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RE: Dr. Zdenek Kostrouch: PhD thesis

To whom it may concern

Dr. David Kostrouch submitted the PhD Thesis "The role of evolutionarily conserved proteins BIR-1/Survivin and SKP-1 in the regulation of gene expression," in September 2016.

The Thesis totals 141 pages and is divided into several sections, which include the Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusions, References, and Supplementary material.

The Thesis was independently carried out by the Candidate under the mentorship and supervision of Drs. Zdenek Kostrouch and Petr Novak.

In the Thesis, the Candidate focused on two evolutionarily conserved proteins, SKP-1 and BIR-1, with special attention to their possible interactions on the protein level using C. elegans.

SKP-1 and BIR1 are two proteins that were found to be functionally connected with the evolutionarily conserved nuclear receptor NHR-23 in C. elegans. SKP-1 is a transcription cofactor that interacts with several nuclear receptors (Notch, TGF beta, and Smad). In C. elegans, this cofactor is very important for normal development and its inhibition results in multiple phenotypes, including defects of larval transition and molting, which is dependent on NHR-23.

BIR-1 is the homologue of the vertebrate protein survivin. Survivin is extremely important in the apoptotic pathways and function as the antiapoptotic protein. Therefore, it is not surprising that survivin is found in fast dividing cells and is upregulated in most cancer cells.

Thus, in C. elegans, it was shown that the BIR-1 and SKP-1 proteins are involved in the regulation of gene expression and development. Therefore, this thesis focused on studying their role in the expression of various genes and proteins and their interactions, as well as their possible new roles in the ribosomal stress pathway, apoptosis, and cell division. Based on Dr. Kostrouch' work, these two proteins may be regarded as proteome sensors.

In the first part of the thesis, which the Reviewer will call the Introduction, Dr. Kostrouch logistically described the most important elements related to the understanding of his experimental work: chromatin and its effect on gene expression; DNA motifs – enhancers, silencers, insulators; transcriptional machinery; nuclear receptors and their coactivators and corepressors; non-coding RNAs; ribosomal stress; and apoptosis. This is also well supported by the justification of the use of C. elegans in this and other experiments.

In the Materials and Methods section, Dr. Kostrouch described the methods used throughout the Thesis. The methods used were appropriate, well thought-out, and correctly applied for the experiments. To identify the BIR-1 and SKP-1 interacting proteins, the author correctly utilized the ProQuest two-hybrid system with gateway technology. Although the Reviewer will not go into details of the numerous methods used in this Thesis, the Reviewer states that the Candidate used all methods correctly. It is evident that Dr. Kostrouch mastered these methods extremely well, including their deep understanding. The Candidate needed to learn many laboratory techniques, including those related to molecular biology, that also consisted of analysis of microarrays, immunohistochemistry, chromatography, mass spectrometry, proteome analysis, and many others. The Reviewer considers these techniques an excellent foundation for any experimental work understanding results and their subsequent synthesis and outcomes to further future experiments and scientific work.

In the Results section, the Candidate described well BIR-1 and SKP-1 interacting proteins (Tables 1 and 2). The yeast-two hybrid experiments indicated that SKP-1 and BIR-1 may influence gene expression through shared pathways, but did not show a direct interaction, further supported by several experiments, including two dimensional comparative chromatography. The results showed that BIR-1-related proteins included ribosomal proteins RPS-3 and RPL-5 and myosin. These proteins were further analyzed for a possible connection with both BIR-1 and SKP-1 together with interacting proteins identified by yeast two-hybrid screens, which indicated shared involvement of BIR-1 and SKP-1 in the ribosomal stress pathway, in apoptosis, and in the regulation of cytoskeleton during mitosis. These results further indicated that SKP-1 and BIR-1 may be a part of functionally linked protein complexes. The author concluded that protein interactions likely occurred in separate cellular compartments and under specific conditions. Therefore, the authors chosen selected protein for additional studies for validation of this proteomic data.

The Discussion section well reflects the results obtained in this study. It is fair and well balanced with appropriate conclusions. The Reviewer appreciates that the Candidate did not overestimate the current results, but rather fairly described that there are possible protein (protein networks) interactions between SKP-1 and BIR-1. However, more work is needed to further exploit these pathways, their function, and their role as very conserved regulatory pathways. To further study how various "external" stimuli can affect these proteins and their pathways (metabolic, developmental, etc.) would be of a great future interest, especially from the developmental point of view and with translation to some human disease, here especially cancer pathogenesis.

In summary, the Reviewer concludes that the Candidate fulfilled all criteria to become an independent investigator and to be given the title PhD.

The Reviewer has these questions:

1. What is the current role or view of the Candidate (based on his thesis) on BIR-1 and SKP-1 in cancer pathogenesis?
2. How does the Candidate view survivin as a potential therapeutic target in cancer or other diseases?
3. What is the role of BIR-1 and SKP-1 in the regulation of stem cells markers?

4. What is the Candidate's view of the p53 function in relation to SKP-1 and BIR-1 protein network?

Sincer

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