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Evaluation of the Ph.D. thesis by Ms. Anna Tejchman, MSc, entitled: "The influence of hypoxia on podoplanin expression in cancer-associated fibroblasts (CAF) and its role in the progression of breast cancer" supervised by Prof. Claudine Kieda and Prof. Maciej Ugorski.

This report is about the scientific work and the achievements that led Ms. Anna Tejchman, MSc, to complete her Ph.D. thesis. The presented work dissects, in several experiments, the role of podoplanin (mucin-type transmembrane glycoprotein) expressed by fibroblasts in breast cancer, especially in conditions of hypoxia.

Breast cancer remains a major public health problem worldwide. Considerable research efforts have led to a better understanding of this cancer and subsequently contributed to its treatment and prevention. However, despite this notable progress our knowledge of the progression and metastasis of breast cancer is still incomplete. It is now well documented that the crosstalk between cancer cells and the tumour stroma plays an important role in the progression of tumours and their metastasis. Among the various cells of the stroma, a specific population of carcinoma-associated fibroblasts (CAFs) plays a crucial role in tumour progression. Therefore, the Ph.D. thesis of Ms. Tejchman is consistent with current trends of breast cancer research. The presented work is very interesting and importantly contributes to our knowledge about the role of podoplanin in the breast cancer progression.

The manuscript is well structured and presented. It is 150 pages in its entirety, with 45 figures, 8 annexes and 185 references. At the beginning of this manuscript, Ms. Tejchman's scientific work is introduced. The results presented in the thesis form part of an original research paper, which has been accepted for publication in Oncotarget (Ms. Tejchman is first author). She is also co-author of two original papers and one review. An exhaustive table of contents is also provided in the thesis, followed by a list of abbreviations and summaries.

Ms. Tejchman introduces her work with a comprehensive and up-to-date review of the literature. The introduction is precise, well structured and focused on the understanding of the thesis objectives and on the rationale of the experiments carried out. The Candidate has demonstrated a thorough knowledge of the scientific field of the thesis.

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The aims of the presented work are clearly and logically defined. First, the role of CAFs expressing podoplanin in migration, invasion, angiogenesis and adhesion properties of breast cancer and endothelial cells was considered. Furthermore, the aim of this work was to identify the factors of the tumour microenvironment that might induce the expression of podoplanin in CAFs, with a specific focus on the influence of hypoxia and the role of specific miRNAs in CAFs. To achieve these goals, a seven-step program of experimental work was designed. The experimental strategy is appropriate and the experimental work is technically sound and makes use of relevant cellular models and advanced molecular approaches. It should be noted that Ms. Tejchman has applied a wide variety of approaches and techniques to answer specific questions.

The results are original, clearly presented and critically discussed with the support of accurate, current literature. The discussion section of the thesis emphasises the contribution of the thesis to the research field taking into account the complexity of the tumour microenvironment in the multifaceted process of the progression and metastasis of breast cancer. Graphical presentations are useful for comprehending the discussion section of Ms. Tejchman's thesis. The conclusion confirms that the objective of the work has been successfully achieved.

To sum up, this study is well constructed and straightforward and has been accepted for publication in an international high-level journal. I have only a few remarks and questions that may contribute to the discussion during the defence of the thesis:

- My concern is with the description of the statistics used. The description appears to be incomplete with respect to several presented results. It appears that tests other than those described in the statistics section have been used or had to be used, i.e. three or more groups/one-way parametric analysis; three or more groups/non-parametric alternative to ANOVA; paired analyses. Unfortunately, the tests used are not specified in the respective figures. In addition, little information is provided as to whether the presented results derive from replicas or only one measure. Furthermore, in Figures 26 and 32 the asterisks indicating significant differences are missing.
- The findings of this dissertation open a multitude of questions for future research. I wonder whether, apart from hypoxia, complex tumour microenvironment components (e.g., oestrogen, inflammatory mediators, hormones secreted by adipocytes and other cells, nutrients) act on CAFs. This knowledge will be of interest in understanding the relationship between the pathophysiological background of patients and cancer progression.
- At the end of her manuscript, Ms. Tejchman discusses some general perspectives offered by this work. However, the limitation of this in vitro research needs to be

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discussed. It would be also of interest to consider short- or medium-term research perspectives on the basis of the obtained results.

In conclusion, the results of the thesis are completely original, have a high scientific value and are important for further research on breast cancer progression and metastasis. The Candidate, Ms. Anna Tejchman, MSc, has achieved all aims of the study and her dissertation satisfies all of the criteria of a doctoral thesis. Therefore, I recommend the thesis to the Council of the Institute of Immunology and Experimental Therapy, Polish Academy of Science in Wroclaw and to the Doctoral Council of the University of Orleans for acceptance.

André Mazur

Directeur de Recherche

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