AKTION ÖSTERREICH - TSCHECHISCHE REPUBLIK

Wissenschafts - und Erziehungskooperation

AKTION ČESKÁ REPUBLIKA - RAKOUSKO

spolupráce ve vědě a vzdělávání

PROJEKT ABSCHLUSSBERICHT / ZÁVĚREČNÁ ZPRÁVA

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***Projektbezeichnung** / **Název projektu**:*

A morphological study assessing the influence of the TNF-derived tip peptide on Na+ channels and membrane characteristics in human epithelial cell lines

Studie vlivu TNF tip peptidu na sodíkové pumpy a morfologii membrán lidských epiteliálních buněčných linií

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Projektdauer von / Trvání projektu od: ***1.June 2006*** *bis / do*: ***31.December 2006***

Beantragte Förderung / Požadovaná podpora:

EUR insgesamt / EUR celkem: 3650 EUR

Kč insgesamt / CZK celkem: 22000 CZK

A morphological study assessing the influence of the TNF-derived tip peptide on Na+ channels and membrane characteristics in human epithelial cell lines

The project aimed at providing financial means to start up a joint scientific and educational venture that involved the Institute of Physical Biology of the University of South Bohemia and the University of Applied Sciences in Krems. Scientific dimension of the project builds upon the evidence recently provided by the project partner Dr. Rudolf Lucas, that the TNF-derived Tip peptide is able to activate lung liquid clearance in healthy lungs and upon the highly desired need for a further pre-clinical evaluation of this substance as an alternative anti-edema agent. In pursuing our scientific goals, we have used the state of the art confocal microscope, atomic force microscope, fluorescence microscope, fluorescence activated cell sorting and Ussing chambers and implemented novel protocols for imaging surface features of live cells, localization of the channels, loci of interaction of the tip peptide and changes that accompany the action of the membrane-inserting bacterial toxin listeriolysin. The educational dimension of the project relied on the fact that both involved institutions provide training for under- and graduate students and thus they can benefit from joint activities both on theoretical platform of numerous general and highly specialised lectures, but also in granting the opportunity to work in their teaching laboratories. In reaching the committed goals, we have united our expertise, technological and methodological resources in a study of Na+ channels in the isolated cell membranes and live cells of the epithelial-like cells lines H441 and Calu-3. The cells lines were initially cultivated in Krems. Later, upon receiving the training in tissue culture, students introduced the know-how to Nové Hrady. Immobilization and labeling of the cells for imaging was trained and perfected in Nové Hrady and consecutively, the know-how was transferred to the partner's laboratory in Krems while cell membranes were isolated solely in Nové Hrady. Confocal imaging of epithelial cells was done in Krems whereas imaging with fluorescence microscope was completed in Nové Hrady. Finally, scientifically valuable results of the AKTION project were exploited in preparation and realization of course of lectures and practical training of "Methods in cell biology" held in Nové Hrady.
This AKTION project assisted in building up and strengthening the collaboration among the research groups based in Krems and the South Bohemia University in Nové Hrady. Funding was provided mostly for the networking of the two partners laboratories, for the doctoral student Alexander Dulebo and the principle investigator of the project David Kaftan's travel expenses and daily allowances while working in Austria. Accommodation and daily allowances were provided also for the project partner Dr. Rudolf Lucas and his students while visiting Nové Hrady. This project opened new opportunities for joint educational and research plans and will be continued on a wide scale. Namely, Dr. Rudolf Lucas has became formally an advisor for PhD student Alexander Dulebo of the IPB USB during the year 2006. Dr. Rudolf Lucas also presented a lecture on intracellular signalling and apoptosis as a part of the Dr. David Kaftan's course on methods in cell biology held in Nové Hrady. Students of the both institutions took part in preparation of the practical courses that accompanied the lectures. In the future, we will carry on with keeping the collaboration vital by lecturers and students exchange. Together with the developed protocols, the realized course provide knowledge and technological base for detection and characterization of single biomolecules in surfaces of biomembranes of living cells thus fulfilling the key tasks of the project.

