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Report on the doctoral thesis presented by Mgr. Markéta Křivánková

„Biological characteristics of polysaccharide-based contrast agents for cancer diagnostics".

The doctoral thesis presented by Markéta Křivánková deals with a biological examination of the novel polysaccharide-based probes intended for tumor diagnostics and detecting sentinel lymph nodes by bimodal optical and magnetic-resonance imaging (MRI). Author demonstrates that biocompatible glycogen- and mannan-based polysaccharide carriers functionalized with near infrared dyes and paramagnetic contrast label were successfully accumulated and visualized in tumors and lymph nodes. Moreover, modification of polysaccharide-based probes with polyoxazoline prevented uptake in internal organs and inhibited accumulation in target tumour tissue.

The thesis is well written and presented in 11 chapters. The used abbreviations are listed in the *Chapter 1* where some of them were missed. The introduction (*second chapter*) consists in a well-illustrated, informative, and detailed review of the literature about cancer treatment, a key role of lymph nodes in metastatic process, and polymer nanocarriers for drug delivery system. Combination of *in vivo* optical fluorescence imaging, which provides only limited quantitative analysis, with highly sensitive MRI in one system represents an interesting approach complementing high resolution and accuracy of fluorescence analysis with a high penetration depth of MRI. For this reason, this aspect is reviewed in detail. A literature review also includes the recent papers that describe an application of polysaccharide-based nanoparticles for theranostic nanomedicine. Nevertheless, some data in the review are not correctly described and thus, the comparison of results among publications is difficult. Objectives (*Chapter 3*) are sufficiently developed where all the results correspond to the aim and problem of the work.

The results obtained in this thesis led to the well-known peer-reviewed publications which are listed in the *Chapter 4*. These papers published in high impact journals: Drug delivery and translational research (IF 4.617), Scientific reports (IF 4.379), Journal of Materials Chemistry B (IF 6.331) and Molecules (IF 4.411). The materials and methods of the work including chemical and biological parts are detailed described in the *Chapter 5*.

The main results of thesis specifying the contributions of the candidate are divided into two parts: Results (*chapter 6*) and Discussion (*Chapter 7*). They are with the same structure as scientific papers, which have already been reviewed and published. In these parts of the thesis, a complex study was carried out on biological characterization of a novel glycogen- and mannan-based polysaccharide contrast system. Both conjugates were surface modified with the paramagnetic gadolinium chelate for MRI and organic fluorescent dyes for optical imaging. All

in vitro and *in vivo* results showed potential of synthesized compound for multimodal imaging of solid tumors and sentinel lymph nodes. Further functionalization of the polysaccharides with poly(2-methyl-2-oxazoline) reduced the elimination from the body and accumulation in organs confirming the well-known significant interest for oxazoline applications. Histological analysis was performed at the end of the experiments confirming the absence of pathological changes in the organs of experimental animals.

The doctoral thesis finishes with a summary (*Chapter 8*) of the most relevant conclusions. All the information was well supported by correctly cited bibliography detailed in the section "References" (*Chapter 9*). The author's CV (*Chapter 10*) and published articles (*Appendix, Chapter 11*) are presented at the end of thesis by the author.

The manuscript contains very interesting information which opens many questions and future research topics. Comments and questions for discussion:

- Comparisons over the past results (past literatures and the other analogue materials) are not sufficiently described.
- Cell viability and cytotoxicity assays with cultured cells are widely used for cytotoxicity results. Nevertheless, it is not clear to me how the discussed cell viability and cytotoxicity in the thesis differs from each other in terms of conjugate toxicity. What's the difference between obtained results and why author separated these terms?
- Compare to the detailed characterized MR relaxometry and imaging, photoluminescence spectra and information about stability in cell growth mediums are missing and should be discuss to allow for a fair assessment of tested conjugates for biological multimodal imaging.
- I am facing difficulties in understanding the following sentence on the p.41: "*Přidání D-glukózy do inkubačního media HepG2 buněk mělo na kolokalizaci glykogenových konjugátů s lysozómy pouze zanedbatelný vliv.*" Clarification of negligible impact may be needed here.
- Page 41: in the discussion of the toxicity results, shown in Figure 7B, I do not fully agree with the statement: the same conclusions (*conjugate is non-toxic*) can also be drawn for GG-GdDOTA-Dy615. Considering the increasing absorbance at low concentrations of conjugates, the 0.6% GG-GdDOTA-Dy615 had a value which was decreased by 30% comparing to the control, low concentrations of this conjugates, or to GG-PMeOx-1-FITC and unmodified glycogen at the same concentrations, are the differences between the results statistically significant?
- The quality of figures 16, 22, 27 and 28 badly support the intelligibility of content and they aren't a benefit to the thesis. Description of scale bar is missed in Figure 5. In the figure 11 and 12 (A, B), it is unclear, which concentrations are given. Some minor typos are presented during the thesis.
- Note that the comparison of the image contrast (at 4.7 T) and relaxivities (at 0.5 T) is not so straightforward, due to the effects of the magnetic field on r_1 and r_2. How does this influence the obtained results?
- The optical and MR properties of the obtained glycogen- and mannan-based carriers should be detailed compared to other polysaccharide for issued from literature.
- Polyoxazoline-modified glycogen- and mannan-based particles demonstrated worse MRI and FI than unmodified ones and commercially available contrast agents. Any modification potentially plays an important role when it comes to MRI (interaction with water protons) as well as photoluminescence (possible quenchers of organic

dyes), which served as a corroborative research tool to supplement *in vivo* MRI. Moreover, photobleaching of NIR dyes prevents long-term imaging and monitoring of fluorescently labeled substances, which reduces the overall fluorescence signal-to-noise. In this respect, conclusion should be justified by results with further recommendations for improvement.

In addition, the author has demonstrated great writing skills as the document is very well written and presented to read with formal standard. The structure follows recommendation for doctoral thesis. I cordially congratulate Mgr. Markéta Křivánková on this achievement and wish her all the best for the continuation of her career. This is an excellent work which fulfills the requirements to get the PhD degree.

Yours sincerely,

Dr. Oleksandr Shapoval

Prague, May 10, 2022