Laboratory of Early Mammalian Developmental Biology (LEMDB)
Faculty of Science, University of South Bohemia,
Branišovská 31c, 37005 České Budějovice, CZECH REPUBLIC.

prof. Alexander W. Bruce *Ph.D.* – Department of Molecular Biology & Genetics
Tel: +420387772291. Fax: +420387772265 (not confidential),
E-mail: awbruce@prf.jcu.cz

Web: http://kmb.prf.jcu.cz/en/laboratories/en-bruce-lab
Twitter: @LEMDB1
Facebook: http:///www.facebook.com/LEMDB/

May 2023

Andrea Hauserová continued on from her Bachelors degree studies (that were in themselves very successful, with Andrea contributing data to two papers published in 2021) in my laboratory as a Masters student from the Autumn of 2021. She continued to work on a closely related project, centred around the classically recognised stress-kinases, of the mitogen-activated -protein-kinase pathway, p38-MAPKs. This project, further investigated the role of active p38-MAPK signalling during the early phase of mouse blastocyst inner-cell-mass (ICM) maturation; when individual blastomeres of an uncommitted cell-fate, specify and then execute their peri-implantation stage fate, either specifying as pluripotent epiblast (EPI – a progenitor pool for the embryonic tissues of the foetus) or differentiating as primitive endoderm (PrE – an extraembryonic tissue, from which the yolk sac membranes are ultimately derived). Andrea was developing the previous observations, of her and colleagues, that a short period of chemical p38-MAPK inhibition during the early blastocyst ICM phase is sufficient to severely impair PrE specification and differentiation from the uncommitted state, without having a profound effect on EPI specification. Specifically, she was testing the hypothesis that this p38-MAPK inhibition phenotype is primarily ICM autonomous and not affected by potential impaired p38-MAPK activity in the surrounding trophectoderm (TE) extraembryonic lineage; for example, by contributing to reduced blastocyst cavity expansion, also observed after p38-MAPK inhibition, that has been reported by Ryan et al., (2019) to affect ICM cell fate derivation.

To this end, it was decided to repeat the p38-MAPK inhibition experiments on isolated ICMs and to compare the potential PrE cell fate specification/differentiation phenotypes with those previous observed; by similar p38-MAPK inhibition on equivalently staged intact blastocysts. This was not a trivial aim, as the “immuno-surgery” technique required to isolate intact early blastocyst stage blastocyst ICMs that could then be further *in vitro* cultured (whereby, under control conditions, an outer PrE layer forms around a specified and encapsulated EPI – assayable by IF staining of lineage marker proteins and confocal microscopy) under p38-MAPK inhibited conditions had not been introduced to the laboratory. However, given Andrea’s previous excellent track record in our group, I was confident that she would be able to step up and learn the necessary “immuno-surgery” technique and then to apply it to testing our central experimental hypothesis; namely would p38-MAPK inhibition in the absence of a TE and expanding cavity still impair PrE specification/differentiation. I am pleased to report that my initial confidence in Andrea has been fully repaid. Not only has she perfected, and introduced the immuno-surgery technique to our group, she has via a process of careful and characteristic methodical experimentation been able to demonstrate the PrE related phenotype associated with p38-MAPK inhibition is primarily mediated by an ICM autonomous mechanism. Moreover, she has (and will continue throughout the Summer, until she starts her Ph.D., in our laboratory in the Autumn) generated a wealth of publication grade data that will form a significant portion of a manuscript currently in preparation (Bohuslavova et al., thus continuing the trend she established in her Bachelors research – but, as one must add, in a much more independent manner than previously undertaken – demonstrating her career development). Therefore, I have a very positive view of Andrea’s time in our group, one that I know is also shared by her colleagues.

Accordingly, during her extended time in our group, I have always found Andrea to be a very dedicated and conscientious worker. She was able to quickly grasp both the theory and practicalities of her project. Moreover, she was able to work with a high degree of independence (that will serve her well in her forth-coming Ph.D. studies) but equally was not afraid to seek advice and counsel when she required it. As already stated, I was particularly impressed that she was able to successfully adopt the completely new, for our laboratory, immuno-surgery technique and apply it to an important hypothesis (highlighted to me by many colleagues at international conferences) surrounding the mechanisms of action of p38-MAPK

and its role in priming uncommitted ICM progenitors for PrE specification and differentiation. Andrea keeps herself well informed with the current literature and can assimilate it in the context of her own work. Her progress in my group is exemplified in her Master's thesis; from which I can clearly see a great progression in quality of writing, understanding and presentation when compared to her Bachelors thesis. She has done a great job, working on an important project/research topic!

In summary, I regard Andrea's most recent time in our laboratory as being very successful. Undeniably, I have a high opinion of her as a research scientist and I am extremely pleased that she will continue her active research career/studies in our group as a Ph.D. student. Andrea is already demonstrating and fulfilling her potential to be an excellent research scientist and I am confident that this trend will continue in its upward direction. Therefore, for all the stated reasons and notwithstanding her charming, relaxed and friendly personality (and the fact she makes the most amazing cakes to celebrate the birthdays of her colleagues), I very highly commend Andrea's Masters' degree thesis to the examining committee.

prof. Alexander W. Bruce, Ph.D. (in České Budějovice)