Dr hab. Joanna Jakubowicz-Gil
Department of Comparative Anatomy and Anthropology
Maria Curie-Sklodowska University
Akademicka19
20-033 Lublin

Lublin, 21.12.2017

Review

of the Ph.D. theses of M.Sc. Urszula Waszut entitled "Assessment of the effect of mitotane on the expression profile of genes involved in mitochondrial metabolism in human adenocortical, lung, breast, and colon cancer cell lines"

Mrs Urszula Waszut completed her doctoral dissertation in England at the Department of Molecular Pathology at King's College Hospital, London, in collaboration with the Department of Cancer Research at King's College London, under the supervision of Dr Dorota Dworakowska, MD (Hons) PhD. The experiments were funded by the Foundation for Polish Science within the grant "Bridge" (POMOST) awarded to Dr Dorota Dworakowska, MD (Hons) PhD.

Substantive assessment

The presented PhD thesis includes a well-planned and -performed laboratory study, in which the Author analyzes changes in gene expression associated with mitochondrial energy metabolism under the influence of mitotane, a compound used to treat adrenocortical carcinoma. Mitotane belongs to insecticides. It was first introduced in adrenocortical cancer therapy in 1960, and no worthy successor has appeared so far. The efficacy of mitotane treatment is still not uniformly considered and many contradictory data on the efficacy of the therapy appear in the literature. The topic is very important because the compound is used in the treatment of a rare but very malignant cancer. Adrenocortical cancer is invasive; it infiltrates the surrounding tissues and gives metastasis to other organs (liver, lung). Therefore, in my opinion, undertaking research to understand better the mechanisms of mitotane action is appropriate and highly advisable.

One of the mechanisms of elimination of cancer cells by the mitotane action is induction of programmed cell death like apoptosis. This process is closely related and controlled by mitochondria, the key energy organelles of the cell. Therefore, the analysis of the expression of respiratory chain genes after mitotane treatment may be very important in understanding the molecular mechanism of the drug action and contribute to the enhancement of the efficacy of programmed death induction in cancer cells.

Another strong point of the work is the series of experiments on the possibility of using adenocarcinoma tumour tissue obtained after tumour resection and embedded in paraffin blocks for genetic studies. Experiments conducted on established cell lines do not fully reflect what is happening in the living organism. They only allow a preliminary assessment of the effect of the studied compound on cells. The use of patient-derived tissue facilitates real evaluation of the level of gene expression, taking into account the effect of the tumour microenvironment on the behaviour of tumour cells. The results obtained in this part of the study extend the methodology of research and provide many new data on the biology of adrenocortical carcinoma. Hopefully, the PhD student will undertake studies to analyze the expression of genes involved in the regulation of mitochondrial energy metabolism as a follow-up to cell line experiments in the future.

Evaluation of work structure

The Ph.D. thesis of Urszula Waszut has 162 pages of manuscript written in English. An Abstract, Acknowledgments, and information of the financial sources written in the Polish language are included as a supplement. The structure of the work is typical for doctoral theses. It begins with a clear table of contents, a list of abbreviations, and the Abstract. On 24 pages of the Introduction, the Ph.D. student characterizes the research model and summarizes the current knowledge about the effect of mitotane on cancer cells, both at the

clinical and molecular level. In an comprehensible way, she presents different ways of elimination of cancer cells by programmed death, which is illustrated by clear figures. The Aims placed after the Introduction are clearly formulated.

The Materials and Methods, in which the Ph.D. student describes the technique used, were prepared in a very detailed and clear manner, which allows performance of the experiments with great accuracy and repeatability. It is clear that the Author is acquainted with numerous modern molecular methods and has mastered the research procedures. It is worth emphasizing that the methodology was appropriately selected for the purposes set.

The Results are developed very carefully and accurately. Numerous figures facilitate understanding and analysis thereof. In the extensive Discussion, the PhD student confronts the results with the literature and maintains an appropriate dose of criticism. Moreover, the discussion is not focused on the presently available data, but presents perspectives for future research.

The Conclusions summarizing the data obtained are clearly formulated and correspond to the aims set. The advantage is the estimation of general conclusions, which easily present the main results of the work, and partial conclusions resulting from the specific data and goals. The work ends with an appendix, list of figures (47) and tables (18) and rich literature, which is up to date and correctly selected.

During the analysis of the dissertation, I noticed that some fragments require revision and clarification.

1. In the Aims, the Author mentions apoptosis induction after mitotane treatment on the basis of the level of gene expression associated with the programmed death process. Further investigation at the protein (marker-specific activity) or cellular (morphological analysis of cells) level would unambiguously confirm the sensitivity of the studied cells to apoptosis induction upon mitotane.

2. In the Materials and Methods section, there is information that H259R cells were incubated with mitotane for 24 hours while the other lines were incubated for 48 hours. In the Results, cells treated with the studied compound for 72 hours are analyzed. This discrepancy should be explained. The cell density should be recorded as $790x10^3$ instead of 790x103, as it appears in the text.

3. In the first conclusion corresponding to the first aim, there is information about the optimization of the test against mTOR, whereas the protein was used in the study of the isolation of paraffin sections (aim 4).

In the work, many language and editorial mistakes appear such as:

- too general specification of the abbreviation TRK described as "tyrosine kinase", while it should be "tyrosine receptor kinase" or "tropomyosine receptor kinase"
- no italicisation of "in vivo" and "in vitro"
- the literature citation in the text, the spelling of HKe-3 cell line (or the Hke-3 synonym). and the units of measure after numerical values are not uniform
- using the Polish phrase "bloki parafinowe" instead of "bloczki parafinowe"
- the translation of the word "Department" as “Departament” (with a spelling error) with regard to the Polish word “Zakład”
- unfortunate wording in the Polish Abstract "mRNA isolation from paraffin tumors” for mRNA isolation from tumors embedded in paraffin blocks
- using a too common expression in the Polish Abstract "nadekspresja VEGFów" to determine the level of VEGF expression
- repeated fragment in Section 3.1 and Section 3.1.1 in the Materials and Methods

I also noticed many spelling errors, especially in the Polish version of the Acknowledgments and Abstract. Moreover, the Polish translation of these chapters does not coincide with the English version.

The above critical remarks do not diminish the substantive value of the work. The Author has shown expertise in molecular research methods, wide knowledge of literature, and the ability to solve scientific tasks independently. The results achieved by the PhD student are her original achievements and expand the knowledge of the molecular mechanism of mitotane action as well as the possibility of the use of the compound in therapy more effectively.

Taking into consideration the arguments mentioned above, I declare that the Ph.D. theses of M.Sc. Urszula Waszut entitled "**Assessment of the effect of mitotane on the expression profile of genes involved in mitochondrial metabolism in human adenocortical, lung, breast, and colon cancer cell lines**" comply with the requirements of a doctoral dissertation and qualify the Candidate to participate in further stages of the Ph.D. conferment procedure.

Joanna Jakubowicz-Gil

Dr hab. Joanna Jakubowicz-Gil