1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title:

The involvement of the actin cytoskeleton and selected actin-binding proteins in collective cell migration – comparative studies of border cells in *Drosophila* and human cancer cells.

1.1. Project goals

The scientific aim of the research project is to verify the potential role of myosin VI (MVI) and other actin-interacting proteins (ABPs) in collective cell migration, based on a model system of border cell (BC) migration in the *Drosophila* ovary, in the context of tumor invasion. The innovative research strategy involves conducting comparative studies simultaneously on two models: (i) migration of BCs in maturing egg chambers of *Drosophila* during oogenesis, and (ii) migration of selected human cancer cells in *in vitro* cultures. The research will be carried out at the Dep. of Cellular and Molecular Biology (FBVS, NCU in Toruń) and at the Dep. of Histology and Embryology (Faculty of Medicine, Collegium Medicum of NCU in Toruń). Collaborative scientific efforts ensure publication activity in research areas encompassing: molecular biology, developmental biology, cellular specialization, carcinogenesis, actin cytoskeleton, and ABPs; methodological proficiency includes cell and molecular biology techniques, such as bioimaging, immunocytochemistry, genetic engineering, RNAi strategy, *Drosophila* cultures, and *in vitro* cell cultures. I anticipate collaboration with leading research institution, whose leader is renowned expert in MVI research and has co-publications with me (Prof. Maria Rędowicz, Nencki Institute of Experimental Biology (NIEB), Polish Academy of Sciences in Warsaw).

1.2. Outline

BCs constitute a subpopulation of follicular epithelium in the *Drosophila* ovary. They exhibit collective movement, wherein mesenchymal-like cells migrate guided by so-called "leader" cells at the leading edge. The migration of BCs is associated with the reorganization of the actin cytoskeleton involving ABPs. Since the mechanism of this migration appears analogous to metastasis, BCs serve as an excellent research model for tumor invasion. MVI is one of the motor proteins within the ABPs group that participates in the morphogenesis and migration of BCs in the *Drosophila* ovary. In mutants with silenced MVI expression, disturbances in collective migration of BCs are observed, along with altered expression profiles or localization of other ABPs, MVI effector proteins, and cell adhesion proteins. Importantly, the expression level of MVI correlates with the aggressiveness of human tumors; its overexpression is observed in ovarian and prostate cancer, while its suppression inhibits the progression of ovarian and breast tumors. However, the molecular mechanism of MVI action in tumor invasion remains unknown. Therefore, understanding the role of this protein in actin cytoskeleton reorganization involving ABPs during cell migration seems fully justified in the context of searching for new targets for metastasis-limiting therapy. The research hypothesis posits that unique ABP complexes regulating the collective movement of BCs in *Drosophila* serve a similar function during tumor invasion.

1.3. Work plan

The proposed project entails the following research activities:

1. Analysis of actin cytoskeleton organization and distribution (location and level) of MVI and other ABPs associated with F-actin remodeling (e.g., Arp3, cortactin, fascin/*Drosophila* Singed), MVI function (e.g., effector protein GIPC/*Drosophila* Kermit), and cell adhesion proteins (e.g., E-cadherin/*Drosophila* Shotgun) in BCs of the *Drosophila* ovary and in human

cancer cells with varying degrees of invasiveness; visualization of F-actin, immunocytochemistry, fluorescence/confocal/electron microscopy, flow cytometry, western blot.

2. Comparative live-cell analysis of actin cytoskeleton organization and selected ABPs, including MVI, during BCs migration in the *Drosophila* ovary and collective movement of cancer cells (life-time imaging, confocal microscopy).
3. Analysis of MVI expression level and pattern in *Drosophila* egg chambers and human cancer cell lines (vs. control cells); *in vitro* methods such as qPCR, dPCR and fluorescent in situ hybridization (FISH).
4. Determination of potential relationships between MVI expression level/pattern and microfilament reorganization, distribution of other ABPs, MVI effector proteins, and adhesion proteins – impact of MVI overexpression/silencing on BCs migration in the *Drosophila* ovary and invasion (collective movement) of cancer cells (transfection of cells with MVI siRNA to reduce the protein levels; visualization of F-actin, immunocytochemistry, fluorescence/confocal/electron microscopy, flow cytometry, qPCR, dPCR and FISH under conditions of MVI siRNA).

Statistical analyses will be conducted, along with the necessary control reactions.

1.4. Literature *(max. 10 listed, as a suggestion for a PhD candidate)*

1. *Bastock R, St Johnston D. Curr. Biol. 2008;18:R1082-7. doi: 10.1016/j.cub.2008.09.011. PMID: 19081037*
2. *Chibalina M.V, Puri C, Kendrick-Jones J, Buss F. Biochem. Soc. Trans. 2009;37, 966-970. doi: 10.1042/BST0370966*
3. *de Jonge J.J, Batters C, O'Loughlin T, Arden S.D, Buss F. FEBS Lett. 2019;593(13):1494-1507. doi: 10.1002/1873-3468.13486*
4. *Friedl P, Gilmour D. Nat. Rev. Mol. Cell Biol. 2009;10:445-457. doi: 10.1038/nrm2720*
5. *Geisbrech E.R, Montell D.J. Nat. Cell Biol. 2002;4:616-620. doi: 10.1038/ncb830*
6. *Karatsai O, Lehka L, Wojton D, Grabowska AI, Duda MK, Lenartowski R, Rędowicz MJ. Biochim Biophys Acta Mol Basis Dis. 2023;1869(6):166748. doi: 10.1016/j.bbadis.2023.166748*
7. *Nowak J, Lenartowski R, Kalita K, Lehka L, Lenartowska M, Rędowicz MJ. Front. Physiol. Sec. Cell Physiology. 2024; 15. doi: 10.3389/fphys.2024.1368416.*
8. *Peercy B.E, Starz-Gaiano M. Semin. Cell Dev. Biol. 2020;100:167-176. doi: 10.1016/j.semcdb.2019.11.010*
9. *Yoshida H, Chen W, Hung J, Montell D, Geisbrecht E, Rosen D, Liu J, Naora H. Proc. Natl. Acad. Sci. USA. 2004;101:8144-8149. doi: 10.1073/pnas.0400400101*
10. *Zakrzewski P, Lenartowska M. Postępy Biochemii. 2014;60(3):323-332*

1.5. Required initial knowledge and skills of the PhD candidate

Basic knowledge of cell and molecular biology is required; additional expertise in *Drosophila* culture and cell cultures will be advantageous.

1.6. Expected development of the PhD candidate's knowledge and skills

Research experience in *Drosophila* genetics, methods commonly used in cell and molecular biology research, a track record of publications, and involvement in both academic and applied activities (external grants) are desired.