Opponent's Report on the Habilitation Thesis of Petr Busek

Analysis of the thesis

General

My report is on the thesis of **Petr Busek, M.D., Ph.D.** from the Institute of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University Prague (2018) entitled

Multifunctional proteases dipeptidyl peptidase-IV and fibroblast activation protein as possible pathogenetic factors, biomarkers and therapeutic targets in cancer.

The thesis describes the role of two related proteases, namely dipeptidyl peptidase-IV (DPP-IV; CD26 and other names) and fibroblast activation protein (FAP; seprase and other names) in two types of cancer, gliomas (brain tumors) and pancreatic ductal adenocarcinoma (pancreatic cancer).

The thesis comprises nine original publications (five where Petr Busek is either first or corresponding author) and five reviews as book chapters (with four as first or corresponding author).

Scope and extend of the thesis

DPP-IV and FAP are two related (more than 50% sequence homology) proteins that belong to the S9B family of "non-classical" serine proteases (inverted sequence of the "classical" catalytic triade, namely Ser-Asp-His). It can be anticipated that both proteins arise from gene duplication. They exist normally as type II transmembrane proteins (N-terminus inside cell) and are located on the cell surface, however, also soluble forms exist. Whereas DPP-IV has a more broad distribution, FAP expression is restricted mainly to activated fibroblasts and some cancer cells.

As enzymes, both liberate N-terminal dipeptides (with proline or alanine in the second position) from bioactive peptides (more pronounced for DPP-IV), whereas FAP has also endopeptidase activity cleaving after these amino acids. As proteins, they can also from complexes with several other proteins implicated and influencing in the immune systems, viral uptake and other in functions.

After summarizing these molecular and functional properties in the introduction, the results of the published original investigations are presented. First, the roles of both enzymes in pancreas and pancreatic cancer are examined. Here, their roles in impaired glucose homeostasis in type 2 diabetes and pancreatic cancer are investigated. Furthermore, the utility of soluble, plasma DPP-IV and FAP activity in pancreatic cancer / cancer-associated diabetes was evaluated. The histological localization of both enzymes in Langerhans islets and pancreatic stroma was specially investigated in this context. As results, the utility of both enzymes as markers and their role in the pathogenesis of cancer-associated diabetes was shown.

Second, the role of both enzymes in the pathology of gliomas (in particular *glioblastoma multiforme;* most fatal brain cancer) was investigated. Main results are the findings that DPP-IV expression / overexpression reduces glioma growth *in vitro* and *in vivo*, which does not rely on its enzymatic activity. In addition, the role of FAP in gliomas was highlighted. Main interesting result is its increased expression in glioma cells of the mesenchymal type (which suggests it utility as a novel biomarker for differentiation of gliomas), and the development of a novel specific inhibitor that may be useful also for treating other types of tumors and chronic inflammations. This should be followed in the future.

Theme elaboration, adequacy of methods / approaches, data processing, presentation and analysis of the results

In the introduction, the various enzymatic and non-enzymatic properties both enzymes are excellently reviewed. There are two instructive figures helping well to understand the background. Good, state-of-the art references are given, including own excellent reviews. These reviews contain further details that place not only the molecular, biochemical and functional properties of both enzymes in a broader context, but high-lighten also the role these and of other proteases in different types of cancer, not only in those investigated here.

The experimental section summarizes shortly the main results of original investigations. The methods used include cell biology, histological and biochemical investigations, including patient's material, furthermore *in vitro* and *in vivo* experiments. This is an excellent approach to elucidate the properties and biological function of both enzymes in cancer. Presentation of the data (in the original publications) and statistical processing (as far needed) are good to excellent. I found the two summarizing graphs in the thesis as particularly instructive and novel.

The results are novel and interesting, not only for specialists in the field, but also for a broader audience in cancer science and biochemistry of proteases. Their general quality is high; measurements and observation are accurate. The thesis adds significant progress to science and in particular, to cancer research.

Main summarizing points are given for both types of cancer investigated. Concluding remarks led to future work and place the results into broader contexts. Overall, this is a well-written thesis based on good and novel experimental data obtained with various modern biochemical, cell biology methods and animal models as further tools.

Statement based on the analysis and overall evaluation of the thesis

The thesis contains new important scientific knowledge!

Conclusion of the opponent

I recommend accepting the habilitation thesis in the present form and, based on this thesis, I recommend granting the

Degree of Docent (Associate Professor) in *Occupational Medicine*

Kiel (Germany), November 1, 2018

[redacted]

(Professor Dr. Rolf Mentlein)