Nové Hrady, 20. February 2007

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Ort, Datum, Unterschrift des Antragstellers
Místo, datum, podpis předkladatele

Krems, 21. February 2007

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Ort, Datum, Unterschrift des Projektpartners
Místo, datum, podpis partnera projektu

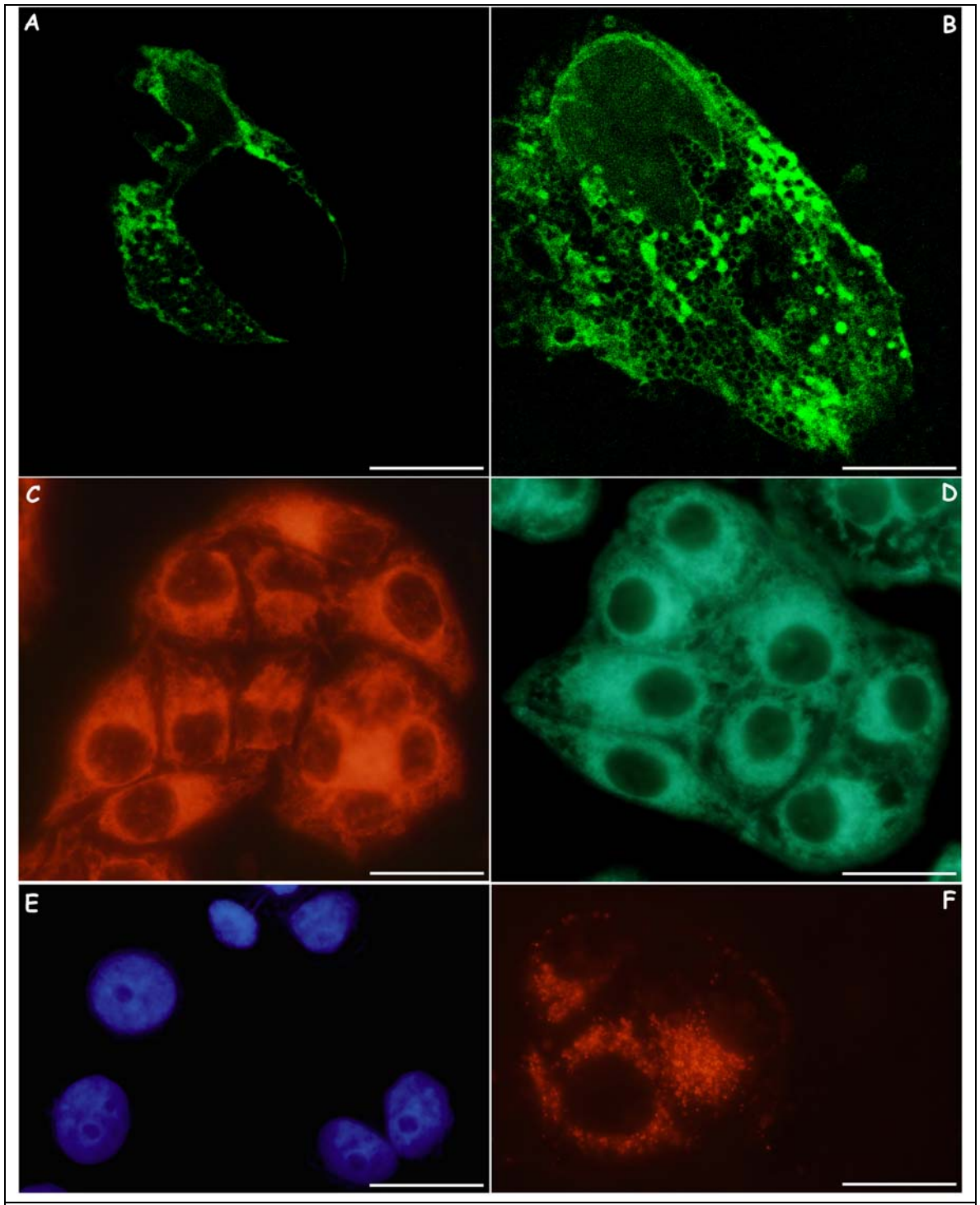

FIGURE. Imaging of H441 cells in a light microscope. A-B: confocal image of the cells labeled with a conjugate of fluorescein and amyloride that inactivates epithelial Na+ channels by binding to one of the subunits of the channel. A shows a cross-section in the upper part of the cell in an equatorial level of the nucleus. B shows labeling of the channel at the basal cell membrane. Both images show non-homogeneous distribution of the channel in small flat patches