Report of the research visit
British Scholarship Trust

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Home Institution: Ruđer Bošković Institute, Zagreb, Croatia

Host Institution: Wellcome Trust Centre for Cell Matrix Research, Faculty of Biology, Medicine & Health, University of Manchester, Manchester, UK

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Introduction

On October 15, 2021., I began my two-month research visit at the Wellcome Trust Centre for Cell Matrix Research, Faculty of Biology, Medicine & Health, University of Manchester, Manchester, UK. I conducted my training and research in the laboratory of Prof. Martin J. Humphries, one of the leading experts in the field of cell adhesion biology. The objectives of my stay were to learn method for isolation and analysis of different adhesions complexes using MDA-MB-435S melanoma cell model. Namely, the previous results showed that these cells use different structures to bind to extracellular matrix proteins, such as focal adhesions (FA), which cells use for migration, but also newly discovered reticular adhesions (RA), which are the only adhesions preserved during mitosis.

Since the composition of RA is still insufficiently known, part of my PhD thesis is to isolate and analyse the protein composition of RA in melanoma cell model using mass spectrometry (MS). Mass spectrometry is a quantitative method for determining the protein composition of protein mixtures and when applied to samples of isolated adhesions can enhance an understanding of the composition of FA and RA and give insight in their role in melanoma. The main objectives of this research are to provide more basic knowledge about cell adhesion but also to detect adhesion protein interrelationship, signalling pathways that they activate and their role in the behaviour of tumour cells as well as potentially select new targets within the cell to improve existing tumour therapies in the future.

Accomplished goals

During my two months visit in the laboratory of Prof. Martin J. Humphries, I had the opportunity to learn and optimize the method for RA isolation as well as preparation of samples for MS. Isolation of RA was performed according to the protocol described in the papers of Jones et al. (2015) and Lock et al. (2018), co-authored by Prof. Martin J. Humphries. During my stay at the Humphries laboratory, I prepared six biological replicas of RA. Four biological replicas were prepared for MS analysis, and two biological replicas were subjected to SDS electrophoresis and western blot and will be used for validation of the results obtained by MS. Samples for MS were prepared by using in-gel tryptic digestion method which is a robust and reproducible method that can be applied to a wide range of samples.

During my stay at the University of Manchester, I visited The Biological Mass Spectrometry Core Facility. Head of the facility, David Knight, PhD, and other employees explained me various methods and instruments for preparing samples of different origins as well as a couple of different mass spectrometers for their analysis (with emphasis on Thermo Exploris 480, Thermo QExactive HF and Thermo Orbitrap Elite). I learned the advantages and disadvantages of the individual instrument, their price and especially the characteristics that the MS instrument must have to provide the most accurate data concerning composition of each sample. I got a clear insight into the course and all steps of MS analysis, as well as awareness of the skills that a person in charge of working on a MS must possess. Since the O-ZIP project (granted by The European Regional Development Fund (ERDF)), envisages the purchase of a mass spectrometer, possibly Orbitrap Elite or its upgrade, I tried to get knowledge how to prepare properly samples for this type of instrument and develop realistic expectations of results we can expect.

After successful preparation of samples and MS analysis there is a need for statistical analysis of data. Therefore, I learned how to use programs such as Mascot, Scaffold and Progenesis, which allowed the display, quantification and statistical processing of data needed for interpretation of the results.

Additional achievements

During my visit I had an opportunity to attend lectures by top experts in the field of cell biology, such as A. Elosequi-Artol (Francis Crick Institute, London) and S. West (Living Systems Institute, Exeter). Lectures were organized by the University of Manchester almost every week in last two months.

In addition to planned activities related to my PhD thesis, I successfully prepared samples for another project that I am working on "Combining clinical and scientific research to improve treatment and increase social awareness about prostate cancer" (Moj Zaba Start 2019) that is active in my home institution. Within this project I made my master thesis in 2020. In the meantime, samples of integrin adhesion complexes isolated from prostate carcinoma cell line DU145 and its radioresistant cell clone were collected and I processed them along with my samples and forwarded them to MS analysis.

Finally, during my stay in Manchester we revised an article that is a result of ongoing collaboration of Ambriović-Ristov group with Prof. Humphries and my co-mentor at the University of Manchester, Jonathan D. Humphries. Upon revision, our article in which I am co-author, was accepted for publication (*Tadijan et al., 2021*). This paper is part of the Research Topic "Integrin Adhesion Receptors in Health and Disease" (*Editors Andreja Ambriović-Ristov, Vassiliki Kostourou and Ben Goult*).

Summary of outcomes and future perspective

The first outcome of my two-month visit to the University of Manchester are results obtained from MS analysis of isolated integrin adhesion complexes in different cancer cell lines. The results of this high throughput MS method is the first step toward detailed analysis of integrin adhesion proteins and their interactions.

The second outcome, thanks to the excellent mentorship, is that I am able to independently prepare and statistically analyse any future samples. Therefore, I am also able to pass on my knowledge to colleagues in the laboratory. I sincerely hope that knowledge acquired during my stay in Manchester will also help me to set up a protocol for sample preparation and analysis in my home institution as soon as MS instrument will be purchased.

Third, and most important, outcome of my visit is establishing new connections with leading scientists in the field as well as with my younger colleagues, PhD students and postdocs. All these further motivated me for my future research.

Literature

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