Abstract

Tooth development in the mouse embryo is an important model of developmental biology for studying not only odontogenesis, but also general organogenesis, and it also has considerable biomedical potential. Tooth shares many developmental features with other epithelial organs whose development initiates from budding of epithelium. The tooth is not only an isolated organ, but it is a part of the organ system - dentition. During dentition development, there is serial initiation of developing teeth. The presumed basic tooth formula in placental mammals comprised three incisors, one canine, four premolars and three molars. Dentition of rodents is already very derived being only formed by one continuously growing incisor and three molars in each dental quadrant. In place of missing teeth between the incisor and molars is a toothless region called diastema. During mouse embryonic development, it is possible to observe the initiation of development of rudimentary tooth primordia in both incisor area and in prospective diastema. In contrast to these morphological findings, the generally accepted assumption is that only the prospective functional incisor and the first molar (M1) develop during initial stages of mouse odontogenesis (ED 11-14) and, consequently, all the molecular signalling events are exclusively co-localised with the same tooth positions corresponding to prospective functional teeth. Some genetically altered mice exhibit the formation of a supernumerary tooth anterior to the molars or in the incisor area. Ontogenetic mechanisms of these supernumerary teeth have not been satisfactorily explained yet. The thesis aimed to experimental determination of the embryonic tooth pattern, and of the developmental dynamics and role of rudimentary tooth primordial during early stages of odontogenesis in mouse mandible. For better understanding of the serial initiation of organ development, we focused on the simpler system of ectodermal organs related to teeth - the development of palatal rugae (ridges on the hard palate). We observed the serial emergence of new rugae by their reiterative interposition in the morphogenetically active area at the posterior boundary of the hard palate. Based on our observations, we proposed a molecular model of temporospatial regulation of new ruga initiation. We showed that rugae development is at the molecular level much simpler system than the regulation of tooth development, but the basic developmental program can be very close. We used the acquired knowledge in the mouse embryonic mandible, where we focused on proving the hypothesis that the rudimentary primordia in cheek area in front of M1 may exhibit their own signalling activity, which could be mistaken for the signalling of the M1 primordium. We demonstrated experimentally that there are serially initiated three signalling centers in the cheek area of the mouse embryonic mandible:. ED 12.5 signalling center is related to the rudimentary primordium MS, ED 13.5 signalling center corresponds to the rudimentary primordium R2, and ED 14.5 signalling center is associated with initialization of the prospective functional M1 development. These findings are in contrast to the generally accepted view that all morphogenetic and signalling activity during early odontogenesis in the cheek area exclusively correspond to the development of M1. Using time-lapse microscopy, we observed the fusion of rudimentary primordium R2 and developing primordium of M1 and consequently their joint development. Thus we provided the first experimental evidence that dental primordia can merge during development, and this mechanism can be responsible for changes of morphology of functional teeth during evolution. In transgenic Sprouty2 -/-, we validated the hypothesis that the emergence of the supernumerary cheek tooth results from revitalization of rudimentary tooth primordium in front of M1. Taking advantage of our knowledge on the cheek area, we analysed the dynamics of expression of Shh as a marker of signalling centers of tooth primordia also in the incisor area. Surprisingly, in contrast to the generally accepted model of development of functional incisor, we found that the first detectable *Shh* expression domain did not correspond to functional incisor primordium, but is associated with a rudimentary incisor of previous tooth generation. *Shh* expression domain of functional incisor appeared later and more posteriorly. Again, it was confirmed that the rudimentary primordia may have partially preserved signalling activity and be confused with functional teeth. Our study opens new dimension of research of regulatory signalling pathways in tooth development by comparing the rudimentary tooth primordia, whose further autonomous development is suppressed, with the progressive development of future functional tooth. The deeper understanding of tooth developmental mechanisms and regulations may be used in the preparation of biological dental replacements in the future.