„The role of Hsp90 in atopic dermatitis"

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Heat shock proteins (HSP) are a highly evolutionarily conserved family of molecular chaperones and proteases present in all studied prokaryotic and eukaryotic cells. Heat shock proteins are classified into families by their molecular weight expressed in kilodaltons (kDa) and include: HSP100, HSP90, HSP70, HSP60, HSP40 and sHSP (small heat shock proteins). Both their adenosine triphosphate -dependent and -independent activity promotes protein maturation, refolding of denatured proteins, stabilizing native protein structures, degradation of misfolded proteins and facilitates peptide transport. In the first half of the 90's the N-terminal domain of HSP90 was recognized as a potential molecular target in cancer. Current research implicates HSPs in the pathogenesis and progression of noncommunicable inflammatory and autoimmune disorders. HSP90 is responsible for the proper function of over 300 proteins, including kinases, ubiquitin ligases, steroid receptors, cyclins and transcription factors, most of which are crucial in the cell's survival, growth, differentiation and apoptosis. In mammals four main isoforms of HSP90 have been described: GRP94 (in the endoplasmic reticulum), TRAP-1 (in mitochondria), and two localized in the cytoplasm: HSP90α and HSP90β. HSP90β expression is constant whereas HSP90α can be induced by various stressors, such as oxidative stress, UV radiation or hyperthermia. Furthermore, HSP90α can be released to the extracellular milieu (eHSP90α), where it acts as a signal transducer and immunomodulating factor, its functions include wound healing, angiogenesis and metastasis. Moreover, eHSP90α can induce humoral response leading to the production of anti-HSP90 antibodies which are elevated in some autoimmune diseases. HSP90's chaperone activity inhibitors, namely 17-AAG and STA-9090, bind to the NTD region with higher affinity than ATP, which leads to the proteasomal degradation of HSP90's protein substrates. Immunosuppressive effects of such inhibition could arise due to: activation of transcription factor HSF-1, which regulates immunoregulatory gene expression i.e. HSP70 and IL-10; expansion of immunosuppressive populations of regulatory B and T cells; inactivation of NF-κB-dependent proinflammatory molecules; and the inhibition of signaling pathways such as JAK-STAT or MAPK.

Atopic dermatitis (AD) is one of the most common chronic inflammatory skin diseases, with reported global incidence of 15-30% in children and 2-10% in adults. AD is classified as an allergic disease characterized by intense neuropathic itch and recurring skin lesions in the form of erythema, dryness and erosion. AD pathogenesis is not clearly understood, however disruption of skin barrier integrity plays a crucial role. One of the better understood genetic factors underlying AD are filaggrin mutations. Filaggrin is a crucial skin barrier protein, whose impaired activity or lowered expression leads to overactive immune response which in turn leads to inflammation which further damages the skin, leading to the imbalance of the skin microbiome and facilitated allergen penetration. Various cell populations, including helper T cells Th1, Th2 and Th17, participate in AD pathogenesis, their excessive activation leads to the increased production of proinflammatory cytokines. T cell activation also promotes the proliferation, migration and activation of eosinophils. Keratinocytes, fibroblasts and dendritic cells also play a significant role, as they secrete numerous cytokines, mediators, chemokines and alarmins. In approximately 80% of AD patients, elevated levels of circulating IgE which can activate mast cells, are observed. Current treatment of AD is individualized and aims to restore the skin barrier function, reduce inflammation and relieve pruritus. It includes the use of emollients, topical corticosteroids or calcineurin inhibitors, with systemic immunosuppression or novel biological therapeutics reserved for more severe cases. Despite the effectiveness of symptomatic treatment, none of the current approaches constitute a cure for AD, and their use is associated with a risk of adverse effects.

The aim of this dissertation was to investigate the role of HSP90 in the development of AD and to evaluate the effect of HSP90 inhibition, using STA-9090 and 17-AAG, on inflammatory processes characteristic of AD, both *in vitro* and in a murine model of the disease.

In the course of this study, a significantly higher concentration of eHSP90α and anti-HSP90α IgE antibodies was discovered in the serum of patients with AD compared to a closely matched control group. Furthermore, a positive correlation was observed, between eHSP90α concentration and the severity of the disease symptoms assessed using the SCORAD (Scoring Atopic Dermatitis) index. The specificity of eHSP90α as a biomarker

of AD was confirmed through comparative analysis with dermatitis herpetiformis (DH, Duhring's disease), which is relevant for differential diagnosis. It was noted that 83.9% (26/31) patients with AD had elevated eHSP90α levels, as compared to 0% (0/26) of patients with DH. Further experiments used a murine model of AD induced by topical application of 1-chloro-2,4-dinitrobenzene (DNCB). Intraperitoneal administration of STA-9090 led to a significant reduction in disease activity (as assessed by SCORAD index), a decrease in epidermal hyperplasia, reduction in leukocyte infiltration, and the amelioration of pruritus. Topical application of STA-9090 also lowered disease activity, decreased serum IgE levels, and increased filaggrin expression in the skin. Both therapies were well-tolerated and did not produce observable adverse effects. Due to greater efficacy and therapeutic potential, further preclinical studies focused on topical inhibition of HSP90 chaperone activity using 17-AAG. This approach significantly reduced disease activity, epidermal hyperplasia, lowered the expression of TSLP, IL-5, IL-6 and NF-κB activity. A reduction in the number of circulating eosinophils and in eosinophil peroxidase (EPX) activity in the skin was also detected. This limiting effect of 17-AAG on the levels of cytokines and alarmins characteristic of the Th-type immune response, and reactive oxygen species production was confirmed *in vitro* in appropriately stimulated cultures of human keratinocytes, $CD4^+$ T lymphocytes, and eosinophils. Notably, next generation sequencing approaches revealed that the 17-AAG topical therapy partially restores gut microbiome balance in AD-like mice. Furthermore, it was demonstrated that 17-AAG reduces biofilm formation capabilities of *Staphylococcus aureus* – an opportunistic pathogen studied extensively in AD due to its role as a source of superantigens and a factor exacerbating disease severity. Increased HSP90 activity (=deacetylation) and elevated EPX activity were observed in leukocytes isolated from AD patients. Additional *in vitro* investigations revealed that the inhibition of HSP90's chaperone activity leads to increased acetylation of HSP90α, which may partially explain the observed immunomodulatory and therapeutic effects.

These results, for the first time, demonstrate a significant role of HSP90 in the pathogenesis of atopic dermatitis. The diagnostic potential of eHSP90α as a biomarker of AD and the symptom-alleviating therapeutic potential of HSP90 inhibition, especially in the form of topical therapy, cannot be overstated. This provides new avenues for the development of more effective, molecularly targeted therapies and diagnostic tools in the treatment of AD.