Twinnig trip report

1. Task number: 1.8

2. Visiting person: Lech Trzeciak

3. Recipient institution: University of Cologne, Institute of Virology (and the German National Reference Center for Human Papilloma Viruses)

4. Recipient/host name: Prof. Herbert Pfister

5. Goal of the visit

Oncogenesis in humans is generally driven by mutations; however, at certain sites the process may be initiated by infectious agents, such as human papillomaviruses (HPV). HPVs are strictly epitheliotropic viruses that infect keratinocytes. Genus A generally prevails in mucosal areas and includes several types known for their substantial oncogenic potential (such as HPV16 or HPV18), whereas genus B types invade skin, usually causing short-lived benign skin changes. However, under certain circumstances (such as immunosuppression) B type infection persists and after prolonged exposure to ultraviolet may lead to a non-melanoma skin cancer (NMSC). Beta-HPV are also suspected of playing a role in the development of NMSC in the general population.

In this respect it is very interesting that rare mutations in two closely related neighbouring genes, TMC6 and TMC8, lead to a strong susceptibility to cutaneous HPV infections, typically manifesting as epidermodysplasia verruciformis (EV) and leading to NMSC. EV is thus regarded as a model for HPV-associated cutaneous oncogenesis. In our former studies on Polish patients with EV we found two characteristic mutations of EVER2 gene; recently, a third patient was identified with atypical presentation of the disease that involved mostly genital areas leading ultimately to neoplastic transformation. We began corroborating these findings in cooperation with a group of prof. Pfister in University of Cologne. Whole exome sequencing of the third patient's DNA, performed in the Department of Human Genetics in Warsaw, revealed a homozygotic splicing site change that according to in silico analysis would lead to splicing aberrations resulting in protein truncation. More studies on both host and pathogen were clearly required.

6. Short description of performed studies.

In order to better characterize the HPV infection in a suspected EV case I visited the Institute of Virology in Cologne for a brief period of 23rd of April to 11th of May 2014 (what constitutes the first half of my twinning experience). There I have successfully performed an extensive genotyping of the course of HPV infection throughout 8 years of disease history of the suspected case, utilizing formalin-fixed paraffin-embedded tissues (FFPE) as a DNA source.

I have gathered hands-on experience on DNA isolation from FFPE, broad spectrum PCR and nested PCR for A types with detection on probe-coated beads using Luminex flow-cytometry-like assay, reverse hybridization assay (RHA) to the Inno-LiPA blots and nested PCR plus LineBlot for B types. I have also measured the viral load of HPV beta at various sites and time-points, utilizing locked nucleic acid (LNA)-based QPCR with Universal Proble Library from Roche (participating also in the design of new assays for HPV types that had never before been studied in the Institute).

These studies demonstrated a profile of infection consistent with the diagnosis of EV. Interestingly, 1) rare strain of HPV alpha continuously dominates in genital lesions despite of a clear exposure to the typical HPV16 strain in the past; 2) while patient's skin is coinfected with multiple beta HPV types at various locations (what is typical for EV), one strain prevails with quite stable viral loads 3) HPV beta viral loads seem to remain unaffected by transplantation of the body skin onto a genital area; so far there are no signs of the dominant alpha type spreading into the transplant, despite the recurrence of skoin changes in this area 4) a dramatic increase of viral DNA of another HPV B type was seen in lesions histologically typical for EV.

In addition, HPV genotyping was performed in 3 more cases: one suspected of EV and two cases of tongue cancer with indirect signs of a high viral load. In the first case only typical strains were identified, while in the

latter two no HPV infection was detected. I have also participied in numerous scientific discussions, including a seminar on studies of viral protein effects on molecular pathways involved in skin carcinogenesis after UV exposure.

7. Photographic documentation

Lech Trzeciak at the entrance to the Departament of Anatomy of the University of Cologne

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Lech Trzeciak with core team members on the balcony of the Institute

Lech Trzeciak at work in the DNA isolation premises of the Institute

Lech Trzeciak discussing the results with prof. Herbert Pfister

8. Future cooperation prospects

There are several areas of possible future cooperation. The results obtained so far need to be supplemented by additional tests, first of all by in-situ hybridization, especially in the area of skin transplant. This method is yet to be established in the Institute, we initially agreed that this topic would be explored during the second part of my twinning visit within next few months. Second, the *TMC8* splice site alteration is not proven to really affect the splicing (or its regulation), what requires further studies. In this respect the method of culturing

keratinocytes, that is working well at the Institute, may be particularly useful, as well as LNA-based QPCR of DNA isolated from FFPE. Third, the methods and techniques used in the Institute for studies on molecular mechanisms of HPV related carcinogenesis (utilizing, among others, transgenic mice models) could be applied to research on TMC8-mutated cells in the future research, this will be a matter of future discussions during the remaining parts of twinning visits.

Fourth, both parties expressed interest in developing closer cooperation in the area of next generation sequencing (NGS) application in virological studies. We have already gained initial experience in detecting HPV susceptibility mutations in human DNA via the whole exome sequencing (WES). Our deparment is also already involved in using NGS in searches for infectious agents in certain diseases. We are now discussing the possibility of making this area a subject of a twinning visit of the Institute team member to Warsaw within next few months.