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Adaptive dynamic artificial polyligand targeting (ADAPT): A Method to identify exosomal proteins from a prostate cancer cell line

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Introduction

In the recent years it was demonstrated that a multitude of body fluids contain substantial amounts of exosomes, extracellular vesicles with sizes ranging between 40 and 100 nm. Those vesicles have protein profiles characteristic of their cells of origin. It was shown that exosomes play a role in cell-to-cell communication making them attractive targets to identify early disease stage biomarkers. Cancer heterogeneity has been known for a long time to be an important clinical determinant of patient outcome. We developed the highly multiplexed ADAPT platform to capture systems-based biological signatures that may reflect the molecular heterogeneity of various cancer types and help to improve diagnosis of the disease[1]. In order to show the potential of the ADAPT Biotargeting System™ on extracellular vesicles, exosomes from two prostate cancer cell lines, VCaP and LNCaP, were used to train ssDNA libraries to discriminate them.

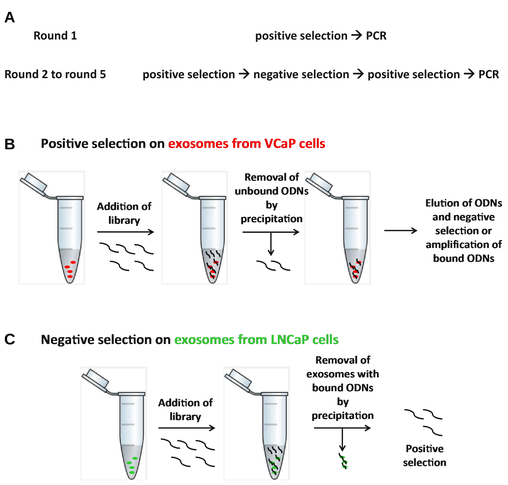
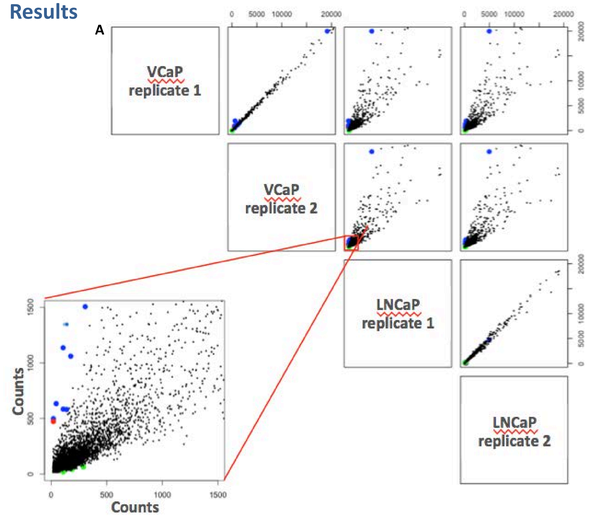
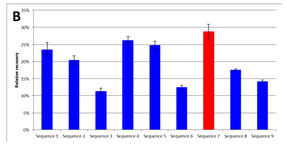
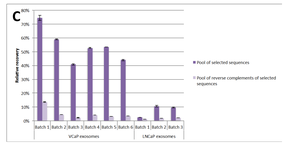


Figure 1 – Selection scheme: A highly diverse library of 10^{12} oligodeoxynucleotides (ODNs) was subjected to five rounds (**A**) of positive (**B**) and negative selection (**C**) against exosomes from VCaP and LNCaP prostate cancer cell lines.
After mixing of exosomes with ODN library the unbound DNA was removed by precipitating exosomes and bound ODN by polymer.

