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Valeriy Kutsyna joined my laboratory as a Masters student in the late Summer/Autumn of 2020 and worked on a project related to mitochondrial biology/morphology in the emerging cell lineages of the peri-implantation mouse blastocyst stage embryo (*i.e.* outer extraembryonic trophectoderm/TE and the two inner cell mass/ICM lineages of the extraembryonic primitive endoderm/PrE and pluripotent epiblast/ EPI); with special emphasis on the ICM lineages. More specifically, his project involved confocal imaging of the morphology and membrane potential (using a combined approach of immuno-fluorescent staining of cell lineage markers, pan-mitochondrial markers and the active mitochondrial labelling probe/dye MitoTracker Red CMXRos). Moreover, assaying these parameters, in a cell lineage specific manner, in response to pharmacological inhibition of the p38-MAPK family of stress-activated kinases; an experimental intervention that has been previously reported by our group to impair blastocyst cavity expansion and the specification/differentiation of the PrE lineage, whilst leaving EPI specification largely intact (and effects both the transcription and translation of many mitochondrial mRNAs, as revealed by our recently published transcriptomics and phospho-proteomic studies – Bora et al., 2021).

Valeriy was able to able to confirm, the findings of others, that the outer TE, of blastocyst *in vitro* cultured under unperturbed conditions, exhibits a more matured tubular mitochondrial network of active mitochondria (displaying an active inner-membrane potential characteristic of OXPHOS) when compared to the ICM lineages (presenting as a fragmented and more immature and spherical mitochondria network); presumably linked to the enhanced metabolic needs of the emerging TE lineage (with particular regard to the required burden to produce ATP to facilitate the osmotic gradient across the TE to contribute to blastocyst cavity expansion). Additionally, that there are no obvious differences in the active mitochondrial population of the emerging EPI and PrE lineages. Furthermore, his data shows p38-MAPK inhibition resulted in a reduced proportion of active mitochondria in TE cells (with a more profound effect on the polar TE, that overlies the ICM and contributes to significant post-implantation extraembryonic tissues, when compared to the mural TE lining the expanding cavity). However, this trend was reversed in the ICM lineages, whereby p38-MAPK inhibition caused comparatively increased mitochondrial inner membrane potential, that was particularly evident in the few cells of PrE lineage that manage to initiate PrE specification/differentiation. Accompanying this data, Valeriy also showed that the average morphological parameters (indicative of establishment, or not, of more mature mitochondrial networks) in p38-MAPK inhibited blastocysts showed a significant trend towards a more consolidated, branched and tubular arrangement and that was driven by dynamic changes in the ICM lineages rather than the TE. Lastly, he was able to confirm that p38-MAPK inhibition during blastocyst maturation (E3.5-E4.5) led to significant decreases in the mRNA expression of multiple components of the complexes of the mitochondrial electron transport chain. These preliminary data are of publication quality and have been used as part of the basis for a grant application currently submitted to GACR/CSF. Additionally, they expand our understanding of the functional metabolic role played by p38-MAPK (in addition to regulation of specific translation/protein synthesis of significant mRNA transcripts) in regulating blastocyst maturation, *per se*, and contributing to the germane emergence of the three blastocyst lineages and the ICM lineages in particular.

During his time in our group, I have always found Valeriy to be an extremely conscientious, diligent and intelligent worker. He was able to understand the theory and practicalities of his project quickly and work with a great degree of autonomy. To exemplify this point, he worked independently and in collaboration with other colleagues outside the laboratory to perfect the necessary and optimised confocal microscopy imaging parameters to derive his data. Additionally, he made an exhaustive literature survey to identify the most appropriate data analyses solutions to derive his data (with particular respect to morphological mitochondrial parameters and the calculation of the percentage pool of active mitochondrial

in each blastocyst cell lineage). In this regard, he really led his project far beyond what one would reasonably expect for a Masters degree level student; and I for one am grateful for this hard work and highly commend him for it. Indeed, he is currently engaged in passing on this knowledge, not just within our group, but also others with our Faculty. Valeriy keeps himself well informed with the current literature (not just related to early mammalian embryo development) and has amply demonstrated his ability to contextualise it within the boundaries of his own project. He has performed well in my group and done a great job!

One further thing I would like to note is that in February 2022, Valeriy suspended his Masters studies (whilst being on the brink of submitting and defending his Masters thesis) and restarted in the late Summer/Autumn of 2022. He had a very good and extremely admirable reason for this suspension, as he was actively engaged in full-time support of his Ukrainian compatriots resisting the full-scale Russian invasion of his homeland. This work involved countless hours of humanitarian work assisting refugees and displaced civilians and actively securing procurement of badly needed resources required by the Ukrainian army to repel their aggressors (that at times brought him right to the trenches at the front-line, that included coming under incoming fire!). Additionally, I know of his involvement in training staff at hospitals to use newly acquired/donated medical apparatus and equipment. Thus, he is to be highly and rightly commended for his efforts. I know it has been a personally difficult time for Valeriy, that has also been coupled with the disruption to his career plans, and I would just like to thank him personally and to express my profound relief he came back to us safely, completed his studies and wish him very well in his future.

In summary, I regard Valeriy's time in our group as being very successful. At the Masters level, he is an excellent research scientist and clearly has a wealth of attributes that would equip him for a successful future research science based career. I know that before the war he had ambitions to pursue a Ph.D. abroad but that after his recent experiences, he is rightly taking some time to reflect (although he now works within our Faculty as a technician). It is not my place to dictate anything to Valeriy but I would only say that it is my opinion that he is more than capable of entering a prestigious Ph.D. programme and continuing his development as a research scientist, should he wish to at some future date; and that he can rely on my to provide him an excellent reference, should he want. Lastly, and not least, Valeriy is a good bloke! He is charming and popular. Therefore, for all the stated reasons I thoroughly commend Valeriy's Masters' degree thesis to the examining committee.

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