University of Fribourg / Cotutelle de thèse University Freiburg im Breisgau / Department of Biology

Influence of the Microenvironment on the Proteome of Skin Fibroblasts

Regine Tölle

The cellular microenvironment is a highly specialized compartment, which is in constant dynamic interplay with its residing cells. It contains structural extracellular matrix (ECM) proteins that provide mechanical support and store soluble growth factors, cytokines and chemokines. The cells strengthen the matrix by secreting matrix proteins or matrix crosslinking proteins, or weaken it by secreting proteases. Connective tissue, i.e. stroma, is particular rich in ECM, with fibroblasts being the main stromal cell type. Cancer-associated fibroblasts (CAF) are tumor stroma-residing fibroblasts that modify the tumor microenvironment and promote cancer cell migration and invasion. CAF contract the ECM and secrete proteases, which facilitates cell migration. To study CAF biology, matrigel-based 3D cultures are commonly employed, since the composition of matrigel resembles that of basement membranes and the activation of the cells is quantifiable by gel contraction. How CAF remodel the ECM and promote tumor progression remains to be understood. Here, a protocol was established to culture SILAC-labeled CAF in a collagen I/matrigel matrix, which can subsequently be subjected to quantitative mass spectrometry analysis. This strategy allows a simultaneous analysis of the cellular proteome, as well as of the ECM secreted by the cells. Cells cultured in 3D showed increased expression of distinct protein groups as compared to classical 2D cell cultures, highlighting the importance for organotypic cultures to study *in vivo*-relevant cell-ECM interactions.

A cancer-promotive role of fibroblasts is also discussed in recessive dystrophic epidermolysis bullosa (RDEB), which is a genetic skin fragility disease caused by mutations in the gene *COL7A1*. The resulting loss of collagen VII leads to loss of dermal-epidermal adhesion and altered ECM. RDEB fibroblasts resemble CAF and patients develop aggressive squamous cell carcinoma. To elucidate the influence the diseased ECM has on the cellular proteome, ECM of healthy fibroblasts and RDEB fibroblasts was decellularized and re-colonized with SILAC-labeled fibroblasts of RDEB and healthy donors, respectively. This allowed the discrimination of cell-intrinsic perturbed pathways from ECM effects.

Loss of dermal-epidermal adhesion in RDEB frequently leads to chronic wounds and fibrosis. Proper wound healing is mediated by platelet-derived growth factor (PDGF), yet PDGF signaling dysregulation has been linked to fibrosis and cancer progression. However not much is known about PDGF receptor (PDGFR) signaling in RDEB. Here, PDGFR induced phosphotyrosine-based signaling was analyzed by mass spectrometry in RDEB and healthy fibroblasts. A general reduced tyrosine kinase signal induction in RDEB was observed, along with increased E3 ubiquitin ligases Cbl-b and c-Cbl protein levels and activation, which might lead to a diminished MAPK signaling upon PDGFR stimulation in RDEB.

Taken together, the protocols established in this study allow further mechanistic studies of ECM-cell interactions. The quantitative proteome analyses of fibroblasts residing in different ECM provide new insights into the crosstalk between ECM and cells. We identify a potential ECM independent dysregulation of TGF-β signaling in RDEB fibroblasts as well as perturbed PDGFR signaling, which might play a role in delayed wound healing.

Jury:
Prof. Jörn Dengjel (thesis supervisor, Fribourg),
Prof. Thomas Reinheckel (thesis supervisor, Freiburg im Breisgau),
Prof. Ulrich auf dem Keller.(external co-examiner),
Prof. Matthias Boll (president of the jury, Freiburg im Breisgau)