2025-03-05

The review of the PhD thesis of HUMMAIRA SADAF, entitled: "The molecular mechanism of PD-L1 overexpression in classical Hodgkin lymphoma (cHL)"
Supervisor: Dr. Elżbieta Sarnowska, professor at the National Research Institute of Oncology, Warsaw
Co-supervisor: Dr. Ryszard Konopinski
PhD was completed at the Department of Experimental Immunotherapy of Maria Sklodowska-Curie, National Research Institute of Oncology, Warsaw

Wydział Biochemii, Biofizyki I Biotechnologii

Uniwersytet Jagielloński

Zakład Biochemii Ogólnej

Prof. dr hab. Jolanta Jura

Hodgkin lymphoma (HL) is a type of cancer that affects the lymphatic system. HL is divided into two distinct categories that demonstrate different pathologic and clinical features: classical and nodular lymphocyte-predominant Hodgkin lymphoma (NLP-HL). Classical Hodgkin lymphoma (cHL) accounts for approximately 95 percent of HL containing an abnormal cells called Reed–Sternberg (HRS) cells. One of the hallmarks of HRS cells is overexpression of genes coding for CD30 and PD-L1 and their presence on the cell's surface. Immune checkpoint inhibitors (ICIs) like nivolumab and pembrolizumab (anti-PD-1 antibodies) have shown remarkable response rates (~65–87%) in relapsed/refractory Hodgkin lymphoma. Thus, understanding PD-L1 regulation could help optimize treatment strategies, identify resistance mechanisms, and explore combination therapies.

Evaluation of the Formal Aspects of the Dissertation:

The doctoral dissertation is written in English and follows a traditional structure. It consists of an Abstract, Introduction, List of Reagents, Methods, Results, Discussion, Conclusions, and References. The Introduction is relatively extensive compared to other sections and is enriched with tables and diagrams that complement the description of topics related to the research objective. The Methodology section provides a description of each experiment conducted by the PhD candidate. The Results section, spanning 14 pages, documents the experiments performed and offers a very general description of the obtained findings. The Discussion section, written over seven pages, contextualizes the results within the existing literature.

The dissertation contains stylistic and grammatical errors. Additionally, the descriptions of several experimental procedures lack specifics on the concentrations of solutions, antibodies, enzymes, or DNA used. While the results themselves are highly interesting, their presentation affects their clarity and reception. Some figure captions are difficult to read due to the use of extremely small fonts. There is also a lack of information regarding the number of experimental repetitions, and statistical analysis has not been performed for key experiments such as Western blot, ChIP, and PCR.

Evaluation of the Scientific Value of the Doctoral Dissertation:

The aim of the project was to investigate the regulatory mechanism of the ***CD274*** gene, which encodes an important immune checkpoint protein, **PD-L1**, and to understand the role of this protein in **HRS cells**. To achieve these objectives, the PhD student applied a range of molecular biology methods, including **PCR, cloning, Western blot, chromatin immunoprecipitation, and the yeast two-hybrid system**. Additionally, **microscopic analyses** and **flow cytometry analyses** were performed. The use of such a wide range of methods demonstrates that the PhD student had the opportunity to learn and apply many advanced research techniques.

In the first phase of the study, three classical Hodgkin lymphoma cell lines were used: KM-H2, L-1236, and L-428. Strong PD-L1 expression was observed only in the L-1236 cell line, as shown in Figures 4.5 and 4.6. Therefore, using flow cytometry, the PhD student demonstrated **PD-L1 and PD-L2 expression on the surface** of this cell line, while PD-1 expression was almost undetectable. In all three examined cell lines, the protein levels of components of the SWI/SNF complex and Polycomb Repressive Complex 2 (PRC2) was analyzed. According to the literature, these complexes are

Adres:

