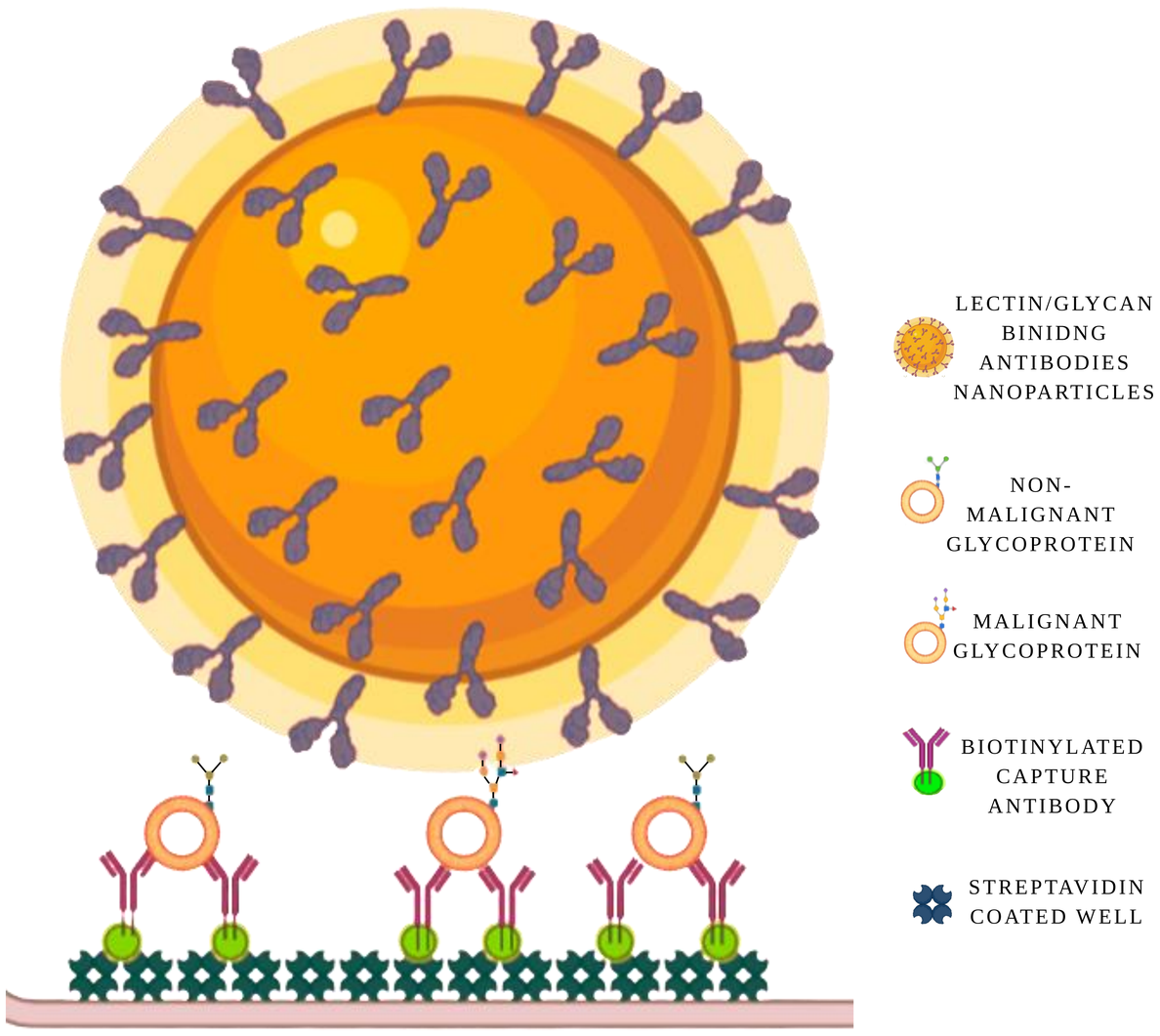


Fig. 1. **Illustration of the structure of exosomes, an EV subclass. Approximately 40-160 nm in diameter, cancer-derived exosomes have vast oncogenic cargo and are enriched in specific glycoproteins.**

MATERIALS & METHODS

Non-Competitive sandwich GV immunoassay



Glycoprofiling of pure EVs and SP fraction

Novel method	Principle	Application
Passive coating	Drying of purified EVs or SPs straight to the bottom of microtiter well & tracing of glycoconjugates with nanoparticles	Profiling of glycoconjugates on the surface of EVs

RESULTS

Signal-to-background (S/B) ratio was calculated to determine the enrichment of glycans. S/B ratio was proportional to amount of glycan, i.e., higher the amount of a glycan on a EV, higher the relation between the signal and background.

