グローバル COE 特別セミナー

代謝生理化学
発生学ジョイントセミナー

（平成 19 年度）

演者:

山田　源　博士

熊本大学生命資源研究・支援センター動物資源開発研究部門

Coordinated organogenesis in embryos; orchestrated growth factor signaling and EMT regulation for reproductive/urogenital organ formation and caudal embryogenesis.

Giovanni Levi 博士

Evolution des Régulations Endocriniennes, CNRS, UMR5166,
Muséum National d'Histoire Naturelle, Paris, France

Role of Dlx5 and Dlx6 genes in the control of limb and craniofacial development.

日時:　2007 年 10 月 9 日（火）16:30ー18:00
場所:　医学系研究科1号館1階講堂

要旨:（別紙）

世話人:　院医・代謝生理化学　栗原裕基（内　23495）

Coordinated organogenesis in embryos; orchestrated growth factor signaling and EMT regulation for reproductive/urogenital organ formation and caudal embryogenesis.

Gen Yamada
Center for Animal Resources and Development (CARD), Graduate School of Medical and Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan

We have been working on the role of several growth factor cascades for reproductive/urogenital organ formation. Coordinated organogenesis is one of the key issues not only for such reproductive organs but a general problem for several organogenesis. By analyzing one of the growth factor cascade, hedgehog pathway, we found immature mesenchyme in the caudal embryos can contribute to several different organs. We also recently found the regulation EMT (Epithelial-Mesenchymal Transition) is crucial for caudal embryogenesis. Our recent observations will be discussed.

Gen (*right*) with Giovanni (*left*)

Role of Dlx5 and Dlx6 genes in the control of limb and craniofacial development.

Giovanni Levi
Evolution des Régulations Endocriniennes, CNRS, UMR5166, Muséum National d'Histoire Naturelle, Paris, France

Dlx genes are homeobox-containing genes involved in the control of limbs and head development. Inactivation of these genes by homologous recombination leads to severe limb and craniofacial lesions. In the first part of the talk the role and regulation of Dlx genes in the control of craniofacial development will be discussed. In particular data supporting the notion of a dosage effect of homeobox genes and their activating signals in the control of head morphogenesis will be presented. The regulation of *Dlx* genes during craniofacial development will then be addressed. In particular the effects of Retinoic Acid (RA) on the activation of *Dlx* genes will be presented. Intake of retinoic acid (RA) or of its precursor, vitamin A, during early pregnancy is associated with increased incidence of craniofacial lesions. The origin of these teratogenic effects remains enigmatic as in cranial neural crest cells (CNCCs), which largely contribute to craniofacial structures, the RA-transduction pathway is not active. Recent results suggest that RA could act on the endoderm of the first pharyngeal arch (1stPA), through a RARß-dependent mechanism. We show that RA provokes dramatically different craniofacial malformations when administered at slightly different developmental times within a narrow temporal interval corresponding to the colonization of the 1st PA by CNCCs. We provide evidence showing that RA acts on the signalling epithelium of the 1st PA gradually reducing the expression of endothelin-1 and Fgf8. These two molecular signals are instrumental in activating *Dlx* genes in incoming CNCCs thereby triggering the morphogenetic programs, which specify different jaw elements. New data will be presented on the role of CNCC on the patterning and

differentiation of head muscles. Similar experiments performed in Xenopus show that treatments as short as one minute of the embryo with RA during the period of 1st PA colonization by CNCC are sufficient to provoke serious craniofacial defects in the larva. Our results might provide a conceptual framework for the rise of jaw morphotypes characteristic of gnathostomes.

In the second part of the seminar the interaction of Dlx5 and 6 with other transcription factors essential for the control of limb development will be discusses. The congenital malformation Split Hand-Foot (SHFM, or ectrodactyly) is characterized by a medial cleft of hands and feet, and missing central fingers. Five genetically distinct forms are known in human; the most common (type-I) is linked to deletions of *DSS1* and the *distalless*-related homeogenes *DLX5* and *DLX6*. As *Dlx5;Dlx6* double knockout mice show a SHFM-like phenotype, the human orthologs are believed to be the disease genes. SHFM-IV and Ectrodactlyly-Ectodermal dysplasia-Cleft lip (EEC) are caused by mutations in *p63*, an ectoderm-specific p53-related transcription factor. These phenocopies may underlie the existence of a regulatory cascade involving the disease genes. We show that p63 and Dlx proteins colocalize in the nuclei of the apical ectodermal ridge (AER). In homozygous *p63*^{null} and *p63*^{EEC} mutant limbs the AER fails to stratify and expression of four *Dlx* genes is strongly reduced; however in *p63*$^{+/EEC}$ limbs only *Dlx5-Dlx6* expression is reduced in spite of a normally stratified AER and normal development. In vitro ΔNp63α activates transcription from the *Dlx5* and *Dlx6* promoters, an activity abolished by EEC and SHFM Type I mutations. ChIP analysis shows that p63 is associated to the *Dlx5* promoter. Thus, *p63* and *Dlx5;Dlx6* take part in a pathway relevant in the aetio-pathogenesis of SHFM.