

**MEASURING COMPLEX *IN VIVO* ENDOTHELIAL CELL RESPONSES:
AN ESSENTIAL TOOL TO COMPREHEND THE DIFFERENCE BETWEEN
THERAPEUTIC SUCCESS AND FAILURE**

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INTRODUCTION: New drugs interfering with specific signal transduction cascades in endothelial cells during inflammatory and angiogenic processes in tumor growth show great therapeutic potential (1). One major hurdle in the development of these drugs is the difficulty to extrapolate *in vitro* data to the *in vivo* situation (2). Endothelial cell behaviour is strongly controlled by their local microenvironment (extracellular matrix make up, local balances in stimulatory and inhibitory growth factors and cytokines, blood flow induced shear stress) and physical interaction with blood borne cells (3). These conditions cannot be mimicked in *in vitro* cell culture model systems.

AIM OF THE STUDY: To develop experimental tools enabling analysis of endothelial cell behaviour and responses to drugs on a molecular level in their actual environment *in vivo*.

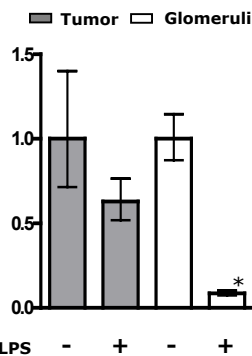
METHODS: 5 µm cryostat sections of B16.F10 tumor and kidney from C57bl/6 mice, either untreated or exposed to endotoxin to induce an acute systemic pro-inflammatory status of the endothelium, were mounted on 1.35 µm polyethylene-naphthalene membranes (P.A.L.M. Microlaser Technology AG). After drying, acetone fixation, and staining with hematoxylin, cells were dissected using the Laser Robot Microbeam System (P.A.L.M.), total RNA was extracted and DNase treated. After cDNA synthesis, quantitative RT-PCR (qRT-PCR) analysis of genes of interest was executed by an ABI Prism 7900HT Sequence Detection System (Applied Biosystems) using Assay on Demand or in house developed primers. Threshold cycle Ct values were analysed using standard software.

RESULTS:

- I. RNA isolated from dissected cells was shown to be intact using RT-PCR analysis for the endothelial marker CD31. The two primer sets, one amplifying at the 5' end and one at the 3' end of the transcript showed similar hybridization signals (data not shown).
- II. Ct values of CD31 versus GAPDH qRT-PCR demonstrate **enrichment of microvascular bed endothelial RNA in microdissected samples.**

Endothelial subset:	~ # dissected structures	Ct GAPDH	Ct CD31
Arteries	30	36.3 ± 0.2	33.3 ± 0.2
Veins	160	31.3 ± 0.2	36.8 ± 1.1
Glomeruli	560	28.3 ± 0.4	28.4 ± 0.2
Control:			
Whole kidney RNA	(whole section)	22.5 ± 0.2	27.6 ± 0.01

III.



Tumor vascular endothelium respond differently to pro-inflammatory stimulus than glomerular endothelium with regard to CD31 expression regulation.

Figure 1: RNA from microdissected tumor endothelial cells and glomeruli from untreated and 4h LPS treated mice were analysed by qRT-PCR for CD31 and β 2microglobulin (B2M). Relative gene expression (y-axis value) was calculated based on the comparative Ct method. Values are mean \pm s.d., n=3.

CONCLUSIONS: The protocols to isolate intact RNA from laser-microdissected microvascular endothelial subsets enable us to study the molecular effects of drugs on their behaviour in their patho(physio)logical context *in vivo*. These technologies facilitate analysis of complex endothelial gene expression profiles in pre-clinical animal tissues as well as in tissue biopsies obtained during clinical testing of new drugs. Only by this means the true *in vivo* pharmacologic effects of drugs on endothelial cell behaviour can be unravelled.

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