



Habilitation Thesis Reviewer's Report

Masaryk University

Faculty

Procedure field

Applicant

**Applicant's home unit,
institution**

Habilitation thesis

Reviewer

**Reviewer's home unit,
institution**

Faculty of Medicine

Pathological Physiology

RNDr. Michal Masařík, Ph.D.

Department of pathological Physiology, Faculty of
Medicine, Masaryk University

**NEW INSIGHTS INTO THE PATHOGENESIS OF
PROSTATE CANCER**

Prof. Dr. Hajo Haase, Ph.D.

Technische Universität Berlin

Dr. Masařík has submitted a cumulative habilitation thesis, compiling total number of 22 articles, thereby 2 as first, 11 as contributing, and 9 as senior author. All were published in peer-reviewed scientific journals of good or even very good quality. Especially the number of senior authorships demonstrates that he has evolved the required autonomous scientific profile required for habilitation.

Within the habilitation thesis, all of the publications are put into the general context by comprehensive introductory paragraphs, and are wrapped up by clearly stating specific hypotheses relating to the relevant findings.

The overall unifying topic of the thesis is prostate cancer (CaP), arising from epithelial acinar cells of the prostate, an age-prevalent disease in men with a great impact on population health, also constituting a considerable economic burden on health care systems. This topic is investigated from a number of different angles:

The thesis starts with the role of the essential trace element zinc, and metallothionein as its major intracellular storage protein, in CaP. The introduction is complemented by two review articles on metallothionein, summarizing the interaction of metallothionein, and thereby zinc, with oxidative cellular processes, and the role of the genetic variation of this protein, based on various single nucleotide polymorphisms, with different pathologies. Subsequently, the specific role of zinc in cancer is highlighted by review articles regarding the specific role of zinc in prostate and breast cancer cells, as well as the role of microRNAs in prostate cancer, with respect to them regulating metallothionein as one possible explanation for altered resistance toward cytostatics.

The specific scientific contribution of Dr. Masařík to this subject starts with two meta analyses regarding the serum and tissue zinc levels as well as metallothionein during various cancers, clearly showing the changes of zinc and its homeostasis as a common event, not equally well pronounced in all types of cancer, with a good potential to be used as clinical marker in some types, including CaP. Changes in zinc homeostasis lead to altered expression

of genes and microRNAs involved in the regulation of apoptosis, which might point toward a therapeutic approach for targeting CaP cells, and another publication investigates the impact of the cells' long-term zinc status in *in vitro* cell culture on resistance and aggressiveness in these model systems.

In the second part the role of amino acid metabolism is investigated with respect to their usefulness as a marker for tumors and possibly for identifying potential metabolic pathways that may serve as therapeutic targets. Notably, this part is well connected to the previous one, as the close relationship between zinc and citrate in CaP cells suggests an intricate connection between zinc and the amino acid metabolism investigated here in chapter 2. Consequently, the first publication deals with a normalization of the cellular metabolism by selection for zinc resistance, showing that long-term zinc supplementation moves the cellular metabolism toward a more malignant profile. This is followed by three publications describing the impact of the amino acid sarcosine, which might very well be one key metabolite in CaP, and testing liposome-encapsulated anti-sarcosine antibodies as a novel therapeutic approach for CaP treatment in mice.

In the third section the thesis covers the redox status of CaP cells. This is also soundly related to the previous parts, by leading back to metallothionein, as this protein is a key interface between zinc and redox homeostasis. In addition to the first part, these data show a diverse reaction of different *in vitro* cell culture models to zinc, in particular with respect to their redox and antioxidant status, with a particular focus on the impact on the effects of cisplatin in relation to oxidative stress. This part is also connected to the section on amino acids, by revisiting sarcosine and investigating its relationship to redox metabolism. In this case, the group around Dr. Masařík was able to exclude a major molecular link between redox stress and this non-proteinogenic amino acid, suggesting that sarcosine should act via a different, unrelated pathway.

The fourth and final chapter is dedicated to prostate cancer markers. As presently used biomarkers, in particular prostate specific antigen (PSA), cannot predict the presence, let alone the clinical severity, of CaP with sufficient precision, clinics would greatly benefit from more advanced and reliable biomarkers. Coherent with the previous parts, metallothionein and sarcosine were investigated, but also other promising molecules such as caveolin-1 and alpha-methylacyl CoA racemase. Experimental data show that while serum levels of the individual markers will probably not find their way into the clinics, a suitable combination might still be worthwhile to investigate further. Also, data show that metallothionein does not necessarily have to be investigated in serum alone, but also in samples such as blood or tumor tissue. This chapter is completed by a paper reporting an elegant FRET-based nanoparticle sensor for sarcosine, which shows promising sensitivity and specificity in first tests in cell lines and CaP patients' urine.

Taken together, this thesis summarizes a coherent and sophisticated body of scientific work, certainly constituting a significant advancement in its field. It is my firm opinion that Dr. Masařík is clearly fulfilling all scientific requirements regarding a habilitation and this habilitation thesis merits acceptance by the Medical Faculty of Masaryk University, which I hereby allow myself to recommend without any reservations.

Reviewer's questions for the habilitation thesis defence

1. What level of long-term nutritional zinc intake (e.g., limitation, unrestricted access with normal food, or even use of supplements) would you recommend to keep the relative risk for CaP low?
2. In the literature, zinc in seminal fluid has been suggested as a biomarker for prostate cancer some years ago. Can you please explain why zinc can be used to this purpose and how you expect this marker to perform in comparison to existing markers, in particular PSA and serum zinc?
3. What distinguishes zinc homeostasis in CaP from observations made regarding zinc in other cancers, e.g., the finding of Kathryn Taylor group in breast cancer?

Conclusion

The habilitation thesis entitled “New insights into the pathogenesis of prostate cancer” by RNDr. Michal Masařík, Ph.D. *fulfils* requirements expected of a habilitation thesis in the field of Pathological Physiology.

In Berlin on June 12th 2018

Prof. Dr. Hajo Haase, Ph.D