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Opponent´s review on the doctoral dissertation prepared by Mrs. Nazila Navvabi

Mrs. **Nazilla Navvabi**, submitted for her defense a doctoral dissertation on "Molecular prognostic and predictive markers in colorectal cancer", which she worked out at the Faculty of Medicine in Pilsen, Charles University, under the guidance of Dr Pavel Pitule. A remarkable topic on the medically important and demanding issue of colorectal cancer (CRC) is very ambitious. The author prepared the dissertation with the necessary thoroughness in the range of 100 pages. She published the original results in the respected scientific journal Cancer Genomics Proteomics. In addition, she has prepared or published two other publications on a topics that are not related to the dissertation, but confirms her research asset in the meantime when she had the opportunity to work at home in Iran.

Colon cancer is one of the most common cancers not only in the Czech Republic but also in many other countries around the world. A prerequisite for its prevention, suppression of its prevalence and successful treatment is reliable early diagnosis of predictive markers of this malignant disease. By predictive markers we mean those that can reliably indicate the onset of synthesis of CRC protein markers circulating in the body as soluble proteins or proteins bound to certain cells. Current knowledge in this area suggests that predictive markers could be the MBNL1 protein and its paralogs 2 and 3. These are RNA splicing proteins that play a key role in the development of Myotonic dystrophy in humans. Lidský MBNL1 is an alternative splicing regulator that harbors dual function as both a repressor and activator for terminal muscle differentiation. In her dissertation, the author demonstrated for the first time the deregulation of MBNL genes in CRC. Due to the physiological role of MBNL proteins, it presupposed a down-regulation of their transcripts in tumor tissue compared to non-tumor mucasa. Based on her hypothesis it can lead to transition of alternative splicing patterns towards a less differentiated phenotype that consequentially induce further tumor development. The results presented by the author of the dissertation confirm the fundamental importance of alternative splicing regulation for tumor growth and propagation.

The author of the dissertation showed considerable invention in solving the dissertation, in verifying the hypothesis about the importance and role of alternative splicing and the involvement of MBNL protein paralogs in the mechanism of alternative splicing by deregulation of MBNL genes. Using advanced methods of molecular biology and also statistical processing of the obtained results, it confirmed the validity of the hypothesis.

The author of the dissertation approached the solution of the problem very comprehensively, formulated a total of 4 hypotheses that she tested, and formulated the conclusions reached.

Hypothesis #1 - evaluation of all 3 MNBL paralogs in CRC - deregulation of their mRNA in CRC tissue _ confirmed!

Hypothesis #2 - CRC is associated with deregulated expression of specific splice variants of selected genes (FOXP1; CD44; EPB41L3) _ confirmed!

Hypothesis #3 - Deregulation expression of the studied genes is related to clinical parameters (Z, N, M, G, UICC, overall/recurrence-free survival) - unconfirmed association between expression of MBNL paralogs and clinical parameters in CRC!

Hypothesis #4 - CRC associated with deregulation of base excision repair glycosylases hOGG1 and MUTYH -_ confirmed decreased expression of both glycosylases!
Hypothesis #5 - Expression of miRNA is deregulated in CRC cells - modulation of sensitivity to oxaliplatin treatment _ confirmed downregulated miR-140 in CRC tissues vs Increasing miR-140 in CRC cell lines resulted in suppressed expression of MRE 11 and increased sensitivity to oxaliplatin treatment.
The author of the dissertation Mrs. Nazila Navvabi published the results as lead author in the prestigious journal *Cancer Genomics and Proteomics* and co-author with a significant share of her contribution in three other research papers in the International *Journal of Molecular Sciences* and *Frontiers in Oncology* and in a book chapter (*Springer Nature*). In addition, she is the author/co-author of two other original research publications with a dissertation topic not directly related, but using her experimental experience with molecular biology methods.
The dissertation is written clearly in all their parts and brings laboratory diagnostics closer to the possibility of using the deregulatory effect of MBNL in alternative splicing to prognostic and predictive diagnosis of impending tumor disease as well as importance of genes coding for the base excision repair glycosylases and miRNAs in CRC pathogenesis and diagnostics. If successful, general medicine would be a desirable early warning molecular tool for the development of CRC.

I have the following questions for the author of the dissertation:
(1) What are the structural and functional properties of MBNL proteins?
(2) How do MBNL proteins deregulate alternative splicing, how does recognition of correct exon splicing work?
(3) What is the difference between constitutive and alternative splicing and the difference in their phylogenetic development?
(4) What we know about repair mechanisms other than glycosylase-based in the cell defense against malignancy in CRC tissues?

In conclusion, Mrs. Nazila Navvabi submitted a dissertation of required quality, and if she fulfielled all other requirements required by law and internal measures of Charles University, I am pleased to recommend her dissertation for defense.

With most cordial regards,

Sincerely yours,

Dr. Libor Grubhoffer

Professor and Director

Biology Centre of the Czech Academy of Sciences