Session 2: Identification and production of active pharmaceutical ingredients

Target identification: general aspects and sphingosine-1-phosphate as an example

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Despite the increasing emphasis on proteomics in target identification, DNA microarray technology is still a powerful technique for identifying genes involved in susceptibility to diseases. Thus, differentially expressed genes may lead to identify genes that play key roles in disease pathways. As an example, up-regulation of sphingosine 1-phosphate phosphatase (SPP) was detected in samples of lesional skin of patients with psoriasis, whereas an up-regulation of sphingosine 1-phosphate lyase (SPL) was detected in patient with atopic dermatitis. It is of interest that both enzymes may lead to a degradation of the lipid mediator sphingosine 1-phosphate (S1P) suggesting that a decreased level of S1P is related in the biology of these diseases. Nevertheless, mRNA does not always correlate with production of protein, and even if more protein is produced it may not be active because it requires post-translational modification or relocalization. Therefore it is of interest to measure also levels of S1P in such patients. Indeed, in correlation with an enhancement of mRNA levels of S1P-degradadation enzymes, S1P levels are significantly decreased in patients with atopic dermatitis. One of the biggest challenges in drug target identification is to understand the underlying physiology of drug targets. The relevance of S1P in cellular processes has been emphasized by identifying its function as a ligand on a family of G-protein coupled receptors (GPCRs) termed as S1P1-5.. S1P exerts diverse cellular effects depending on the expression of the specific S1P receptor subtypes and their coupling to separate G proteins. But the role of S1P in skin cells has not been examined very well. Therefore, a characterization of the expression profile of S1P receptors in skin cells has been performed. Indeed, all investigated cell types including keratinocytes, fibroblasts and dendritic cells show a significant mRNA expression of all five S1P receptor subtypes. Moreover, the complex biology of S1P in skin cells has been explored in an efficient manner indicating that S1P is a crucial molecule in the homeostasis of both keratinocytes and dendritic cells. Thus, S1P has been identified as a bioactive molecule in keratinocytes as it inhibits their growth and initiates differentiation. Further experiments by downregulation of S1P receptors and the use of agonists/antagonists clearly indicate that the S1P2 receptor is dominantly involved in the S1P-induced keratinocyte growth arrest. Dendritic cells play a pivotal role in inflammation as they carry haptens from the skin through afferent lymphatic vessels to draining lymph nodes, where these haptens are presented to T cells. Our results indicated that S1P inhibits the function of dendritic cells leading to a decreased immune response. It is of interest that the S1P1 receptor subtype is essential to mediate this action. Thus,

it seems likely that S1P is an appropriate candidate for the treatment of hyperproliferative and inflammatory skin diseases. To prove this hypothesis, animal models of psoriasis and contact dermatitis were performed. Indeed both models indicate that S1P may beneficial in the treatment of inflammatory skin diseases.