

**Reviewer's Report on Doctoral Thesis**  
“Alteration of the redox signalling in liver cancer cells  
by non-thermal plasma and laser radiation”  
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The Doctoral Thesis of Barbora Smolková is devoted to *one of the focal problems* of application of the physics methods in biomedicine, namely using non-thermal plasma (NTP) and laser irradiation as possible means in cancer therapy. It is focused on studies of redox signalling in three types of liver cancer cells, carcinoma cells Huh7 and Alexander (PLC/PRF/5), and hepatoblastoma cells HepG2. Redox signaling species (ROS) are the communication centers, which signal to the organism if something is going wrong and there is a need of a change for restoring the normal actions of cells. Thus, the ROS signalling alteration can be a very good indicator of positive or negative changes upon different actions on living cells. In the PhD work of Barbora Smolková, the ROS responses are used as markers for NTP and laser light actions on hepatocellular carcinoma and hepatoblastoma cells. Although some works in these directions have already been known in literature, still the applications of novel, more efficient methods for cancer treatment and diagnostics of treatment effect are of high demand, and this Thesis makes a very good contribution to the field.

The Thesis is logically structured into chapters, subchapters, and Appendixes, which represent the publications co-authored by Barbora Smolková. The first Chapter presents a very thorough review of the state-of-the-art in the main subjects of doctoral research, redox signalling in cells, non-thermal plasma action on cancer cells, and light-cancer interaction. This part of the Thesis demonstrates a deep understanding by the candidate of both the subject and the methods of the interdisciplinary research, which includes cell biology, medicine, chemistry, and physics. The list of literature consisting of 218 references justifies very good knowledge of the topic under study. In the second Chapter, the aims of the doctoral study are presented in concise but clear and convincing way. The third Chapter overview the experimental methods, which have been applied in the investigations, ranging from cell cultivation to several types of microscopies and spectrofluorometric and statistical analyses.

The fourth Chapter describes the rich experimental data on NTP and laser light actions on liver cancer cells, which are very well presented in the form of tables, graphs, spectra, and figures. The Chapter is mostly focused on manifestations and mechanisms of ROS signalling. By now, the ROS signalling has not still completely been understood for different types of cell damage, in particular cancer ones. Against the background of information flow on this topic, the contribution presented in the Doctoral Thesis by Barbora Smolková is impressive and adds considerable novelty to understanding of ROS signalling as a whole and in particular a response of ROS to NTP and laser actions on cancer cells. The following *new observations* reported by Barbora Smolková can be specially highlighted:

- The molecular mechanisms of the action of air non-thermal plasma on liver cancer cells have been identified. It has been shown that NTP plasma action leads to accumulation of redox signaling species in liver cancer cells. For Huh7 and Alexander cancer cells, ROS accumulation was demonstrated to induce apoptotic death, which is mitochondria-mediated and p53 dependent. The HepG2 cells occurred to be resistant to NTP. The resistance is explained by the elevated level of Bcl-2 protein in HepG2 cells.

- It was shown that blue and red laser light at low power can be an efficient tool for inducing the apoptotic death of Huh7 and Alexander cancer cells. Interesting aspect of this finding is that the mechanisms of blue and red light action are different in spite of almost similar results on the apoptotic death. It has been revealed that blue light inhibits cytochrome c oxidase (COX) in electron transport chains of mitochondria while red light excessively activates COX. Interestingly, both these mechanisms lead to ROS overproduction resulting in apoptosis.
- Similarly to the NTP observation, action of either blue or red laser light on HepG2 did not lead to apoptosis. It was again explained convincingly that the resistance of HepG2 cells to laser light is explained by elevated level of Bcl-2 protein.

As a whole, the experimental results obtained in the frames of the doctoral work are of valuable fundamental and practical importance as they give indications for the possible routes for identification of specific cancer cells for whose treatments NTP and/or by low-power laser irradiation are applicable. The main results of the Thesis have been published in 4 papers in high-IP journals. It should be noted that the whole number of publications of Barbora Smolková is 17 and additionally 3 papers are now under revision that is impressive.

Upon reading this Dissertation, some *remarks arose*. In the Thesis, not so clear is why green laser light does not have any effect on any of cancer cell lines studied. Another question is about personal contribution of the author in this very rich collective work, which involves numerous investigation methods and techniques. However, according to the Thesis description, it is clear that the candidate well mastered the methodology of research and was deeply involved into the reported investigations, that resulted in an excellent Doctoral Thesis.

Summarizing, the candidate for PhD degree has performed large amount of insightful research and obtained new original results, which broaden our understanding in non-thermal plasma and laser light action on a number of liver cancer cell lines. The doctoral work has been performed at a high scientific level. All important results presented in the Doctoral Thesis are published in peer-review journals. Judging by the Dissertation, the candidate Barbora Smolková merits the PhD Degree.

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