REPORT OF THE RESEARCH VISIT: BRITISH SCHOLARSHIP TRUST

LUCIJA LULIĆ

OVERVIEW

1. Institutions and duration

Home institution: Ruđer Bošković Institute, Zagreb, Croatia

Host institution: Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, United Kingdom

Duration: February 01 – March 31, 2021

2. Introduction

After receiving a grant from British Scholarship Trust, I started a two-month long internship at the Institute of Cancer and Genomic Sciences, University of Birmingham on February 01, 2021. I was a part of team in the laboratory of Dr Jo Parish, one of the leading scientists in the Human papillomavirus (HPV) field. Dr Parish is working on virus-host interactions important for HPV-induced carcinogenesis which is directly bound to my PhD research. Namely, HPVs are strongly implicated in the development of various cancer types and HPV infection is considered the most common sexually transmitted disease among both males and females. High-risk HPVs are associated with almost 100% of cervical and around 40-60% of oropharyngeal carcinomas *(Sastre-Garau and Harlé, 2020)*. However, the process of primary infection to cancer development is well defined in cervical area; studies have shown that oncogenic capacity of HR HPVs relies on collaboration of both viral oncoproteins, E6 and E7 that inactivate tumor suppressors, p53 and pRb, respectively *(Smith et al., 2015)*. In addition to inducing p53 degradation, numerous studies have indicated that E6 has many other targets including proteins which contain PDZ domains, ubiquitous protein interaction modules involved in maintaining cellular homeostasis. Some of the best characterized targets are PDZ-domain containing proteins from the Scribble complex: SCRIB and DLG1 required for cell polarity. Studies have shown that SCRIB and DLG1 can complement each other *(Bilder and Perrimon, 2000)*, and since they

are both targeted by HPV E6, cells expressing E6 form weaker cellular contacts and grow in more disorganized manner *(Nakagawa and Huibregtse, 2000)*. Therefore, these interactions could contribute to the development of later stages of HPV-induced malignancies. One of the subtopics of my PhD thesis is enlightening the effect of HPV16 on cell polarity regulatory in head and neck cancers. So, the aim of my research visit was to learn a technique of 3D organotypic raft cultures which are tissue culture systems that permit full differentiation of keratinocyte monolayers via culturing of the cells on collagen gels at the air-liquid interface. Considering that the productive phase of the HPV life cycle is dependent on epithelial differentiation, learning this in a group of Dr. Parish would be of great value to further investigate whether the E6 oncoproteins' preference for DLG1 and SCRIB changes depending on raft origin.

3. Accomplished goals

During these two months of research in the laboratory of Dr Jo Parish, I had the opportunity to learn a technique of establishing 3D organotypic raft cultures. Those are tissue culture systems that permit full differentiation of keratinocyte monolayers via culturing of the cells on collagen gels at the air-liquid interface. To do so, I have grown keratinocytes in a monolayer, and then lifted cells onto a solid support and cultured them at the air-liquid interface for about two weeks which is enough for basal keratinocytes to fully differentiate. During my stay at the Parish lab, I prepared three biological replicas of HPV16 infected human foreskin keratinocytes raft cultures which will be used for further analysis of E6 effect on DGL1 and SCRIB proteins during viral life cycle. I got a clear insight in all steps of establishing raft cultures and am now trained to transfer my knowledge to my home institution.

4. Additional achievements

Apart of successfully conducting all experiments I reported to obtain BST grant, I had an opportunity to become a part of the team of truly amazing scientists. All of them, from graduate and PhD students, postdocs to lab managers, selflessly invested their time to discuss obtain data with me, to propose additional experiments, to elevate my research form back home on an additional level. Because of their selflessness and openness, I had an

opportunity to learn how to perform, analyze and discuss RT-qPCR data which enable me to investigate how HPV affect DLG1 and SCRIB on transcript level too.

5. Acknowledgments

First and foremost, I am genuinely thankful to all the people from Parish and Roberts lab. If it weren't for them, I would have never become the only person in my host institution knowing this method. Acquired knowledge will greatly help to investigate the impact of HPV on cellular proteins during epithelial differentiation. More importantly, by getting this opportunity to work and get to know them, only deepened my desire and love to stay in science.

I am also grateful to British Scholarship Trust which recognized the importance of my research visit and enabled me to meet great people and scientist. Because of BST I was able to establish my own professional and personal network and I will always appreciate that. To all of you considering application, I say go for it because this kind of experience will enrich not only your professional CV but your life as well.

6. Literature

1. Sastre-Garau, X. & Harlé, A. Pathology of HPV-Associated Head and Neck Carcinomas: Recent Data and Perspectives for the Development of Specific Tumor Markers. *Front. Oncol.* 10, 528957 (2020).
2. Smith, E. A., Matrka, M. C. & Wells, S. I. HPV Virology: Cellular Targets of HPV Oncogenes and Transformation. in *Human Papillomavirus (HPV)-Associated Oropharyngeal Cancer* (eds. Miller, D. L. & Stack, M. S.) 69–101 (Springer International Publishing, 2015). doi:10.1007/978-3-319-21100-8_4.
3. Bilder, D. & Perrimon, N. Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. *Nature* 403, 676–680 (2000).
4. Nakagawa, S. & Huibregtse, J. M. Human Scribble (Vartul) Is Targeted for Ubiquitin-Mediated Degradation by the High-Risk Papillomavirus E6 Proteins and the E6AP Ubiquitin-Protein Ligase. *MOL. CELL. BIOL.* 20, 10 (2000).

Lucija Ilić