Dominika Zacharski

Summary

The epidermis is a dynamic renewing tissue, principally formed by highly specialized keratinocytes. During a carefully choreographed program of differentiation keratinocytes undergo complex morphological and biochemical changes (Eckert *et al*., 2004).

As chitooligomers, which are β-1,4-linked oligomeric glucosamins, were shown to stimulate cellular differentiation (Deters *et al*., 2008), also β-D-glucans can be hypothesized to induce human keratinocyte differentiation. The aim of this study was to identify glucans as inductors of cell differentiation, to establish an *in vitro* test system for distinct structure-activity relations and to identify the molecular targets of differentiation inducing glucans in keratinocytes.

Using a combination of microarray analysis and qPCR for quantification of differentiation-specific genes, western blotting for differentiation-specific proteins and laser-scanning microscopy for verification of results on cellular differentiation two glucans were identified with a high and promising potential for inducing cellular differentiation.

Lichenan (β-1,3/1,4-glucan) from *Cetraria islandica* and xyloglucan (β-1,4/1,6-glucan with defined xylose and galactose side chains) from *Tropaeolum majus* turned out to be inductors of epidermal differentiation in a concentration-dependent manner (10 and 100 µg/mL) as determined by the differentiation-specific marker proteins cytokeratin 10 and involucrin on protein level.

Microarray analysis revealed p38 MAPK signaling and different cytokines to be likely involved in the signaling pathways induced by lichenan and xyloglucan.

Both polysaccharides were FITC-labeled for further investigations to gain more information about the molecular interaction of the glucans with cell surface structures or subcellular compartment structures.

Preparations of soluble and membrane protein fractions from keratinocytes were analyzed in a dot blot assay concerning binding of FITC-labeled glucans (FLG). A clear

association of the FLG with the membrane protein fraction was observed. Subsequently the membrane protein fraction was separated by polyacrylamide gel electrophoresis, blotted and incubated with the FLG showing strong binding of FLG to several distinct bands or spots which were excised and subjected to MS analysis. Results indicate i. a. cell adhesion-related proteins like integrins, EGFR and actin to be involved in binding of FLG as well as a member of the Ras signal transduction pathway and protein disulfide isomerase A3. Furthermore, xyloglucan – containing galactose residues – showed binding to galectin-7, a protein with a binding specificity for β-galatoside sugars.

Thus, this thesis presents lichenan from *C. islandica* and xyloglucan from *T. majus* as the first β-glucans which were identified as inductors of cellular differentiation in human keratinocytes and provides information of potential molecular targets and signaling pathways of the polysaccharides as basis for further detailed investigations.