

Expert opinion report on dissertation thesis of Rosana Mateu Sanz, M.Sc.

### **“Role of fibroblasts in wound healing and cancer”**

The thesis of Rosana Mateu Sanz, M.Sc., was written in 114 page and expanded by another 88 pages of annexes containing 5 publications in which Rosana Mateu participated. The thesis is accompanied with 26 figures and 2 tables documenting the main results. 267 references added to the end of the thesis documented excellent knowledge of the recent literature related to the topic of the thesis.

The thesis has the usual structure. In the introduction, the author provided information on fibroblasts incl. their development, markers, activation signals, functions, and ageing dermis. Then she paid attention to the role of these cells in the process of wound healing and then focused on cancer stroma and cancer-associated fibroblasts participating in construction of a microenvironment promoting tumour cell growth and progression. Finally, she explained principal hypotheses of her study and specified the objectives.

The chapter „Material and Methods“ comprised 7 pages with a brief description of procedures performed incl. cell isolation and cultivation, functional assays, experiments on animals, immunohisto/cytochemistry, Western blotting, qPCR, transcriptome analysis, and statistical analysis.

Results of the thesis were described in 24 pages. The first part of Results was focused on comparison of neonatal and adult fibroblasts and keratinocytes from the point of view of their phenotypic and functional characteristics as well as gene expression pattern. The second part analysed differences between neonatal cleft lip fibroblasts, older children cleft lip fibroblasts and expression profiles of adult skin fibroblast isolated from face, breast and scar. Results identified differences in  $\alpha$ -SMA expression and transcriptional activity of genes participating in TGF- $\beta$  signalling. The third part documented that simultaneous blocking IL-6 and IL-8 with neutralizing antibodies can suppress melanoma cell invasiveness (which otherwise is supported by cancer-associated fibroblasts). Final

part of original results clarified the origin of cancer-associated fibroblasts in experiments with xenotransplantation of human tumours to immunocompromised mice by providing evidence that these cells were recruited from the host tissue.

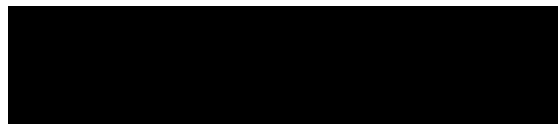
In the discussion, the Ph.D. student further discusses the results of the analyses performed and the findings from his own experiments with the data available in the literature. The most important conclusions are commented separately in the final Summary; their content clearly shows that all the stated objectives of the dissertation have been met.

Regarding a formal processing, the thesis submitted by Ms. Rosana Mateu Sanz, M.Sc., had a common layout and brought novel data. The thesis is written well, with occasional minor grammatical and spelling mistakes. My two criticisms concern: i) inclusion of a review article into the Results of the thesis (this part belongs in the Discussion), and ii) low quality of histological microphotographs (worst in the case of Fig. 24c). These are, however, minor issues. Results of the study are promising and important for the advance of detailed characterization of fibroblasts and their changing properties in ontogenesis with emphasis on new-born fibroblasts and signalling that controls their activity. A part of the study describing cancer-associated fibroblasts not only provides their characteristics but also clarifies their origin from host cells. The quality of a submitted thesis is high, and it is focused on the actual topic. I am impressed by a broad spectrum of sophisticated methods and approaches used in this thesis incl. transcriptome analysis that point out that a site of the cell origin also contributes significantly to the fibroblast heterogeneity. I am convinced all the results described by Mateu Sanz and her colleagues have a positive impact on better understanding of molecular crosstalk between covering epithelium and underlying stromal cells or cancer cells and their stroma which are crucial for targeted therapeutical interventions in modulation of wound healing and cancer progression.

I have two questions for the candidate:

- In the experimental settings described in your thesis you confirmed the cancer-associated fibroblasts were derived from the host organism. Would you be able to suggest conditions under which exogenous cells could also be recruited to tumour stroma?
- When you compare neonatal fibroblasts to their adult counterparts it seems that you consider each population as homogenous although this is not a case. What factors can contribute to intra-organ heterogeneity amongst the fibroblasts?

In my opinion, Ms. Rosana Mateu Sanz, M.Sc., was fully conversant with the subject of the study and showed the capacity to conduct a creative scientific work. She was well trained in sophisticated methodology incl. morphological approaches, cell cultivation as well as methods of molecular biology to receive relevant data. Furthermore, she demonstrated the ability to analyse, interpret and publish her findings. The candidate received rich practical experience from her study in the laboratories of the Department of Anatomy at 1<sup>st</sup> Medical Faculty in Prague. Based on my examination, I judge that the submitted thesis corresponds in its character, extents as well as quality to demands put on the Ph.D. thesis and it merits the award of the degree of Ph.D.



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