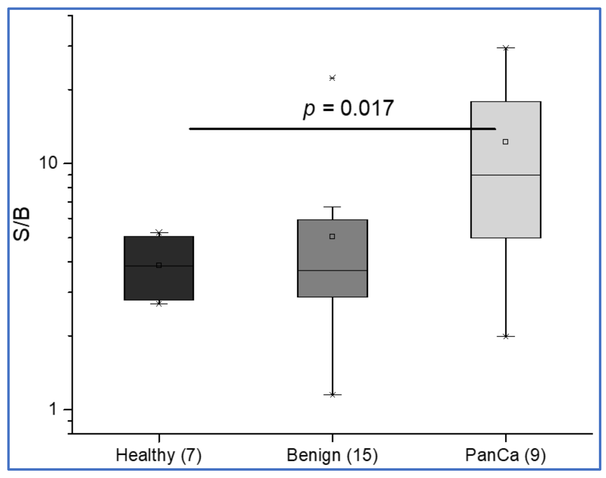


Fig. 2. **Discrimination of PanCa (n = 9), benign pancreatitis (n = 15), and healthy controls (n = 7) used in the initial $CA125^{WGA}$ glycovariant assay.**

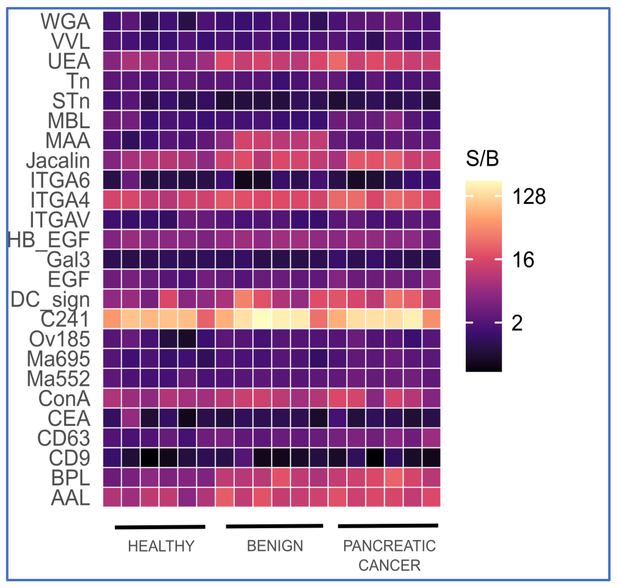


Fig. 3. **Glycoprofiling of the soluble protein (SP) fraction of purified EVs. Each sample type was purified with six different FastEV conditions 2, 4, 6, 8, 9 and 11 which correspond to one column, read from left to right.**

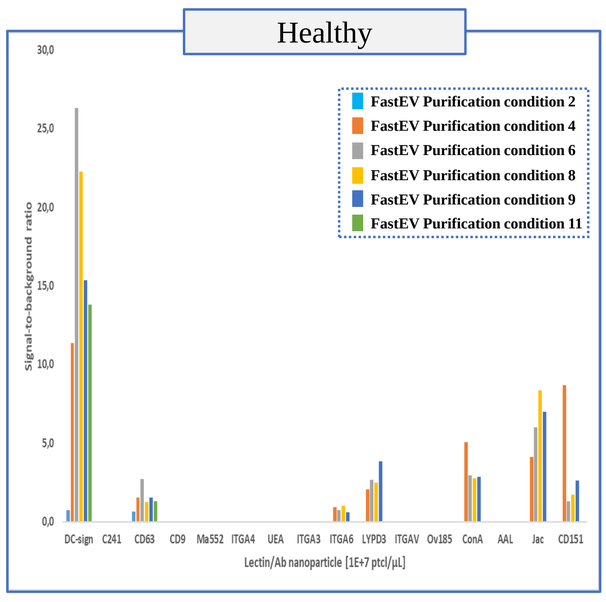


Fig. 4. **Glycoprofiling of purified EVs from healthy control serum pools. Each sample type was purified with six different FastEV conditions indicated by column color. Missing column is due to insufficient sample volume, i.e., sample diminished before planned assay was ran.**

