**Evaluation of new therapeutic targets in circulating tumor cells and exosomes isolated from small cell lung cancer patients**

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## Background & objectives

Small cell lung cancer (SCLC) is a rapidly growing and metastatic type of cancer with a poor prognosis. Since most of the tumors are unresectable, reliable liquid biopsy biomarkers are very important for SCLC patients. Our research group showed that overexpression of JUNB (transcription factor) and CXCR4 (chemokine receptor) in breast cancer patient's circulating tumor cells (CTCs), is associated with clinical outcome.

The aim of the current study was to develop a novel protocol for the parallel assessment of the aforementioned biomarkers in CTCs and exosomes derived from SCLC patients.

## Methods

CTCs from 40 SCLC patients were isolated using Ficoll density gradient. Cytospins were stained using CK, JUNB and CXCR4 antibodies. Slides were analyzed with VyCAP platform and ACCEPT software. Plasma exosomes derived from the same patients and 10 healthy donors (HDs) were isolated using the EXO-Prep® kit. Exosomes were characterized regarding their morphology by transmission electron microscopy, molecular profiling by Western blots, and RNA-sequencing.

## Results

Most of the patients harbored $JUNB^+$ CTCs (93.9%), while $CXCR4^+$ was detected in 75.8%. The most frequent CTC-phenotype was the ($CK^+JUNB^-CXCR4^-$): 62.8%, with subsequent

phenotypes referring to ($CK^+JUNB^+CXCR4^+$): 12%, ($CK^+JUNB^+CXCR4^-$): 14.4% and ($CK^+JUNB^-CXCR4^+$): 10.8%. Regarding protein levels in patients' exosomes compared to HDs, one-way ANOVA reported significant differences for JUNB (p-value=0.005) and CXCR4 (p-value=0.039). Furthermore, both biomarkers showed high predictive ability derived from the Receiver Operator Characteristics curve ($C_{JUNB}$=0.82, $C_{CXCR4}$=0.77). Notably, CXCR4 exosomal protein levels were positively correlated with $CXCR4^+$ CTCs (p-value=0.012). Similarly, CXCR4 expression in exosomes and $CXCR4^+$ CTCs were associated with PFS (p-value=0.007, p-value=0.049), while only CXCR4 exosomal expression was associated with OS (p-value=0.033). RNA-seq analysis in exosomes revealed 248 differentially expressed microRNAs (5 related to JUNB and one to CXCR4), implicated in TP53, G1/S transition, and AKT-mediated pathways.

## Conclusion

Simultaneous analysis of both CTCs and exosomes could unveil prognostic biomarkers and provide potential therapeutic targets, such as JUNB and CXCR4 for SCLC patients.