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Introduction

Tumor angiogenesis plays a crucial role in tumor development and progression. Genome analyses of endothelial cells identified genes specifically expressed by tumor endothelial cells. These so called tumor endothelial markers (TEMs) are discussed as potential new therapeutic targets or as biomarkers for monitoring anti-angiogenic therapies. In non-small cell lung cancer (NSCLC) the role of TEMs is not yet investigated. Therefore the aim of our study was the evaluation of different TEMs in NSCLC cell lines and tumor samples of NSCLC patients.

Patients and Methods

The expression of different TEMs (Robo4, Clec14 and ECSCR) was evaluated by qRT-PCR and Western blot analyses in three NSCLC cancer cell lines (A549, Calu1, Colo699) compared to human umbilical vein endothelial cells (HUVEC), endothelial colony forming cell clones (ECFC) and human bronchial epithelial cells (HBEpC). Then, the expression of TEMs was measured in resected tumor tissue of NSCLC patients (n=63) by qRT-PCR and compared to adjacent non-cancerous lung tissue (n=52). Immunohistochemical analysis of Robo4 expression in tumor tissue (n=33) and adjacent non cancerous tissue (n=27) was performed. Further correlation analyses of expression levels with clinical course were performed using the data from a lung cancer register.

Results

In vitro experiments

NSCLC cell lines do not express TEMS (Robo4, Clec14, ECSCR) on mRNA nor on protein level

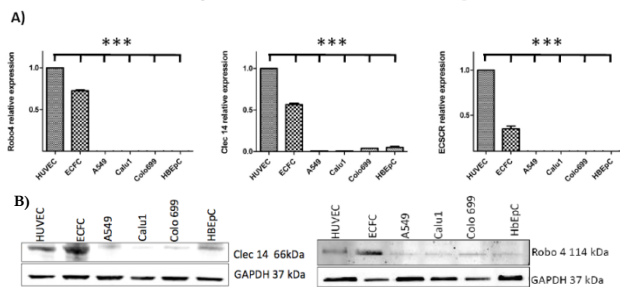


Figure 1: TEM expression levels of NSCLC cell lines (A549, Colo699, Calu1) and HBEpC are shown compared to the expression of control cells HUVEC and ECFC. Abbreviations: HUVEC: human umbilical vein endothelial cells, ECFC: Endothelial colony forming cells, HBEpC: primary Human Bronchial epithelial cells, ***: $p < 0.001$; B) TEM expression measured by Western Blot. GAPDH served as control.

Tumor sample Analysis qRT-PCR

TEMs are higher expressed in tumor samples in comparison to adjacent tissue

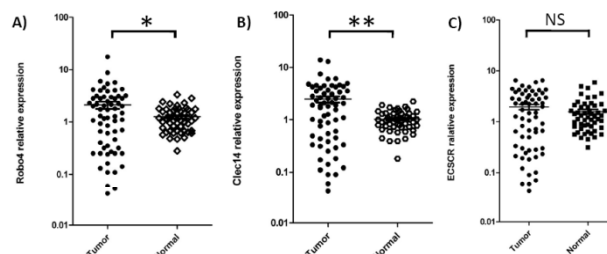


Figure 2: Expression level of TEMs Robo4 (A), Clec14 (B) and ECSCR (C) by qRT-PCR in tumor tissue compared to normal adjacent lung tissue. * $p < 0.05$, ** $p < 0.01$, NS: not significant

Tumor sample Analysis Histology

Robo 4 is higher expressed in tumor tissue in comparison to the adjacent tissue

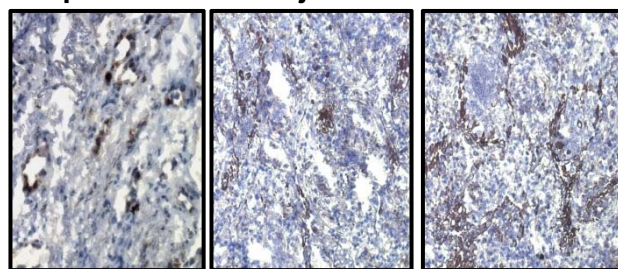


Figure 3: Robo 4 expression of 33 NSCLC patients and 27 adjacent tissues (not shown) was analyzed on protein level by immunohistochemical analysis.

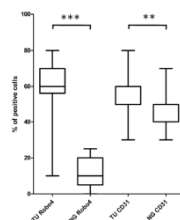


Figure 4: Immunohistochemical analyses of Robo4 and CD31 expression in NSCLC cancer tissue and adjacent normal lung tissue. **: $p < 0.01$, ***: $p < 0.001$

Correlation with Overall Survival

Increased TEM expression correlates with prolonged overall survival in NSCLC

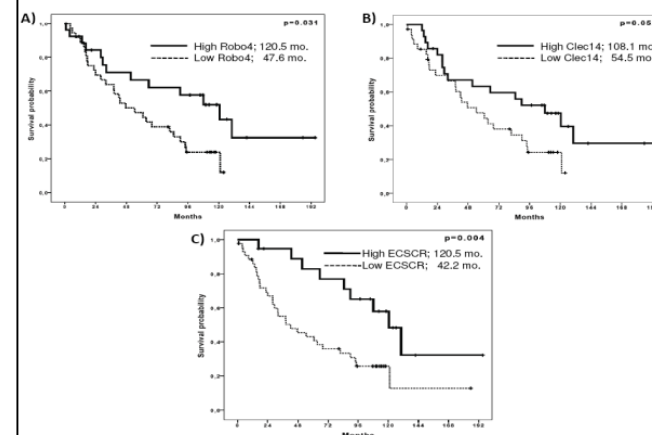


Figure 5: Correlation of qRT-PCR expression levels with overall survival (OS). A) Robo4; B) Clec14; C) ECSCR

Prognostic factor	RR	95% CI	p
Age in years (<63 vs. ≥63)	6.541	0.234 – 0.825	0.011
Gender (female vs. male)	2.221	0.826 – 4.063	0.136
Robo4 qRT-PCR low vs. high	0.024	0.291 – 2.869	0.877
Clec14 qRT-PCR low vs. high	0.082	0.312 – 2.382	0.775
ECSCR qRT-PCR low vs. high	2.967	0.192 – 1.112	0.085

Table 1: Multivariate analyses (Cox regression) of prognostic markers (n=63), censored cases n=22 (34,9%)

Conclusion

- TEMs are not expressed in NSCLC cancer cell lines and primary bronchial epithelial cells, compared to HUVEC.
- TEMs are upregulated in stromal tissue including tumor vessels.
- Increased TEM expression levels correlate with superior clinical outcome (prolonged OS) possibly due to vascular stabilization.