Evaluation of the Doctoral Thesis

„Molecular basis of the phenotype alterations of melanoma cells in vitro"

p. mgr Mariusz Ł. Hartman,

completed in the Department of Molecular Biology of Cancer

Promotor: p. Prof. dr hab. Małgorzta Czyż

1. Description of the merits of the dissertation

As pointed out in the introduction of the thesis, melanoma persists as a deadly kind of cancer with limited chemotherapeutic treatment options after progression to the metastatic stage. Tumor heterogeneity and the emergence of resistance complicate therapies, and both phenomena are related to the phenotypic plasticity of melanoma cells, meaning that these cells are not invariable entities, but living structures able to react to changing conditions including therapeutic stress.

It is exactly this phenotypic plasticity which is addressed by the research carried out in the Department of Molecular Biology of Cancer and to which Mag. Mariusz Hartman contributed with his work. One of the major ways for any cell to react to changing life conditions is the adaptation of gene expression. Apoptotic stimuli are counteracted by the upregulation of anti-apoptotic proteins, drug exposure stimulates the expression of drug exporter proteins, etc. Using a clever in vitro cell culture model, in which a simple change of the growth medium has dramatic consequences on the growth characteristics of patient derived melanoma cell lines, the effects of environmental changes on gene expression were investigated. The identification of molecular regulators which enable melanoma cells to adapt to changing conditions and which drive phenotypic heterogeneity increases our understanding of acquired

drug resistance and treatment failure. Therefore, the choice of the research direction was fully justified.

As described in one of five publications included in the PhD thesis, Mag. Hartman analysed the results of a comprehensive gene expression study comparing melanoma cells grown in monolayers with melanospheres. In serum-free growth medium, melanoma cells derived from some patients form large, multicellular spherical structures, called melanospheres, which can be converted into monolayer cell cultures when grown in the presence of serum. Analysis of the gene expression profiles of melanospheres versus monolayers pointed at members of the WNT signaling pathway, the MITF transcription factor, as well as anti-apoptotic proteins as key molecules involved in the adaptation to the changing growth conditions. Monolayer cell cultures upregulated DKK1, an inhibitor of the WNT signaling pathway. The WNT pathway plays a role in carcinogenesis and embryonic development including cell fate specification. Interestingly, silencing of DKK1 increased the percentage of monolayer cells with self-renewing capacity, which is a defining feature of cancer initiating cells.

In contrast to the monolayers, several WNT pathway components were upregulated in melanospheres indicating the preferential activity of this pathway in the multicellular structures. One of the target genes of the WNT pathway is MITF, a transcription factor important for melanocytes and controlling critically aspects of melanoma. In melanospheres, more than 70 MITF target genes were found to be upregulated. MITF contributes to the apoptosis resistance of melanoma cells by upregulating antiapoptotic proteins from the bcl-2 family, and other proteins involved in releasing cellular stress induced by DNA damage, reactive oxygen species or hypoxia. The pro-survival activity of MITF was portrayed in a review article which is part of the thesis. Two more review articles are included, one depicting the pro-survival molecules which protect melanoma cells from apoptosis including Bcl-2, Mcl-1, Bcl-X(L), among others. The third review article deals with pro-apoptotic proteins of the BH3-only type and with strategies employing BH3 mimetics aiming at tumor targeting. Practically, Mag. Hartman analysed gene expression on mRNA and protein

levels in the two cell culture models, partially after silencing of specific proteins. The gene expression study comparing monolayers and melanospheres confirmed that melanosphperes better preserve of the original tumors in terms of the intra-tumoral heterogeneity and the expression levels of key regulators like MITF and DKK1.

In the following part of his work, Mag. Hartman focused on the changes of the expression of five anti-apoptotic proteins during the adaptation to monolayer cell cultures. He could show that the transfer to serum-containing medium is accompanied by transient increases in pro-survival proteins which helps the cells to survive in the altered environment. He further demonstrated that the increased levels of some pro-survival proteins were based on enhanced transcript stability.

The five publications comprising the PhD thesis are complemented by a short introduction and a description of the results including a graphical abstract summarizing the most important findings. The text illustrates a firm knowledge of Mag. Hartman in his research field which became already evident from the publication of the three review articles.

In conclusion, Mag. Hartman presents results which improve the understanding of the phenotypical plasticity which lets melanoma cells adapt to changing growth conditions and eventually complicates melanoma therapies. In addition to the five publications included in the PhD thesis, Mag. Hartman is co-author of eight additional articles (in half of them as first author), showing an outstanding scientific activity for such an early phase of the career.

2. Evaluation research methodology and data analysis

The experiments employed several cell lines which were established from patient derived melanoma samples. Gene expression data were successfully analysed using various bioinformatics tools. Mag. Hartman used quantitative RT-PCR and ELISA to validate microarray data and to measure protein levels of secreted proteins. Protein expression was further analysed by Western blotting which was also

used in combination with fluorescence microscopy to visualize cell death. Gene expression was manipulated by the transfection of siRNAs. Mag. Hartman might have acquired more different techniques during his PhD studies, however the applied methodology perfectly matched the tasks and yielded very convincing results.

3. Formal aspects of the PhD thesis

The PhD work of Mag. Hartman comprises 5 publications published between 2012 and 2015, including 3 review articles. The articles are published in journals with impact factors in the impressive range from 3.4 to 6.1. The five publications are preceded by an abstract in English and Polish languages, a list of own publications, and an abbreviation list. The list of abbreviations is not complete, for example MCAM is not included. Remarkably, Mag. Hartman also functions as Principal Investigator in a PRELUDIUM project. The topic of the PhD thesis is described in a short introduction followed by a description of the results including their discussion and a reference list. The text is written in a very good English language, mistakes are rare. The reference list is appropriate and up to date. In my opinion, the introduction should contain more information about the WNT pathway. A little misleading is the title page preceding the five publications, as not only the texts, but also figures are shown. Participation in national and international conferences, including one invited oral presentation, further demonstrate scientific activity at a high level. In summary, the layout of the PhD thesis was prepared in accordance to all requirements.

4. Final conlusions

In conclusion, according to my opinion the Doctoral thesis of mag. Mariusz Hartman, prepared under supervision of Prof. dr. hab. Małgorzata Czyż, fulfills all the requirements for the acquisition of the doctoral degree. Based on the outstanding quality and quantity of the results obtained for the thesis and the impressive additional achievements during the same period, I strongly recommend to award the candidate by being graduated with 'distinction'.

Uważam, że rozprawa doktorska spełnia warunki określone w art. 13 Ustawy z dn. 14 marca 2003 r. o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki (Dz. U. nr 65, poz. 595 z późniejszymi zmianami). Mam zaszczyt przedstawić Wysokiej Radzie Naukowej Wydziału Lekarskiego Uniwersytetu Medycznego w Łodzi wniosek o przyjęcie rozprawy doktorskiej p. mgr Mariusza Hartmana i dopuszczenie Go do dalszych etapów przewodu doktorskiego.

Biorąc pod uwagę charakter i zakres badań przeprowadzonych przez Doktoranta proponuję wyróżnienie rozprawy doktorskiej.

/Prof. dr hab. Markus Duechler/

Łódź, 3 września 2015 r