STSM Report

STSM Title: Carcinogenesis in occupational skin diseases

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Host: Research Division for Skin Biology and Pathobiology Department of Dermatology Medical University of Vienna.,

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Background and objectives: In recent years, skin cancer incidence has been increasing. By far the most significant factor is the UV radiation. It was recently acknowledged that in outdoor works occupational sun exposure represents a major risk factor for skin cancer . Despite this, there are few reports of cases of occupational skin cancers, although the number of patients with UV induced skin cancer is increasing. For this reason effective ways to prevent these diseases are necessary.

Our purpose for participating in this STSM was to learn new techniques in the Research Division for Skin Biology and Pathobiology Department of Dermatology Medical University of Vienna, Vienna General Hospital, in order to be able to study carcinogenesis in occupational skin disease.

The objectives of my stay in the department were: to learn to retrieve antigens from paraffin embedded tissue, to perform immunofluorescence and double immunofluorescence staining on tissue sections and to analyze immunofluorescence staining on conventional immunofluorescence and laser scanning microscopy.

Work carried out: During my stay I got familiarized with different aspects of fundamental research which I plan to use for further research of carcinogenesis in occupational skin diseases.

Together with Professor Erwin Tschachler's team, I analyzed sections of paraffin samples from patients with basal cell carcinoma, squamous cell carcinoma. I prepared slides for analysis with conventional immunofluorescence microscope and with laser confocal microscope.

For conventional fluorescence microscope 5 µm thick sections were used whereas for the confocal microscope we learned to prepare and analyze "thick" sections (i.e. 30 µm) .

The samples were stained as following: squamous cell carcinoma- factor VIII, keratin 2, keratin 10; basal cell carcinoma- factor VIII.

In order to retrieve antigens from paraffin embedded tissue first we deparaffinized and rehydrated the sections, afterwards we heated the samples that were submerged in 250 ml citrate buffer solution 10 mM.

For the immunofluorescence staining we used two types of antibodies, and for counterstaining we incubated the sections with Hoechst, a blue fluorescent dye used for DNA staining.

First, we analyzed the sections with conventional immunofluorescence microscopy. We used UV light in order to observe the cell nuclei of the cells from the tissue, and green light, to see the structures we targeted with the fluorescent stained antibodies. Second, we analyzed the sections using the laser

confocal microscope. We learned how to calibrate the microscope, how to choose the correct parameters for analyzing the sections, acquiring the images and building 3D models with the images we obtained.

I analyzed the basal cell carcinoma sections and observed enlarged nuclei of the tumor cells in tumor islands, with retraction of stroma and with the staining for factor VIII, I saw the endothelial cell from the blood vessels inside the tumor tissue.

The squamous cell carcinoma sections were stained with factor VIII, for endothelial cell and I observed rich vascularization inside the tumor tissue; also, I used staining for keratin 2 and 10 and identified nests of atypical cells inside the dermis. The sectioned analyzed showed features of low grade squamous cell carcinoma

Benefits: To conclude, this STSM has achieved the purpose of learning new techniques, which I believe will be very useful for further research projects on the topic of occupational carcinogenesis. I would like to analyze skin samples taken from occupationally exposed patients by using the conventional immunofluorescence microscope and the laser confocal microscope, in order to see the changes that appear in different sun exposed sites. I would also like to compare the results with what we observe in vivo, by means of dermatoscopy and in vivo confocal microscopy, so as to identify potential aspects that could be early markers of accelerated carcinogenesis in order to prevent the development of skin cancers in occupationally exposed workers.