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Reviewer comment to the PhD dissertation by Mgr. Michaela Rothová: Cell proliferation and tissue dynamics during development of teeth and tooth related palatal rugae

The dissertation has been presented as a short comprehensive overview of recent knowledge, thesis aims, results, discussion, and major conclusions followed by a collection of related IF papers, which Michaela (co)authored. The style is clear and concise, the sequence of the chapters is logical, presented data are well supported by figures and schematic displays. Most of the work originated in the international cooperation with the Department of Craniofacial Development and Orthodontics, King´s College London. The author neatly combined expertise, know-how and facilities of both groups and focused her thesis on challenging topics in craniofacial biology.

The first part of the thesis deals with further development of knowledge on rudimentary teeth (supervisor is an expert in this field) and also the molecular background of supernumerary tooth development. Michaela, as a member of the research team, moved the studies on morphogenesis of rudiments to a detailed molecular level. The findings published in PNAS (2010) revealed three signalling centres sequentially patterned and collocalized with tooth rudiments related to the first molar (M1). The rudiments therefore seem to have their own signalling centres, resembling the primary enamel knot of molar teeth. Moreover the R2 rudimentary bud was clearly shown to integrate with second molar (M2).

Revitalization of the diastemal tooth primordial is the research subject of the second part of the thesis. Michaela cooperated in a study based particularly on qualitative and quantitative evaluations of proliferation and apoptosis. As it was previously shown that the arrest of a rudimentary tooth bud could be rescued by inactivation of Spry2 (FGF signalling antagonist), this research focused on an analysis of Spry2 deficient embryos. Increased level of FGF signalling led to decreased apoptosis and increased proliferation in the rudimental bud. Consequently, this bud was involved in formation of a supernumerary tooth primordium.

To view the dynamics of tooth development, cell fate maps after labelling, tissue grafting and modern methods of organs slices, introduced by Michaela´s co-supervisor, were used in the further parts of the thesis. Here the author demonstrates amazing data showing clearly the origin of the dental papilla not only from the cells of the condensed dental mesenchyme.

Invasion of several non-neural crest cells was observed in the forming dental papilla. Moreover, the dental papilla cells were shown to contribute to the formation of the dental follicle already from the cap stage of odontogenesis.
The closing experimental part of the thesis contributes to the knowledge on patterning of palatal rugae and reveals anterior/posterior boundaries in palatal development.
The second half of the thesis book is created by five IF papers in their full version. Michaela is the first author of two of them and contributed as a co-author to the others. All papers have been published (one of them submitted) in respected IF journals, some of them already cited and support the superior quality of the dissertation.

Based on this evaluation, **I am happy to recommend the thesis by Mgr. Michaela Rothová** for defence discussion. The thesis **fulfils all criteria for graduation** of the author as the Doctor of Philosophy.

Questions for discussion:
1) The author states that "the signalling role of the primary and secondary enamel knot centres is terminated by apoptosis" (p. 12) – does it mean that absence (inhibition) of apoptosis leads to persistent signalling?
2) The author refers to the cervical loop in plural (loops). How many cervical loops has one tooth germ?
3) The author mentions embryonic staging ED 12.7 or 13.3 (p. 22). How was such exact timing achieved and how much was the set of embryos homogenous?
4) How was reasoned selection of Ki67 as the proliferation and PH-3 as the mitotic markers for double staining? Were there expected (and found) temporospatial differences?
5) The author emphasizes the importance of the findings for molecular dentistry (bio-engineered teeth). What direct contribution does the author think her results might have? How is the expected extrapolation of findings in the mouse for the human?