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Institutet**

Institutionen för Medicin, Enheten för Reumatologi

STUDIES ON MOLECULAR REGULATION OF INFLAMMATION IN CUTANEOUS LUPUS ERYTHEMATOSUS

AKADEMISK AVHANDLING

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ABSTRACT

Lupus erythematosus (LE) is an autoimmune disease with a wide range of clinical manifestations. This spectrum spans from limited cutaneous disease to life-threatening rheumatic disorder involving vital organs. Sun exposure is an evident exogenous trigger of both cutaneous (CLE) and systemic LE (SLE). CLE-resembling skin lesions can also be experimentally induced using artificial ultraviolet radiation (UVR). Skin is an organ which is physically available for clinical observation and biopsy acquisition. This provides a possibility to relate the clinical appearance of developing and healing lesions to the molecular and cellular events observed in the skin specimens.

In the studies included in this thesis we aimed to define the molecular regulation of cutaneous inflammation in CLE and to evaluate if a standardized photoprovocation is a suitable method to study CLE in a multicenter study. Firstly, we explored what cytokines are involved in the regulation of inflammation in UVR-induced CLE lesions; secondly, we investigated if the autoantigen Ro52 is expressed in UVR-induced and spontaneous CLE lesions and lastly, if UVR and reactive nitrogen species (NO) could modulate the expression of autoantigen Ro52 in the LE-target cell keratinocyte. We also wanted to examine which domains within the Ro52 protein that determine its subcellular localization, and if Ro52 can interact with ubiquitin conjugating enzymes residing in different cell compartments.

To achieve our goals we used skin biopsy material derived from spontaneously occurring CLE lesions, and also a longitudinal collection of cutaneous specimens acquired from experimentally UVR-induced LE-specific lesions. We established patient- and healthy control-derived primary keratinocyte cultures in order to investigate Ro52 expression under the influence of UVR and NO. Furthermore, by constructing green fluorescent protein-Ro52 (GFP-Ro52) mutants and transfecting HeLa cells with them, we investigated the sequences of importance for subcellular localization of this autoantigen.

In paper I we demonstrated that a standardized photoprovocation allows inducing CLE-resembling lesions in approximately half of the patients and is a safe and reproducible method suitable for multicenter studies.

In paper II we demonstrated that HMGB1, an alarmin with cytokine-like functions, is upregulated and translocated to the extracellular space in UVR-induced CLE lesions, and that its highest expression coincides with the peak of clinical activity of the lesions. Other investigated cytokines TNF- α and IL-1 β seemed to be of less importance.

In paper III we showed that Ro52 is strongly expressed in the epidermis and dermis of spontaneous and UVR-induced CLE lesions and is predominantly located in the keratinocyte cytoplasm. Moreover, our results of *in vitro* experiments indicate that UVR can upregulate the expression of this autoantigen in the cytoplasm of keratinocytes.

In paper IV we determined that NO can modulate the subcellular localization of Ro52 in human keratinocytes and HeLa cells *in vitro*. We have also demonstrated that Ro52 is expressed in close proximity to iNOS and is located in both cytoplasmic and nuclear compartments of the cells present in CLE skin lesions. In addition, we proved that the sequence located within the leucine zipper/coiled coil domain of Ro52 is the one that retains the protein in the cell cytoplasm while the B30.2 domain is important for the nuclear translocalization of Ro52. We have also demonstrated that Ro52 can interact with both nuclear and cytoplasmic ubiquitin conjugating enzymes.

In conclusion, the studies presented in this thesis provide novel insights into the molecular events that occur in the skin of CLE patients during lesion development. Our findings indicate that UVR and NO can modulate the expression of the autoantigen Ro52 in keratinocytes, which are the target cells of autoimmunity in LE. We demonstrate that standardized photoprovocation is a safe and reproducible method to study UVR-induced CLE in multicenter studies.