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Identification and implications of transcription factors in circulating microvesicles from cancer patients

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Abstract

Circulating microvesicles (cMVs) contain proteins and RNA molecules which can be used to detect specific diseases. The presence of transcription factors (TFs) within cMVs has only recently been established. In 2008 researchers detected higher levels of two TFs in urine exosomes from patients with acute kidney injury; ATF3 and WT-1. TFs have also been identified within cancer-associated cMVs including c-Myc, p53, AEBP1 and HNF4a.

In this study three TFs were evaluated in cancer-associated cMVs: STAT3, a Y-Box TF (YB-1) and SPDEF. STAT3 was detected in VCaP exosomes and found to be twice as prevalent in cancer plasma cMVs. YB-1 was 18-times higher in breast cancer cMVs compared to controls. Using a bead-based ELISA, SPDEF expression for PCa (n=80) was two-fold higher than controls, including benign (n=39), inflammatory disease (n=29), cellular atypia (n=8), HGPIN (n=21). Other TFs were evaluated in multiple studies showing 8 to 21-fold increases between cancers and controls. Interestingly, low SPDEF has been associated with aggressive cancer phenotypes suggesting that PCa cells may actively shuttle this TF into cMVs as part of the progression of the disease. Like miRs, TFs can regulate multiple gene networks perhaps mediating the "field effect" seen in cancer patients.

Methods

STAT3 expression was determined for VCaP-derived MVs (Fig A1, top panels) or cMVs from patient plasma (Fig A1, lower panel) and co-stained for CD9 expression. As indicated MVs were permeabilized using life technologies' Fix and Perm® cell fixation and permeabilization kit without washing steps and analyzed using a Beckman Coulter MoFlo XDP flow cytometer. cMVs were isolated using 150kD Pierce concentration columns.

To evaluate transcription factor expression in multiplex bead-based ELISA /flow cytometry assays (Fig A2, A3 and A4) sets of beads with individual internal infared dye concentrations were coated with the indicated antibodies, washed and blocked according to the manufacturer's instructions. MVs were incubated and unbound MVs removed by washing. Then a second fluorescently labeled "detector Abs" (anti-CD9, -CD63 and –CD81-FITC) for A2 and A4. For A3 patient cMVs were captured with anti-PCSA and detected with FITC-conjugated anti-SPDEF antibodies.

GeneCards® website was interrogated for the Interaction Network analysis of several identified transcription factors.

Results

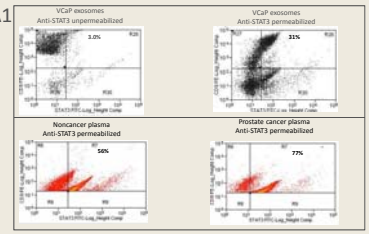
A1) These studies show that STAT3 can only be found on VCaP-derived MVs after permeabilization implying internal localization of this TF.

A2) Shows standard curve for breast cancer cell-derived MVs for YB-1 and that breast cancer plasma has higher levels of YB-1+ MVs compared with healthy female controls.

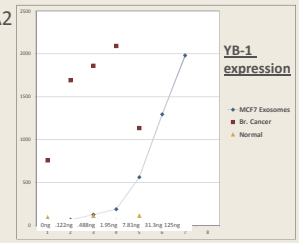
A3) Summarizes SPDEF expression on prostate tissue-derived cMVs from men with a range of prostate disease diagnoses.

A4) Summarizes TF expression on cMVs from prostate or breast cancer plasma and the ratio compared with controls.

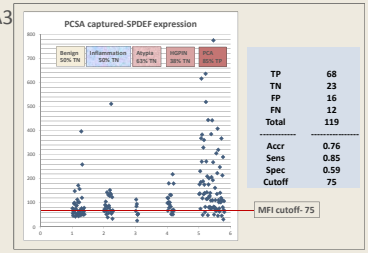
A1



A2



A3



TP	68
TN	23
FP	16
FN	12
Total	119
Accr	0.76
Sens	0.85
Spec	0.59
Cutoff	75

A4

Marker	Ave MFI Normal	Ave MFI PCa Cancer	Fold Increase	Multiple studies Ave Fold increase
STAT3	221.8	1954.9	8.8	9.6
EZH2	127.5	1372.8	10.8	11.8
p53 (Ab1)	117.6	1194.2	10.2	12.5
p53 (Ab2)	121.6	994.3	8.2	9.0
p53 (Ab3)	117.0	1403.9	12.0	10.4
MACC1	137.8	1472.4	10.7	only once
SPDEF	104.5	1201.6	11.5	only once
RUNX2	24.2	510.8	21.1	20.2
AURKA	116.9	1045.7	8.9	8.6
AURKB (Ab1)	140.3	1725.1	12.3	13.3
AURKB (Ab2)	114.4	1336.6	11.7	10.7
Breast Cancer screen 5 BCa vs. 5 controls				
YB-1	80.5	1509.0	18.7	only once

Conclusions

Identification of TFs in microvesicles

These studies demonstrate that TFs can be identified in exosomes and microvesicles from cancer cell lines and from cancer patients. STAT3 was identified in prostate cancer associated MVs and YB-1 was identified in breast cancer-associated MVs. Importantly, the transcription factor SPDEF was identified in prostate tissue-associated cMVs from plasma from men at risk for prostate cancer and the level of expression increased with increasing malignant diagnosis indicating transfer of this TF to MVs may be associated with the process of tumorigenesis.

Implication of TFs in microvesicles

Like miRNAs and lncRNAs transcription factors can influence the expression of multiple proteins and can have a major impact on cell biology. TFs can directly alter the transcription rate of specific mRNA (Target genes) and have also been shown to interact with other proteins that have significant biologic impacts. These impacts include cancer associated properties such as epigenetics, cell cycle regulation, DNA repair, anti-apoptosis, differentiation, proliferation, angiogenesis and even steroid hormone response.

Of all the TFs evaluated their levels were higher in cancer-associated MVs than in controls. This higher level of TFs in cancer-associated cMVs may contribute to the well documented "field effect" seen in normal tissue surrounding tumors, promote invasion/metastases and may contribute to cancer progression in patients.