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Prague, March 22, 2013

Reviwer's Report on Mgr. Vladimír Čermák's PhD thesis „Regulation of Transcription by Proteins of the Early Growth Response and Myb families".

Dissertation thesis of Mgr. Vladimír Čermák was carried out under supervisioon of RNDr. Michal Dvořák, CSc. at the Institute of Molecular Genetics AS CR in Prague. The PhD thesis is presented as a shortened version of four published manuscripts and one submitted manuscript currently under review. The PhD thesis contains general introduction and extended results and discussion sections pertinent to appended manuscripts. As concerns the contribution, the candidate is the first author on manuscript published in *Cellular and Molecular Life Sciences* and corresponding author on manuscripts published in or submitted to *Gene* and *PlosOne*, respectively.

In the presented thesis Mgr. Vladimír Čermák focused on the function of transcription factors EGR and Myb in chicken model. A recurring theme in this work is the ability of transcription factors to determine cell fate. The presented data suggests that the cell fate decision process clearly depends not only on transcription factors variation, or mutation, but also on overall cellular context. From my point of view the key and a very interesting part is the role for EGR transcription factors in metastatic spread of transformed cells and myofibroblast differentiation and dedifferentiation. The majority of applicant's published work concerns this area and it seems that EGR research is the center of mass of this PhD thesis.

This work brings novel findings that substantially and non-trivially broaden our knowledge about the function of EGR and Myb transcription factors and the signaling circuits they regulate and how this signaling is implemented in cell fate decision. From the perspective of the opponent I consider crucial fact that the results of this dissertation are part of five original manuscripts, including one with first authorship and two with corresponding authorship, published in – or submitted to - respected scientific journals. This work also demonstrates that the author has mastered advanced methods in molecular and cellular biology, and is able to design experimental procedure and interpret the data. High level of achievement is further underscored by the fact that Mgr. Čermák is corresponding author on two manuscripts.

Presented PhD thesis is very well written and I really enjoyed reading it. The introduction part has a logical structure where the author presents findings

concerning the EGR and Myb families of transcription factors, their regulation and biological functions. This part reads very well although it is, in my opinion, maybe too general. For example, the induction of EGR transcription factors by ETS family of transcription factors depends on functional inactivation of ERF repressor. In my opinion this regulatory role of ERF should be mentioned, as well as the fact that individual EGR members may regulate their own expression and also the expression of other family members. Some parts of the results and discussion section, concerning the microarray results, read not so well because the text contains huge number of systematic gene names. This is clearly consequence of interpreting the microarray data, however, using the gene common names more frequently would ease the reading.

I appreciate the fact that the thesis is written in English although this became the standard lately. As far as I can judge, presented thesis contains minimum number of grammatical errors, typos and stylistic clumsiness that are otherwise typical for this kind of work. The errors I found are stated in the appendix to this report, and they are intended for the author's information and reflection. I do not consider it necessary to be answered during the thesis defense. I would also appreciate if the published Supplementary data are included in full with the thesis as they are integral part of the original manuscript.

Given that the results are presented in the form of peer-reviewed manuscripts I feel that it would be splitting hairs to look for the potential flaws in the experimental procedures or interpretation of obtained results as is expected in such a report. Thus, majority of my questions are of more general nature:

1. The author states that both PR9692 and PR9692-E9 cell lines form rapidly growing primary tumors although the size of the tumor of PR9692-E9 cells is smaller. Is there any difference in proliferation between PR9692 and PR9692-E9 cells grown *in vitro,* i.e. on plastic?

   How does the fact that EGR1 increase metastatic potential of transformed cells relate to the recent finding that ERK-ERF-EGR1 signaling regulates cell migration of MCF10A cells? Is the migration speed of PR9692 cells higher than of PR9692-E9?

2. EGR proteins control the expression of both positive and negative regulators and initiate feedback signaling at various levels to accelerate or dampen given cellular response. This dynamic network is shown in a scheme in Appendix C, Figure 5. However, several other factors may play, and definitively they play, the important role in this complex signaling network. Brief look at the original microarray data revealed deregulated expression of Sprouty protein (Spry1), additional regulator of the ERK pathway signaling. In addition, EGR1 has previously been shown to regulate its own transcription. Could the author expand the scheme and speculate if Sprouty and EGR1 autoregulation affect the signaling network?

3. The sustained expression of TGF-β is able to efficiently induce myofibroblast diferentiation in EGR4 expressing chicken cells. This raises the question about the level, or strength of TGF-β signaling required to restore myofibroblastic phenotype. Could the author hypothesize what is likely to happen if TGF-β is expressed at different levels e.g. from inducible promoter? Would the myofibroblast differentiation be switch-like (i.e. when TGF-β reaches threshold level the cells will restore myofibroblastic phenotype)? Or, alternatively, would it be gradual process of constant accumulation of phenotypical and expression changes with cells displaying intermediate phenotypes?

Conclusion:

Despite abovementioned comments regarding the shortcomings of a formal nature the methodological and professional level of work is outstanding. From the results presented it is clear that the goals of this study have been achieved. The multiple experimental approaches applied, many procedures and techniques, as well as decent analysis of obtained results, show applicant's independence in conducting research. Taken together, it indicates that the author is fully prepared for the scientific carrier if he has chosen so. Based on the quality of Vladimír Čermák's PhD thesis I recommend this thesis to be fully accepted as the fulfillment of the requirements for the degree of *philosophiæ doctor*.

Tomáš Vomastek, PhD

Appendix to PhD thesis of Mgr. Vladimír Čermák „Regulation of Transcription by Proteins of the Early Growth Response and Myb families“.

Editing and typos:

There are very rare editing and typographical errors otherwise typical for this kind of work. These are mentioned for author's information and it is not necessary to answer them during public defense:

In the appendix A the supplementary figures 1-2 are not included. They should be shown as they are integral part of the original manuscript.

Pg. 30, Typo "…the expression o p53…"

Pg. 68. Typo "*different from TGF-β could be involvedSignaling pathways*"

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