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Evaluation of the PhD thesis
"Characterization and biological significance of STAT2-dependent but STAT1-independent type of interferon-mediated transcriptional responses" by Ms Katarzyna Błaszczyk

The PhD candidate has addressed a very timely and interesting topic, namely biological and molecular characterization of ISGF3 complex containing STAT and IRF transcription factors and gene expression networks that are crucial for directing inflammatory responses against viruses and other pathogens. In recent years, a huge numbers of studies have been carried out on identification of transcriptional targets of ISGF3 and STAT targets and components in different cell types and under various conditions. Introduction of NGS sequencing allowed to enter the higher level and analyzed cell responses in a global scale. It is becoming clear that application of those methods and comparison of different states/patterns will be more informative about real transcriptional networks activated by inflammatory cytokines. The questions addressed in this PhD thesis are very important to understand basic mechanisms of inflammation.

Mgr Błaszczyk's work addressed this important issue employing state of the art cell biology techniques and modern molecular biology methods to isolate characterize mechanisms and signaling pathways underlying cell responses to interferon alpha (IFNα) using global transcriptomic and chromatin immunoprecipitation approaches, and computational methods to derive relevant information about common or distinct set of genes activated in the specific context. To identify the role of specific STATs or IFRs she used different cell lines either lacking distinct STAT proteins or overexpressing specific transcription factors. There are many aims of this study as the author addressed several molecular aspects regarding the role of specific components of ISGF3 that are based on prior studies in the supervisor laboratory. The scope of studies was very broad and ambitious. It encompasses:

1) identification of IFNα upregulated genes in 2fTGH fibrosarcoma cells and overrepresented classes of genes based on Gene Ontology terms;
2) identification of the ISRE motif in all significant clusters of IFNα upregulated genes in 2fTGH cells;
3) analysis of gene occupancy by STAT2, IRF9, STAT1 at the promoters of *OAS2* and *NANOG* genes (a negative control);
4) mapping genome occupancy by STAT2, IRF9, STAT1 in a whole genome scale by ChIP-seq in IFNα treated cells, selection of the overlapping regions and identification of gene functional networks;
5) correlation of genome occupancy by STAT2, IRF9, STAT1 with gene expression data;

6) generation of human fibrosarcoma U3C-STAT1-KO cell lines with overexpression of STAT2 and IRF9;
7) identification of ISG3-dependent and ISG-independent gene networks in human fibrosarcoma cells STAT1 (U3C-STAT1-KO) and in clones with overexpression of STAT2 and IRF9.
8) global analysis of STAT2 and IRF9 gene occupancy and gene networks in human fibrosarcoma cells ST2-U3C clones;
9) comparison of global expression profiles and STAT1 /STAT2 occupancy between different cells (NB4, ST2-U3C, 2fTGH) varying in induction of ISGF3 components by IFN.

To achieve her goals mgr Błaszczyk employed a very broad set of methods. It is important to underlie that the appropriate and state of the art methods have been used in this study, antiviral functional assay to characterize IFN responses. Several of implemented methods such as chIP-seq and following computational analyses are really modern and this is a rare example when they are implemented with such success. The methods description section is complete and very precise, all information required to control those experiments is provided.

The thesis is well constructed, according to general rules and with good proportions, it contains a long list of relevant references. Introduction provides a lot of information regarding the structure, binding capacities, roles and interactions between different STAT and other transcription factors induced by interferons. It shows how much work has been done and what are urgent questions in the field.

A section of Methods presents the applied procedures clearly and in most cases in sufficient details. It is noteworthy that a wide scope of different techniques has been used in this study, such as cell culture, antiviral response test, Western blots, molecular biology techniques of gene expression analysis and transcription factor binding (ChIP-seq) in a whole genome scale. These molecular biology methods are assisted by well selected and appropriate computational analyses. Numerous computational analyses have been performed including GO terms enrichment, gene co-regulation analyses, gene network/pathway finding and DNA motif enrichment. These are indeed “state of the art” methods allowing to make global comparisons and identify target genes. The wet lab and computational methods are well described, nicely illustrated and fit to the objectives very well. Proper techniques have been employed to verify author’s queries and hypotheses. The author took a good care for quantitative analysis of data. The quality of figures is good, layout of the text is suitable. The sample size fits the objectives. Information regarding a number of experiments for microarrays, ChIP-seq, Western blotting and q-PCR studies was provided. Sufficient care has bene taken to verify results, microarray analyses have been complemented by qPCR verification (however I missed information if those experiments were performed on the independent material or the same as used for microarrays).

The findings are original, very interesting and provide a plethora of new information about STAT targets and functions in different types of cells. The identification of ISGF3 dependent and independent gene network brings a lot of new information**.** Mgr Błaszczyk has demonstrated a new set of genes that have been upregulated in in clones with overexpression of STAT2 and IRF9, and have been depleted of the classical ISRE motif displaying instead a variety of IRF-like motifs. The expression of selected genes *CCL8* and *CX3CL1* was shown as IRF9 dependent in ST2-U3C cells treated with IFN.