of cell membrane. The apparent dark circular pores in the images are due to indentations of the membrane (see the image of whole cell obtained by AFM). Groups of cells were also imaged in fluorescence microscope, under staining against mitochondria (C), lysosome (D), DNA (E) and endoplazmatic reticulum (F) to yield additional information about the physiological status of the cells. Scale bar represents 5 um in A and B, 10 um in C and D and 25 um in E and F

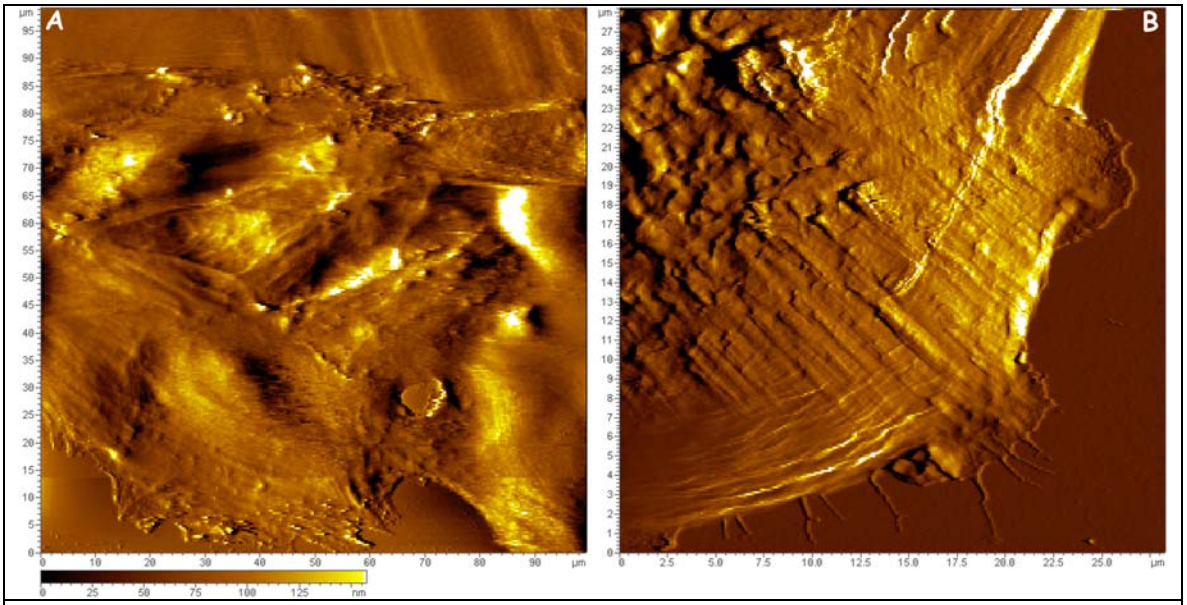

FIGURE 2. Imaging of human lungs epithelial cells (H441) in atomic force microscope. A: surface topography obtained in a contact mode AFM. Bright regions of the image represent high structures of the cells, namely the part containing nucleus. Surface indentations along with cytoskeletal elements are clearly visible in the image B.