MASARYKOVA UNIVERZITA

Habilitation Thesis Reviewer's Report

Masaryk University Faculty	Faculty of Science
Procedure field	Genomics and proteomics
Applicant	RNDr. Martin Falk, Ph.D.
Applicant's home unit, institution	Institute of Biophysics of the Czech Academy of Sciences
Habilitation thesis	DNA Damage and Repair upon Cell Exposure to Different Types of Ionizing Radiation ? the Importance of Chromatin Context and New Perspectives of Cancer Radiotherapy
Reviewer	Prof Dr rer nat Kai Rothkamm, Dipl Phys
Reviewer's home unit, institution	Department of Radiotherapy and Radiation Oncology, University Medical Center Hamburg Eppendorf

Report summary:

The habilitation thesis submitted by Dr Martin Falk collates, summarises and discusses the extensive body of research undertaken and published by Dr Falk and his research team over the past 18 years. Over this period, Dr Falk has contributed to a number of fields, all of which are associated with the biological effects of ionising radiation and their implications for radiation carcinogenesis as well as cancer radiotherapy. The role of chromatin structure and DNA damage induction, repair and misrepair have been very much in the focus of his research, together with various approaches to modify the cellular response, e.g. through the use of densely ionising particle beams, radiosensitzing metal nanoparticles, extreme conditions (cell freezing) or pharmacological substances. Thirty-seven peer-reviewed journal articles co-authored by Dr Falk, which form the scientific basis of this well-written thesis, clearly demonstrate that this cumulative work by Dr Falk fulfils all the requirements expected of a habilitation thesis.

Specific comments

The abstract is succinct and provides an excellent brief overview of the various aspects covered by Dr Falk’s work over the past 18 years as well as the broader scientific context. The summary is a useful guide through the thesis chapters, which also introduces the different scientific hypotheses and technological advances – especially in the field of chromatin structure and dynamics – that have shaped Dr Falk’s research progress over the years. The thesis aims reinforce the importance of the chromatin perspective in Dr Falk’s overall research strategy.

The Introduction (chapter 1) kicks off with the basic macroscopic effects of radiation exposure, but quickly homes in on DNA damage and, more specifically, DNA double-strand breaks as the most deleterious molecular lesion induced by ionising radiation. Following an introduction to DNA damage response and repair pathways, again with a focus on DNA double-strand break repair mechanisms. The question about the mechanisms driving repair pathway choice could have been covered a little bit more comprehensively (based on the recent literature on the importance of DSB end protection vs. resection), and the alternative end joining pathway could have been introduced, which is favoured in some tumours. Section 1.4 on DSB complexity and LET nicely explains the direct vs. indirect effect; however, a clear description of what complex DSB look like, and of clustered DSB and their biological consequences, would have been useful. Section 1.5 on the dynamic interactions between chromatin and DNA repair exhaustively covers this topic from all possible angles. Following this detailed molecular excursion, section 1.6 takes a step towards translation and looks at the application of ionising radiation in cancer therapy, the main remaining challenges, such as tumour radioresistance, and strategies to overcome treatment failure, including ion beam therapy, the utilisation of high Z nanoparticles for radiosensitisation, a freezing/thawing approach proposed by his group, and options for normal tissue protection. The structure/order of the remaining sections (1.7 Conclusions and Perspectives; 1.8 Methodology; 1.9 Detailed Explanation on the Principles of Higher-Order Chromatin….) has left me somewhat puzzled, but they certainly present sound concepts, meaningful thoughts and a plethora of relevant information, especially once again on chromatin structure and dynamics.

Chapter 2 contains a detailed discussion of the specific aims, methodologies, results and conclusions of all 37 papers that contribute to this thesis. Clustering of several related papers into common themes and short introductory sections for each of these themes have contributed enormously to making this chapter an enjoyable read rather than a daunting task. I would like to congratulate Dr Falk on this achievement!

The concluding part of the thesis summarises the main results obtained by Dr Falk and his team, again split into the aforementioned themes. While some of these are somewhat confirmatory, others represent genuinely new findings that have certainly advanced our understanding of higher order chromatin structure and its dynamic interactions with the DNA damage response and repair machinery. Finally, some of Dr Falk's research findings may support the development of combined treatment approaches to enhance the efficacy of radiotherapy.

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

- Which other DNA double-strand break repair pathways may be of relevance, in addition to canonical NHEJ and HR?
- Which factors influence DNA double-strand break repair pathway choice?
- Why does high LET radiation induce more deletions compared to low LET radiation? Are there different mechanisms for small and large deletions?
- Are there any generic/principal differences between higher order chromatin structure and dynamics in tumour vs. normal tissue cells? Could these be exploited for tumour-specific targeting?

- The introductory sections on nanoparticles mention lysosome-mediated biological effects that may contribute to radiosensitisation via enhanced apoptosis. What might be the underlying biochemical mechanism? How tumour-specific would such an effect be? How could tumour specificity be enhanced?

Conclusion

The habilitation thesis entitled "DNA Damage and Repair upon Cell Exposure to Different Types of Ionizing Radiation ? the Importance of Chromatin Context and New Perspectives of Cancer Radiotherapy" by RNDr. Petr Falk, Ph.D. ***fulfils*** requirements expected of a habilitation thesis in the field of Genomics and proteomics.

1.4.2020

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