Faculty of Science
CHARLES UNIVERSITY IN PRAGUE

Synoptická meteorologie

Opponent assessment of PhD thesis

Opponent:	RNDr. Karel Drbal, PhD	**Date:** 14. 2. 2014
Author:	Mgr. Michaela Krausová	
Title:	Molecular mechanisms and components controlling the Wnt signaling pathway output	
Institution:	Charles University in Prague	**Discipline**: Biochemistry (31-250)
Advisor:	RNDr.Vladimír Kořínek, PhD	**Mentor:**

Prologue:

Immediately, when reading through the preface, I have realized this thesis is an exceptional one. At midnight I had started and could not resist the temptation to read the 60 pages Introduction chapter at once until I finished in the morning, just as it would be the best novel I have read.
The day before, I went to see Michaela's presentation at the PhD cover stories of the annual Advances in Molecular Biology and Genetics at her home institution IMG. And I was astonished by her easy and fluent diction in the lecture she gave. Science, language, patience, ambition - all merged together in a beautiful concert.

Summary:

Scientifically, the topic of the thesis encompasses the central question of tumor and developmental biology today: the role of stem cells in the adulthood and the interplay between the plastic and hardwired signaling pathways in their developmental cues.
Formally, there is absolutely no objection I can raise. High quantity of 187 pages written in excellent English are filled with high quality science. The extensive introduction (60 pages) was organized and cited correctly, followed by the aims of the study and discussion on 4 primary publications. Usually absent introduction and up-to-date discussion to each of the inserted publications precedes it correctly in an extensive way followed later by statement of the author contribution in Conclusion chapter.
I have learned form this thesis many details of a modern view on tumorigenesis with many details in the intestine tissue. With Michaela's deep understanding of the topic and her personal attitude for Science, she succeeded to narrate us clearly and undeniably through the complicated story. Simply, I can only express my admiration. The already strong Wnt community in the Czech Republic seems to get an additional boost in quality.

Epilogue:

Michaela's list of publications in not an exhaustive one and I put her example forward. PhD thesis must be only a starting point of our careers, not an ultimate goal. One has to learn the scientific discourse, the methodical nuances and the humility in front of experimental data tangles, all in a fast and appropriate manner, in analogy to Wnt itself – just-right dosage.
Michaela has started her career with nobility and I really hope her example will soon be followed by other talented and dedicated young scientists, who I can see around not only at the Faculty of Science. Remember your Alma Mater in your future scientific life and be proud of where your footsteps originate.
In respect to Paul Ehrlich I would like to finish with his words to wish Michaela to achieve the four big Gs in Science: *"Geduld, Geschick, Glück und Geld" (patience, ability, luck and money)*.
The first two Michaela has already achieved the later two she deserves. It is more important to learn the technology of financial support and most importantly the team leadership to really become respected investigator. I truly believe in Michaela's future dedication to Science in all aspects of that term, and I wish her the best career she might ever envisage.

Recommendation:

The submitted thesis is one of the best I have ever read. Without any doubt I can recommend it for successful admission of the PhD degree.

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Details:

In the introduction the author has described carefully the historical perspective and mode of function of Wnt signaling. Many details of structural features, biochemical processes and genetic screens as well as posttranslational modifications of individual players have been discussed and put into a complex overview of embryogenesis and tumorigenesis roles of Wnt signaling in multiple model species.

It would be beneficial to bring it one step further to provide reader with a comprehensive list of Wnt ligands, receptors followed by their effector / signaling function and affinities, knowing that almost 20 *Wnt* genes have been described. The receptors of the Frizzled family together with LRP co-receptor and LGR/RNFcomplex should have been graphically described in signaling schemes to illustrate the information-rich text.

Despite the title of the thesis focusing on: „Wnt signaling pathway output", there is only a brief description of transcription network (mainly the canonical β-catenin) without a comprehensive scheme of important target genes and their regulation. The gene expression programs have been described only very shortly in fragments in more chapters, such as: "3.9 Malignant conversion of the intestinal tissue". It would deserve a separate chapter since the particular gene in/activation (very often an epigenetic one) is the major driver of tumor phenotypes and clearly discriminates between the described non/canonical pathways including other interconnected programs (e.g. TGFβ, Hippo, Foxo, NFκB). Moreover, the Wnt target gene identification has been one of the major aims of the study and I would recommend the author to concentrate more on the final goal of and this is in my opinion the word OUTPUT: define the output, describe it and discuss with the data obtained. I know it is obvious that such links are difficult to draw through the myriad of signaling branches of Wnt with all the cellular fates of multiple cell lineages involved in the tumorigenic process. Heterogeneity of tumor mass was and its convergent evolution was not put into a fully complex picture with respect to tumor genotype and phenotype, the involvement of external inflammatory and stromal cells and their regulation of Wnt signaling.

