
Title of doctoral thesis: Identification and functional characterization of C/EBPα targets in normal and malignant hematopoiesis

Title in Czech: Identifikace a funkční charakterizace cílových genů C/EBPα v průběhu normální a maligní krvetvorby

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Study program: Molecular Biology, Genetics and Virology

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The thesis reports the results, which were obtained within the research carried out in departments of Hematooncology, Leukocyte Signalling and Molecular Immunology of the Institute of Molecular Genetics of the ASCR in Prague. The aims of this doctoral thesis were: 1) to find a critical set C/EBPα target genes, and to identify the way to restore their expression in cells with mutated C/EBPα; 2) to describe the regulation of the chosen target gene of transcription factor C/EBPα - EVI2B by wild-type C/EBPα and mutant form C/EBPα, and to identify the role of EVI2B in myeloid differentiation; 3) to characterise the interplay between C/EBPα and its target miR-182 and to find the role of this microRNA in myeloid differentiation. All these aims are without any doubt of a great importance and their successful solution shows ways for future investigation.

Theoretical part (pages 11-31) covers all studied areas except miR-182. This microRNA is then discussed in detail in paragraph 7.3 in Discussion. Paragraph 5 (Materials and Methods) presents all methods used in the project solution. Among them there are difficult modern methods used in prominent world-wide laboratories.

The author elected to use K562 cells because they do not express endogenous C/EBPα. In addition, they are a human cell line derived from a patient with chronic myelogenous leukemia and represent a very early progenitor capable of multilineage differentiation. The author introduced the wild-type and mutant C/EBPα proteins in the form of an estrogen receptor fusion protein. The C/EBPα-estrogen receptor fusion proteins were expressed but remain cytoplasmic. Their function as transcription factors was induced by addition of β-estradiol which led to translocation of C/EBPα-estrogen receptor fusion proteins to the nucleus. The author identified 33 upregulated genes after activation of C/EBPα. These genes are listed in Table 3 on the page 41. EVI2B and TRIB1 are among these 33 upregulated genes. The author analyzed gene expression profiling (GEP) also in samples from 525 newly diagnosed AML patients. I did not find whether mononuclear cells or another cells were used for this analysis. Altogether 110 samples clustered demonstrating downregulation of 33 target genes mentioned above. These 110 AML patients was further referred as the C/EBPα dysfunctional subset and contained 26 AML patients with bi-allelic and 12 AML patients with mono-allelic mutation. It is interesting to note that 17 out of 26 AML patients with bi-allelic
and only 2 out of 12 AML patients with mono-allelic mutation clustered into this C/EBPα dysfunctional subset.

The author employed the connectivity map establishing correlations between gene expression levels and certain drugs and identified several histone deacetylase inhibitors (HDAC inhibitors: Trichostatin A, suberoylanilide hydroxamic acid and valproic acid) capable to upregulate the C/EBPα signature. The results of clinical trials with HDAC inhibitors in AML patients have not been encouraging. Therefore, the results of the doctoral thesis are very important because they showed that only a subgroup of AML patients with the C/EBPα dysfunctional subset responded to treatment with HDAC inhibitors.

The author described EVI2B as a novel C/EBPα target gene. However, the expression of EVI2B was not altered in CEBPA knockout mice compared with wild-type control. This finding indicates that C/EBPα positively regulates EVI2B expression, but is not absolutely required. The author also observed the downregulation of EVI2B expression in a subset of AML patients characterized by mutated or silenced CEBPA.

The author also discovered negative regulatory loop between C/EBPα and miR-182. Enforced expression of miR-182 impaired myeloid differentiation in vivo in sorted mouse fetal liver hematopoietic stem cells.

I would like to address the author with three questions listed below:

1) What is the role of Tribbles family of proteins in myeloid differentiation and in leukemogenesis and how these proteins affect C/EBPα degradation?

2) What cells of AML patients were used as source of RNA for GEP analysis?

3) What further target genes of miR-182 have been detected and described? What mRNAs are regulated by this microRNA?

The results were published in two papers in international journals with high impact and the third paper is in revision in Nature Communications (IF=11.329). Polina Zjablovskaja, MSc. is author or co-author in all these articles. This is very good outcome of a doctoral study. Since all of these articles went through a thorough refereeing process, it warrants a high level of the work. The doctoral thesis proves the author's ability for an independent creative scientific activity. From the above reasons I recommend to accept the thesis of Polina Zjablovskaja, MSc. and to award the scientific degree PhD. to Polina Zjablovskaja, MSc.

In Prague, June 15, 2017

Ing. Ota Fuchs, CSc.