Ruprecht-Karls-Universität Heidelberg

Medizinische Fakultät Mannheim

Dissertations-Kurzfassung

Establishment and characterization of an in vitro coculture model to study functional interaction between porcine keratinocytes and sensory neurites involved in cutaneous nociception

Autor: Leslie Ponce Moya

Institut / Klinik: Institut für Molekular- und Zellbiologie der Hochschule Mannheim

Doktorvater: Prof. Dr. M. Hafner

Cutaneous nociception was defined for more than hundred years ago by Sherrington, who postulated: "It is fair to infer that the species of nerve-ending excited is that which may be termed the nocicipient, and certainly that division of it which lies in the more superficial layers of the skin" … "These nerve-endings when still connected with the sensorium (using that term simply to mean the neural machinery to which consciousness is adjunct) on excitation evoke skin pain". With those statements, he recognized the cellular machinery and the nature of the stimulus that are able to evoke the sensation of pain. Nowadays, it is known that activation of sensory receptors in the skin triggers a cascade of reactions that end with the sensation of pain. Dorsal root ganglion (DRG) neurons express a family of transmembrane receptors (thermoTRP) responsible for sensing noxious stimuli (i.e. TRPV1). Thus, peripheral nerve endings encode those stimuli into action potentials and send the information to the central nervous system where it is integrated and transmitted back to the skin to generate a response. Moreover, keratinocytes convey in close contact with those peripheral nerve endings in the skin, secrete neuromodulatory substances and express some of those receptors as well (i.e. TRPV1, "pain receptor").

In this study, a reliable system for the investigation of functional interactions between sensory nerve endings and keratinocytes was developed. Therefore, a compartmented culture chamber was employed for the establishment and characterization of a coculture of porcine DRG neurons and porcine keratinocytes. The compartmented chamber allowed the spatially segregation of neurites from their somata, differential medium supplementation between compartments and coculture of keratinocytes and nerve endings distant from the somata. Prior to the development of the coculture, keratinocytes were successfully isolated from pig skin and a cell culture procedure for porcine keratinocytes was established. The keratinocyte cell culture was characterized using assays based on conventional and fluorescence microscopy. Porcine keratinocyte isolation reached a yield of $1{,}73 \times 10^6 \pm 16$ % viable cells per cm^2 tissue with a viability of $88{,}01 \pm 2{,}04$ %. Human collagen type I and EpiLife keratinocyte growth medium supported a clonal growth of keratinocytes seeded at a density of 500 viable cells/cm^2 (P0) and 100 viable cells/cm^2 (P>0). Population doubling (PD) and generation time (tg) values were plating density dependent. Keratinocyte differentiation was induced with either 1 mM calcium or 5% serum. The results showed that optimal isolation and culture conditions for keratinocytes from piglets differed from those of adult pig and of human tissue.

The investigation of the nociceptive nature of DRG neurites and keratinocytes in monocultures was performed by the application of 1 µM capsaicin and recorded by live cell imaging of cytosolic calcium increases. Capsaicin-dependent TRPV1 activation in NGF-dependent neurons reached a level of 87,2 % (34 out of 39; 4 animals) whereas the GDNF-dependent population reached a level of 67,4 % (29 out of 43; 3 animals). Thus, the proportion of responsive neurites in the peptidergic population (NGF) was significantly higher compared to the non-peptidergic population (GDNF) ($p < 0.05$). Capsaicin-responsive keratinocytes only reached a level of 2,6 % (34 out of 1441).

Atomic force microscopy (AFM) combined with fluorescence live cell imaging allowed to mechanically stimulate single cells and to monitor transient calcium changes simultaneously. The induced calcium responses in DRG neuron monocultures demonstrated for the first time the ability of that approach to mechanically stimulate single cells and simultaneously to detect signal propagation to surrounding neurons. In cocultures, close contact between keratinocytes and neurites was topographical visualized using AFM.

Finally, functional interaction between keratinocytes and sensory neurites was investigated by a i) chemical activation of neurons or ii) mechanical activation of keratinocytes in the coculture system. Thus, i) depolarizing concentrations of KCl were applied to the somata in the central compartment and resulted in a transient calcium increase in neurites and adjacent keratinocytes in the lateral compartment. ii) AFM-epifluorescence imaging approach was successfully implemented in the coculture resulting in a precise mechanical activation of single keratinocytes and resulted signal propagation in surrounding neurites and keratinocytes. Altogether, both results confirmed a direct cross-talk between sensory nerve endings and keratinocytes.

Further investigations are needed to clarify the role of keratinocytes in cutaneous nociception, which can be achieved using the compartmentalized coculture system described in this work. They should focused on elucidating the mechanism by which neurites and keratinocytes communicate with each other and on classifying the involved mediators.