Gronostajowa 7

30-387 Kraków

tel. +48(12) 664 63 59

fax +48(12) 664 69 02

email: jolanta.jura@uj.edu.pl

involved in PD-L1 expression regulation. However, no clear correlation was found between the levels of selected subunits of these complexes and the amount of PD-L1 protein.
Interestingly, the PhD student demonstrated that **PD-L1 is present in the nucleus** and used mass spectrometry to identify nuclear proteins that potentially interact with PD-L1. The identified proteins are mainly **associated with mRNA maturation and processing**. For one of these proteins, FUS, the yeast two-hybrid system was performed. However, in my opinion, the interaction was not confirmed, although the results presented in Figure 4.15 are difficult to interpret.
PhD student performed co-immunoprecipitation of endogenous PD-L1 with selected subunits of the PRC2 and SWI/SNF complexes and revealed **interactions with INI1, BRM, BAF155, and EZH2**. Additionally, chromatin immunoprecipitation analysis showed a very interesting result: **BAF155, EZH2, and the nuclear fraction of PD-L1 bind to the site of methylated and acetylated histone H3 in the *CD274* gene promoter**. However, using tazemetostat which inhibits H3K27 methylation (EPZ-6438) and blocking the active site of EZH2, had no effect on the levels of PD-L1, BAF155, BRM, or the histone methylation levels.
The Discussion and the Conclusions subchapter are written in a very interesting manner and significantly better than the previous chapters. The obtained results are placed in the context of literature data. Moreover, the PhD candidate formulates a hypothesis on how obtained results can be interpreted in the light of applied therapies and what their practical applications might be.
The dissertation addresses a significant scientific problem and contributes valuable insights to the field. The research is based on a well-designed set of experiments, aiming to elucidate the mechanism of PD-L1 regulation in cHL. The study provides new data that may have implications for understanding the molecular pathways involved in this disease. The research incorporates advanced molecular biology techniques, demonstrating the candidate's ability to conduct complex analyses.
However, despite the significance of the results, some limitations in data presentation and analysis reduce the clarity and impact of the findings. The lack of statistical analysis in certain experiments and missing methodological details may affect the reproducibility and reliability of the conclusions. Improvements in data presentation, methodological transparency, and statistical rigor would enhance its contribution to the field. Addressing these issues could strengthen the scientific value of the dissertation.
Considering my concerns, I would like the PhD student to address the following questions and comments:

1. In my opinion Figure 4.1, showing cells under a light microscope, is unnecessary.
2. Which proteins are shown in Figure 4.3 (Western blot)?
3. In the mass spectrometry analysis, were only 12 potential PD-L1 partners identified? How many times was this experiment performed? Does the PhD student plan to validate the interaction with all potential candidates?
4. Considering that MS mainly detected proteins related to mRNA processing, what could be the role of PD-L1 in this process?
5. Figure 4.13 shows the interaction of PD-L1 with INI1, BRM, BAF, and EZH2. However, in the figures displaying the presence of these proteins in immunoprecipitates, PD-L1 is not shown.
6. How many times was the chromatin immunoprecipitation (ChIP) experiment performed? Statistical analysis should be added to proof the data.
7. Based on Figure 4.15, can the PhD student explain which proteins interact with PD-L1? Is there a confirmed interaction with the FUS? Quality of the picture is very low.
8. Considering that inhibition of EZH2 activity does not affect the levels of the studied proteins or histone H3 methylation, what is the role of these proteins in the regulation of the CD274 gene encoding PD-L1?

Summary:
I conclude that the doctoral dissertation presents an **original solution to a scientific problem**. The aim of the study was to explain the mechanism of action of PD-L1 and its regulation in cHL. **A comprehensive research plan was designed — a unique set of complex experiments that enabled the identification of the potential role of this protein in cHL**. The PhD candidate conducted a series of intricate studies, through which she became familiar with various molecular biology techniques and gained experience in independently conducting analyses.

Praca doktorska Hummairy Sadaf spełnia warunki określone w art. 187 ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (Dz.U. 2018 poz. 1668 z późn. Zmianami). Wnioskuję do Rady Naukowej Narodowego Instytutu Onkologii im. M. Skłodowskiej-Curie-Państwowego Instytutu Badawczego o dopuszczenie Hummairy Sadaf do dalszych etapów przewodu doktorskiego i popieram wniosek o nadanie Jej stopnia naukowego doktora.