Results







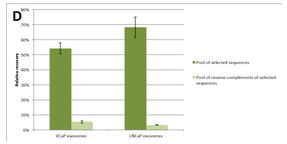
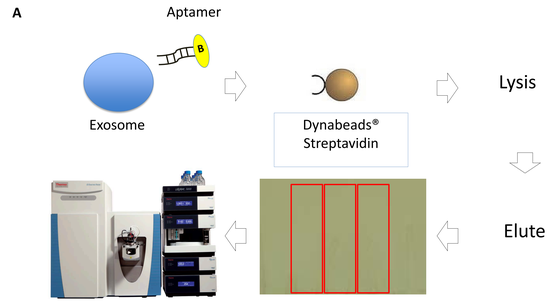


Figure 2 – Sequence identification and verification: Individual ODNs that bound preferably to exosomes from VCaP cells (blue and red) or LNCaP cells (green) were identified by NGS (**A**); each dot represents one unique sequence.
Sequences were resynthesized and binding of co-precipitated ODNs to VCaP exosomes was verified by qPCR (**B – D**). (**B**) Binding of nine unique sequences to exosomes from VCaP cells; red represents Sequence 7 that was used for target ID (**Figure 3**). (**C**) Binding of pool of nine unique sequences (dark) and pool of their reverse complements (light) to multiple batches of exosomes. (**D**) Binding of pool of three unique sequences (dark) and pool of their reverse complements (light) to exosomes.



B

Protein	Description	Significance	Reference
CHMP1B	Charged multivesicular body protein 1b	Part of ESCRT machinery that plays role in exosome biogenesis	2
CHMP2A	Charged multivesicular body protein 2a		
CHMP4B	Charged multivesicular body protein 4b		
VPS28	Vacuolar protein sorting-associated protein 28 homolog		
Syntenin-1	Syntenin-1	Associated to ESCRT machinery that plays role in exosome biogenesis	3
I-TAC	C-X-C motif chemokine 11	Chemokine that is overexpressed in blood and tissue of men with advanced prostate adenocarcinomas	4
hnRNP-1	Polypyrimidine tract-binding protein 1	Cancer associated splicing factor	5
RNPL	RNA-binding protein 3	Cold shock proteins. Knock-down of these proteins has been shown to enhance chemotherapeutic cell killing of prostate cells	6
A18 hnRNP	Cold-inducible RNA-binding protein		

Figure 3 – Target ID: Affinity purification of target proteins bound to biotinylated Sequence 7 in combination with LC-MS/MS detection identified exosomal binding partners of the aptamer (**A**). Gel in red boxes was used for digestion and mass spec analysis. Lane 1: no DNA control; bare beads. Lane 2: pull-down with Sequence 7. Lane 3: pull-down with reverse complement of Sequence 7. (**B**) List of proteins identified in exosomes from VCaP cells after pull-downs with Sequence 7.

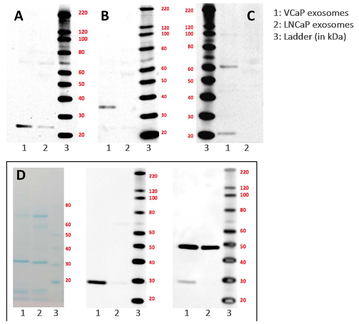


Figure 4 – Target verification: Confirmation of higher expression of proteins in exosomes from VCaP cells compared to exosomes from LNCaP cells.
Proteins tested: **A** – VPS28 (28 kDa), **B** – Syntenin-1 (33 kDa), **C** – CHMP4B (24 kDa) and **D/middle** – CHMP1B (28 kDa) with Memcode staining (**D/left**) and stripping/re-probing with anti-TSG101 (**D/right**) as loading and transfer controls.

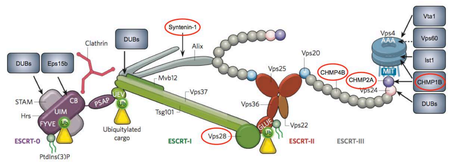


Figure 5 – Structure of the ESCRT machinery: modified from reference 2. Proteins in red circles were found in exosomes from VCaP cells as part of this study.

Conclusions

- Successful selection of ODNs that bind preferably to exosomes from one cell line.
- Identification and verification of proteins as binding partners for selected ODNs.

→ ADAPT is an unbiased profiling platform that identifies proteins expressed on exosomes. This platform can be deployed against multiple sample types and offers broad potential applications in biomarker discovery.

References

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