STSM REPORT

STSM Title: Confocal microscopy evaluation of cutaneous tumors developed after occupational exposure

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Host: Research Division of Biology and Pathobiology of the Skin which is part of the Department of Dermatology from the University of Vienna Medical School.

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Skin cancer developed after occupational exposure is more frequent than recognised. I am interested in studying the development of precancerous and cancerous lesions in people exposed to occupational agents and also evaluation of subclinically affected skin. Early detection of subclinical changes at a cellular level and early treatment might help prevent the development of malignant lesions.

Confocal microscopy is a non-invasive imaging technique that analyses the morphology of the different skin layers. It can be used both in vivo, without having to perform a biopsy and in vitro, on biopsies of lesional tissues. Harvested tissues can be analysed by confocal laser scanning microscopy after imunostaining, a technique that allwos to reconstruct 3 dimensional images of specific cells. Visualising the image of previously stained cells and their relationships to each other in a 3D mode, can help achieve a better understanding of events that take place when various cutaneous lesions are developed.

Combination of in vivo and in vitro confocal assessment of the same cutaneous area or lesion could prove to be an excellent insight for premalignant changes that occur at cellular level.

The goal of the stay was to learn sample analysis using the technique of confocal microscopy for cutaneous inflammatory and malignant lesions.

Work carried out:

There I benefitted from hands-on training on preparing tissue samples for analysis with the confocal laser scanning microscope. The analysed samples were fragments of basal cell carcinomas, squamous cell carcinomas and inflammatory lesions that were brought from Romanian patients treated in the 2nd Department of Dermatology at Colentina Clinical Hospital in Bucharest, Romania.

I had hands-on training for every step of the process in order to obtain valid specimens for confocal laser analysis:

a. cutting paraffin blocks (thin sections of 5µm for immunofluorescence analysis and thick sections of 30 µm for confocal laser scanning analysis)

b. immunofluorescence staining of specimens. The basal cell carcinoma and squamous cell carcinoma samples were stained for keratins (keratin 2 and keratin 10) and endothelial markers.
   - The following sequence was used: heating and deparaffinization of samples, demasking of antigens, sequential incubations and washing of samples and controls for adequate staining with antibodies (double immunofluorescence staining – same sample stained with two antibodies)

c. validation of staining at the conventional 3 channel immunofluorescence microscope
   - after staining, the thin specimens were analysed at the immunofluorescence microscope in order to validate the markers for keratins and endothelium. Photo documentation of the samples was performed.

d. 3D analysis at the confocal laser microscope.
   - after validation of staining, the thick specimens were analysed at the confocal laser microscope.
   - basic training for confocal laser scanning consisted in review of method principles, calibration of the laser, choosing the appropriate parameters for analysis, performing various analysis patterns and building 3D images of the samples, with visualisation of keratins and vessels in the analysed tumours in conjunction with surrounding cells.

Benefits: I consider the visit at the Research Division of Biology and Pathobiology of the Skin in Vienna and the hands-on training on basic confocal microscopy useful for my future projects of correlating between clinical and confocal microscopy aspects of benign and malignant cutaneous lesions developed after occupational exposure.

As future research I want to investigate correlations between histopathological, in vivo and in vitro confocal microscopy aspects of cutaneous benign, malignant and premalignant tumours developed after occupational exposure to various agents. The goal is to establish a set of characteristics for in vivo laser microscopy in order to asses noninvasively skin tumours, precancerous lesions and subclinically affected skin in field cancerization, with evaluation of field margins, in an attempt of decreasing the number of biopsies needed, early treatment and evaluation of response to treatment.