The results are highly innovative because this study is one of the first comprehensive characterization of STATs and IRF9 occupancy at the promoters in IFN-stimulated cells and their gene expression networks. The achievements and findings may provide a rationale for development of novel drugs targeting specific transcriptional regulators and signaling pathways in inflammation.

In the Discussion part, the Author summarizes briefly her findings and presents their biological meaning that allows to make reasonable and original conclusions. The conclusions are based on interpretation of results and are well supported.

Minor comments:

1. INTRODUCTION. This section is in general well written but a section of innate /adaptive immunity is too laconic and simplified. The sentence: *a rapid onset "innate immune response ....involves the synthesis of cytokines called interferons and stimulation of "natural killer" lymphocytes* contains some shortcuts. In responses to viral infection numerous cytokines are synthesized besides IFN.

   P2. NK cells are natural killer cells, not neutral killer cells.

   This section is overloaded with numerous information concerning STAT structure, interactions at the promoters that is delivered in a chaotic way and would benefit from better selection of relevant information.

   I am surprised that the author while referring to numerous papers on STAT dependent genes overlooked the study on identification of STATs-dependent genes in inflammatory innate immune cells or cancer cells using ChIP-on-Chip combined with transcriptome analyses (Przanowski et al. J Mol Med 2014).
2. OBJECTIVES AND SCOPE OF THE THESIS. Instead of stating clearly the rationale behind the studies, hypotheses and approaches, the author summarized here what she did and wrote it in a very elaborated manner. This was unnecessary at that stage and should be presented at the end as a summary of results.

   I do not fully comprehend the idea of comparing human fibrosarcoma cells and promyelocytic leukemia NB4. While according to the information provided ST2-U3C, 2fTGH share common ancestor, NB4 is a leukemic cell line. From published studies it is clear that STAT-dependent transcription is cell type specific. The rationale behind such comparison escapes me.
3. RESULTS section. Reading of the results section would be much easier without repetition of information and digressions about cell lines (a half of the first page in relevant sections of the Results). The information about cell lines should be in Methods. I do not appreciate the discussion of results in the Results section as it has been done while presenting antiviral response test results (p.39), it is repetition of information from and makes this section difficult to read.

   It is not explained to my satisfaction why STAT2 phosphorylation and IRF9 increase are missing in IFN treated U3C-STAT1-KO and how that can impact results.
4. DISCUSSION. In general, this part summarizes well the achieved results and discusses their relevance. Mgr Błaszczyk has a deep knowledge and is capable of seeing her results in a broad context. The discussion shows well her ability to analyze a complex problem. The presented schemes help to recognize the importance of the achieved results. However, this part is not written very well, again it is overload with

information about other studies instead of a more concise summary of results and discussing their significance.

Some interpretation or hypotheses are far-fetched, i.e. while trying to explain differences gene responses in cell lines, Mgr Błaszczyk elaborates on posttranslational modifications of STAT1 (acetylation or sumoylation), which is not relevant as she did not study those modifications and has not information about their status in tested cells. The more likely interpretation would be a replacement of one STAT by another at the common ISRE site as it has been described by many authors.

5. General comments: The text is overloaded with facts and it would be much easier to read if discussion of results would be presented in more concise way, some findings in tables or in graphical manner.

   It would help a reader if gene names in the entire text were in italics as it is widely accepted.

   A list of abbreviations would very useful, as the author used numerous acronyms.

Summarizing, I would like to stress that the presented goals of the study have been achieved and the PhD candidate has collected a very impressive set of original results and reported several important findings. The thesis is well written and clearly delivers an important message. Altogether, the findings presented in the thesis are very important, significantly contribute to our understanding of molecular mechanisms of antiviral responses.
This reviewer assesses that the thesis entitled **"Characterization and biological significance of STAT2-dependent but STAT1-independent type of interferon-mediated transcriptional responses" by Ms Katarzyna Błaszczyk** fulfills all requirements for PhD thesis and this work is sufficient to get Doctor of Philosophy (Ph.D.) at the Biology Department of the Adam Mickiewicz University in Poznań. The candidate is well prepared for independent work and seems to be a good candidate for scientific carrier.
I consider the results presented in dissertation as an excellent achievement worthy to be honored with distinction.
Rozprawa doktorska spełnia wszystkie warunki określone w art.13 Ustawy z dnia 14 marca 2003r o stopniach naukowych i tytule naukowym (Dz.U.nr 65, poz.595 z późn.zm.) stawiane pracom na stopień doktora nauk biologicznych. Wnoszę więc do Rady Naukowej Wydziału Biologii Uniwersytetu Adama Mickiewicza w Poznaniu o dopuszczenie mgr Katarzyny Błaszczyk do dalszych etapów przewodu doktorskiego. Ze względu na wybitny charakter osiągnięcia naukowego prezentowanego w rozprawie doktorskiej chciałabym również złożyć do Rady wniosek o wyróżnienie pracy.

Sincerely yours

Prof. dr hab. Bożena Kamińska-Kaczmarek