The author proved an extraordinary ability for synthetic work. There is much more work ahead in Michaela's scientific career to conquer. The starting point in terms of her PhD degree prerequisites has been achieved with honor.

Questions:

I would like the following questions to be discussed during the thesis defense:

1. Questions related to the 1st publication:
   a. Lipid modifications are important for Wnt ligand function.
      Is it the effect of Wnt membrane attachment and partitioning (lipid rafts) or rather physicochemical properties of Wnt (hydrophobicity) to be more important for Wnt signaling? Is the O- or S-acylation dominant for particular Wnts driving the canonical and non-canonical signaling, respectively?
   b. How the disulfide competition over the reversible S-acylation regulates the function of Wnts?
      Is there a molecular regulation of reversibility of Wnt S-acylation?
      Can we attribute this dynamics to Wnt signaling activity and correlate it with the redox potential? Is it related to the extracellular oxidative environment and even enhanced in the pro-oxidative inflammation?
      What is the result of real-time imaging of Wnt dynamics (Holzer *et al.,* 2012)?
   c. What is the tumorigenic role of long-range Wnt traveling in exosomes vs. carrier proteins (Swim/Lipophorin/Flotillin2)? Is there any?
   d. Are the Wnt morhogen gradients deposited on ECM typical only for developmental processes and do these regulate the tumorigenesis as well?
2. Questions related to the 3rd publication:
   a. Is there a clear difference of tumorigenic potential in respect to slowly and fast cycling intestinal stem cells?
   b. TNFRSF19 (TROY) potential ligand LTA is driving the normal lymphoid tissue development including the tertiary nodules with inflammatory signature based on NFκB signaling. Moreover, other inflammatory signatures such as TLRs have been attributed to CRC pathology. In this respect, IL-6 mediated phosphorylation of STAT3 (mentioned as the LEF1 target) is a well known point of convergence in many tumor types.
      Can you comment on the importance of this inflammatory scenario in CRC?
   c. While in mouse tumor models with clear upregulation of TROY mRNA and protein levels (with no apparent phenotype in $TROY^{-/-}$ mouse) there is observed both up- and downregulation of TROY levels in patients with advanced tumors. The collected samples of human sporadic colorectal cancer indicated a different pattern of TROY deregulation in human tumors.
      Is this species difference related to the positional information of the tumor origin or to diverse mutations? Moreover, the author has mentioned that C/EBPs do repress TROY. Is there any evidence for epigenetic silencing of C/EBPs in CRC such as in AML and other leukemias?
   d. Is there a potential use for TROY being a true classifier in CRC patient stratification? Could you, please, summarize the current knowledge on other progenitor signature genes under your study (LGR4/LGR5, AXIN2, ASCL2, SOX9, BMI1) to serve as valuable classifiers for CRC on the background of major drivers such as APC, TP53, KRAS, PTEN, SMAD4 mutations?

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3. It was stated in the introduction that the author concentrates on canonical Wnt signaling.
   However, is the ultimate function of Wnt signaling in early tumorigenesis dependent more on canonical Wnt3a- or non-canonical Wnt5a-mediated signaling?
   Does the ratio between these signaling pathways change between normal homeostasis and early tumorigenic process or aging in the intestinal stem cells?
4. A frequently mentioned context-sensitive alternative regulation of Wnt signaling was not finally summarized.
   Is there any connection between Wnt and TGFβ, Hippo, Foxo, NFκB signaling pathways in the fine tuning of the context (stroma)-dependent tumorigenic potential of Wnt pathway?
5. Currently, there is an established system of histological analysis of excised and fixed solid tumors in clinical practice.
   My final question is directed to the potential use of live biopsies for *in vitro* or *in vivo* analysis of their heterogeneity and therapy susceptibility: would it be possible to test their true complex phenotype within each patient including the follow-up analysis of tumor development and evolution *in situ*?

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