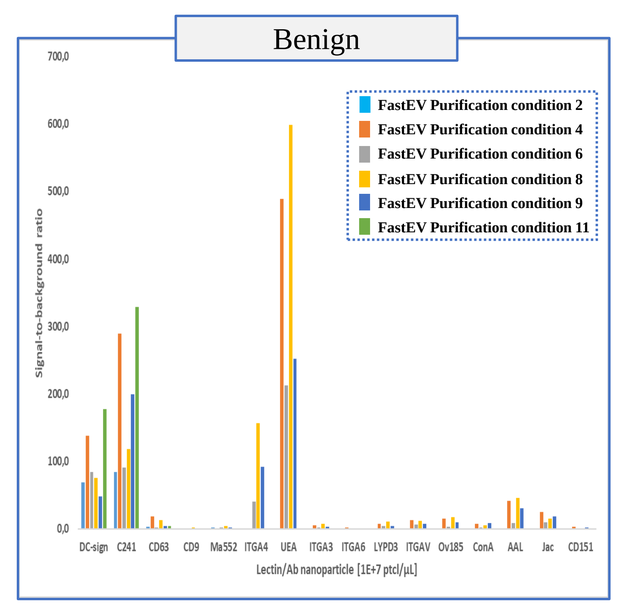


Fig. 5. **Glycoprofiling of purified EVs from benign pancreatitis serum pools. Each sample type was purified with six different FastEV conditions indicated by column color. Missing column is due to insufficient sample volume, i.e., sample diminished before planned assay was ran.**

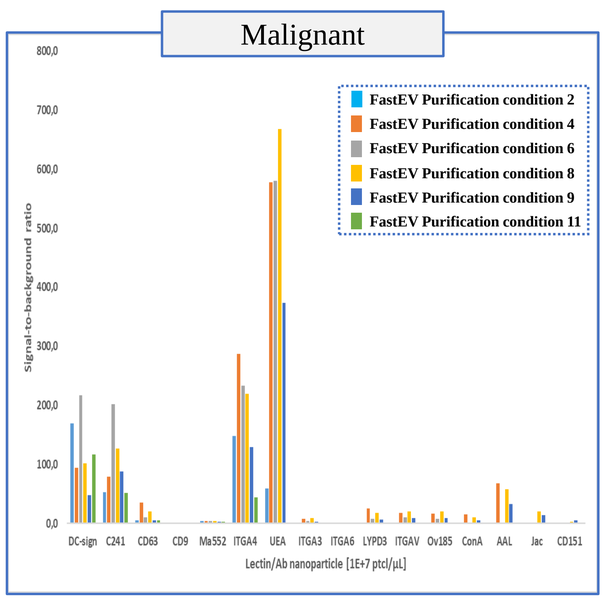


Fig. 6. **Glycoprofiling of purified EVs from malignant PanCa serum pools. Each sample type was purified with six different FastEV conditions indicated by column color. Missing column is due to insufficient sample volume, i.e., sample diminished before planned assay was ran.**

Tab. 1. **Complementation of $CA125^{ITGA4}$ GV assay to conventional CA19.9 EIA. Conventional assay detected two benign samples as false positive and two malignant samples as false negative, while $CA125^{ITGA4}$ detected them as true negative and true positive, respectively.**

Sample	Conventional CA19.9 EIA S/B ratio	$CA125^{UEA}$ GV assay S/B ratio
BCP-12*	240	5
BCP-14*	116	1
PanC-7**	2	32
PanC-9**	2	41

* BCP indicates benign sample
**PanC indicates malignant PanCa sample

CONCLUSIONS

Compared to conventional methods, glycovariant assays have shown superior performance and improved specificity in detection of cancers. With cancer-specific modifications on glycoprotein expression, and their reflection on EVs, our study suggests that glycovariant assays could be used complementary in combination with conventional assays.