PhD Thesis by Polina Zjablovskaja, 
Opponency by Prof. Tomas Stopka MD, PhD

The opponency of Polina Zjablovskaja

Author describes C/EBPα targets and elaborates on their roles in leukemia, focuses on one of them: Evi2b more deeply. The work summarizes and comments data of important publications such as Liss et al 2014 in Hematologica (3rd coauthorship of the applicant), Zjablovskaja 2017 in Cell Death & Differentiation (1st), and unpublished MS: Wurm et al (2nd). Additional two IF-publications were not included in the Thesis.

Major 1st authorship work describes transmembrane glycoprotein Evi2b expressed at the HSPC membrane as a target of C/EBPα. Moreover, the author led by her Mentor, Dr. Meritxell Alberich-Jorda, generated mouse knockout model and confirmed importance of Evi2b for progenitor biology and G-CSF dependency. Several types of experimentation was applied such as in vitro estradiol-dependent p42 as well as p30 C/EBPα activation. ChIPseq data were verified by ChIP-PCR to establish C/EBPα binding to Evi2b gene and this was confirmed by reported assays with involved mutagenesis of the Evi2b regions. Thus functional relevance was confirmed for C/EBPα binding sites again supporting direct Evi2b transactivation by p42 C/EBPα. Next, the Evi2b sh-knockdown was shown to block granulocytic differentiation using 32D/G-CSF-R system as well as CFU activities using primary BM cells. Not surprisingly, the Evi2B has been shown to be downregulated in a subset of 36 out of 529 AML patients with CEBPα mutation or promoter hypermethylation. Then the authors asked how and whether Evi2b has any function at the HSPCs level and observed that HSPCs from Evi2b mutants were more quiescent and tend to undergo apoptosis.

In both two coauthor manuscripts the applicant plays significant roles. Role of microRNA-182 appears to be dependent on C/EBP activity as identified using global RNA analysis in the K562 cell system with CEBP activation. Indeed, miR-182 expression relates to the AML prognosis like CEBP expression. The relationship, as was later evidenced, between miR-182 and C/EBP is mutual and miR-182 was revealed to be inhibiting C/EBP and its downstream effects on granulocytic differentiation. Another coauthor manuscript describes global search for C/EBP targets in AML. A signature of 33 targets was identified as reexpressed upon activated C/EBP in an AML line. Interestingly, this gene set responds to
HDAC treatment implicating its role in AML supporting the role of epigenetic silencing during leukemogenesis of AML.

The dissertation is well written. The publications are high quality papers that underwent peer review. Together the work presented by Polina Zjablovskaja successfully documents the experimentation. The dissertation is therefore recommended to the PhD defense with my fullest support.

Questions:
How the author explains the role of Evi2b in HSC quiescence?
CFU assay can also document effect of Evi2b-sh on differentiation - was myeloid-granulocytic differentiation assessed in the CFU cultures (?); defect in any other lineage: erythroid, megakaryocytic, lymphoid?
I did not find the peripheral blood counts for the Evi2b k.o. mice – are there present any differences in granulocytes (?); similarly, bone marrow findings such as cytology or histology are not provided.
Is EVI2B mutated in any cancer subtype?

Sincerely,

Dr. Tomas Stopka