THE NON-CODING RNA, PRINS AFFECTS AIM2 INFLAMMASOME ACTIVATION IN KERATINOCYTES

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The non-coding RNA, PRINS was previously described by our research group as a differentially expressed transcript in psoriatic uninvolved and healthy skin. The expression level of PRINS in cultured keratinocytes is altered after exposure to various stress factors and silencing of it decreases the viability of keratinocytes during stress stimulations suggesting its role in stress response of the cells. A potential stress signal in psoriatic skin may be the extracellular DNA, which activates the AIM2 inflammasome. The activated inflammasome cleaves the precursor proIL-1β form into ma-ture, functioning IL-1β. The role of the AIM2 inflammasome and the IL-1β cytokine in psoriasis has been described re-cently.

The aim of our study was to investigate if the PRINS non coding RNA affects the expression and activation of the inflammasome members and IL-1β in normal human epidermal keratinocytes (NHEK) after exposure to extracellular DNA.

The expression of PRINS was transiently silenced by a vector based RNA interference method in cultured NHEK cells. Silenced and non-silenced NHEK cells were primed with the cytokines TNF-α and IFNγ and transfected with the synthetic DNA analogue poly(dA:dT). The expression of PRINS and inflammasome members was detected by real-time RT-PCR and the secreted IL-1β was measured by ELISA.

Poly(dA:dT) treatment caused a moderate increase in PRINS expression and IL-1β secretion as well, whereas priming with a combination of TNF-α and IFN-γ before poly(dA:dT) transfection resulted in a highly elevated PRINS expression and higher secreted IL-1β levels. The silencing of PRINS decreased the amount of secreted IL-1β, but did not affect the expression of the proIL-1β or AIM2.

Our results suggest that the PRINS non-coding RNA regulates the IL-1β production of NHEK cells, but not through the regulation of proIL-1β expression, rather contributing to inflammasome-activation.

DEVELOPMENT OF A NEW METHOD FOR PERMEABILITY MEASUREMENTS

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Background: Increased endothelial cell permeability plays an important role in the pathomechanism of several immunologically relevant diseases, for example in hereditary angioedema, allergy, or sepsis. Numerous methods are known for measuring the permeability changes, such as the protein transport through transwell inserts, impedance measurement, or immunostaining of adhesion molecules, just to mention a few. These methods are widely accepted and used, but expensive, unreliable or labor-intensive. To overcome these problems, we aimed to develop a cheap, simple and highly reliable technique, which allows to test several samples simultaneously.

Methods: In our experiments, we used confluent human umbilical vein endothelial cell (HUVEC) culture as a model. HUVECs were prepared from fresh umbilical cords, and were kept in AIM-V medium, completed with FCS, EGF and bFGF. VE-cadherin, PECAM-1 and ZO-1 adhesion molecules were stained with the corresponding primary antibodies, followed by fluorescently labeled secondary antibodies. Samples were observed and images were recorded using an Olympus IX-81 inverted fluorescence microscope mounted with an Olympus XM10 digital camera.

Results: HUVECs were grown on 96 well plates precoated with biotinylated gelatin. After treating with a given agonist, we added a strepatvidin-Alexa 488 (a straptavidin conjugated fluorescent dye) to each well, which tightly binds to the biotinylated gelatin in the gaps between endothelial cells. After paraformaldehyde fixation, paracellular gaps were visualized using fluorescent microscopy. To evaluate these images, we performed quantitative image analysis to define the size of the stained area on each photograph. The new method was validated by known permeability-increasing factors; thrombin and bradykinin, which increased the size of the stained area as expected. To confirm our permeability test results, we stained key adhesion molecules (VE-cadherin, PECAM-1, ZO-1) after thrombin and bradykinin treatment, which showed that endothelial cells became separated from each other.

Conclusion: Consistent with the literature data, thrombin and bradykinin increased endothelial permeability in our novel permeability test. We could also confirm our results by immunostaining of adhesion molecules, therefore we found the new method suitable for permeability measurements. Based on our results, we can conclude that we successfully