Summary

The skin is one of the most complex organs in the human body. It is a barrier, which protects the body against environmental influences. An optimal balance between proliferation and differentiation of human keratinozytes is essential for preserving a stable barrier function. Due to a dysbalance, resulting in skin diseases like psoriasis or ichthyosis or skin injuries the barrier can lose its protective character. Recovering the healthy state by using substances that can influence or support the differentiation and/or proliferation process may represent an optimal treatment strategie. New active compounds are therefore necessary.

In this project, several natural products were analysed regarding their effect on terminal differentiation and three compounds were pinpointed for further detailed investigation.

The polysaccharides Xyloglucan (XG) form *T. majus* L., the semisynthetic γ-Propoxysulfolichenan (γ-PSL) (based on Lichenan from *C. islandica* L.) and the quinoid diterpene Cryptotanshinone (CTS) from *S. miltiorrhiza* Bunge. were investigated in detail concnerning the respective influence on keratinocyte cell physiology.

Previous studies have shown that XG induces the process of terminal differentiation on gene and protein level, but the underlying mechanism was not understood until now. The present work indicated that XG interacts with the cell surface by blocking the epidermal growth factor receptor (EGFR), which reduces the activity of *cAMP* response element-binding protein (CREB). Furthermore, the signals for terminal differentiation of human keratinocytes is induced by XG on gene and protein level. Similar effects ca be observed for lichenan and its semi-synthetic structure derivative γ-PSL.

γ-PSL (100 µg/mL) induced the terminal differentiation on protein and gene level within 7 days. The marker proteins involucrin, cytokeratin 1 and 10 were used for charcterisation of the differentiation process. The mode of action of γ-PSL is still unclear, but it it seems that it is comparable to the way XG interacts with the keratinocytes.

In contrast to the previous mentioned polysaccharides, the diterpene CTS from *S. miltiorrhiza* influenced the process of differentiation completly different. CTS (1 µM) acts as a selective inhibitor of the Cytokeratin 1 and 10 expression. This was proven on protein level by Western Blot analysis as well as on gene level by qPCR. Other differentiation specific proteins as involucrin, fillagrin, loricrin and transglutaminase 1 were not affected. The effect of CTS and the transforming growth factor β (TGFβ) on the cell cycle (NHEK) and cell migration (HaCaT cell line) was investigated by use of PI-staining/flow cytometrie and scratch assay. The keratinocytes were just slightly influenced by both substances, but CTS depicted an opposite direction regarding its effect on the cell behavior compared to TGFβ. Using a combination of Drug Affinity Response Target Stability Assay, a proteomic approach and bioinformatic methods for target fishing the peptidyl-prolyl-cis-trans-isomerase FKBP1A (the target of inhibitors as Tacrolimus or Rapamycin) was addressed as potential molecular target of CTS. The interaction of CTS with FKBP1A was additionally proven by thermal shift assay and enzymatic activity assay of the enzyme. Interestingly CTS served as activator of FKBP1A, which leads to a reduced activity of the TGFβ receptor pathway and therefore to a diminished Cytokeratin 1 and 10 expression. From these data a potential dermatological use of CTS and CTS-containing plant extracts (e.g. hydroalcoholic extract from the roots *of S. miltiorrhiza*) for keratinopathic ichtyosis, a disease characterized by overexpression of cytokeratins 1 and 10, is discussed.

In summary, the present work shows how natural compounds can influence the complex process of differentiation of human keratinocytes. The study showed that on the one hand it is possible to trigger keratinocytes into the terminal differentiation by interaction of XG and γ-PSL with the cell membrane and on the other hand, that the process can be disrupted by the low molecular compound CTS, which interacts on a cytosolic level by boosting the activity of FKBP1A. This leads to a manipulated signal cascade involving the TGFβ pathway.