OFERTA PROYECTO PARA AYUDAS PARA CONTRATOS PREDOCTORALES PARA LA FORMACIÓN DE DOCTORES 2018 (Antiguas FPI)

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<td>INVESTIGADOR PRINCIPAL (IP)</td>
<td>María A. Ros</td>
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<td>TÍTULO PROYECTO</td>
<td>Descifrando las redes génicas regulatoras de SPS y HOXC en el ectodermo de la extremidad</td>
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RESUMEN PROYECTO/PROJECT SUMMARY (Máximo 3.000 palabras)

Hox genes are a family of important developmental regulators critical for the establishment of the basic body plan of bilaterian animals (Lewis 1978). In vertebrates, Hox genes are organized in four clusters (HoxA, -B, -C, and -D) two of which, HoxA and HoxD clusters, play essential roles in the outgrowth and patterning of the tetrapod limb morphology providing cellular identities (Woltering et al., 2014; Kmita et al. 2005). During limb development members of the HoxA and HoxD clusters are activated sequentially in time and space, following their genomic topography in the chromosome, a feature referred to as temporal and spatial collinearity (Lewis, 1978). Both Hoxd and Hoxa genes show complex and dynamic patterns of expression that correlate with the morphology of the different limb segments (Davis and Capecchi, 1996; Zakany and Duboule, 2007). In contrast, no specific function for the Hoxc or HoxB clusters has been identified during limb development and to date no obvious limb morphological abnormality has been reported in the complete absence of either of these clusters (Suemori and Noguchi, 2000).

Regarding Hoxc genes, it was reported that some members of the cluster (Hoxc9-Hoxc12) were specifically expressed in the hindlimb but not in the forelimb (Nelson et al., 1996) leading to the suggestion that they could contribute to differences between fore- and hindlimbs. It was also shown that the knock out (KO) of some individual Hoxc genes (Hoxc4, Hoxc8 and Hoxc9) had rib malformations and mild vertebral transformations but no specific limb phenotype (Suemori et al., 1995; Boulet and Capecchi, 1996). Of particular interest was the phenotype of the Hoxc13 null mice characterized by absence of all pelage and long and twisted nails (Godwin and Cappechi, 1998, 1999). The claw defect was linked to defective keratin gene expression, as Hoxc13 is known to be an important regulator of various keratin genes (Awgulewitsch, 2003). However, unexpectedly, the removal of the whole HoxC cluster had no phenotypic consequences and although null mice died at birth because of respiratory problems, the morphology of the skeleton and internal organs was almost normal (Suemori and Noguchi, 2000). Therefore, the phenotype of the individual mutations of Hoxc genes was interpreted as resulting from the specific gene disruption strategies (i.e. insertion of a positive selection marker gene cassette) and the general assumption was that Hoxc genes were dispensable for limb development.

Recently, we generated the temporal expression profile of the limb ectoderm uncovering a collinear activation of Hoxc genes that we have validated by in situ hybridization. Intrigued with this expression, we have reexamined the HoxC cluster null mice and found that they present anonychia (absence of nail/claw). Investigating the function of Hoxc genes in the limb ectoderm is the core of this project.
The nail/claw/hoof

The nail, claw and hoof are homologue structures and integral components of the digital tip in most terrestrial tetrapods. They are keratinized appendages of ectodermal origin adapted for a variety of purposes according to the lifestyle of the species (Hamrick, 2001 and 2003). The skin or integument, which constitutes the interface between the organism and its surrounding environment, has evolved several organs such as hairs, feathers, glands, teeth, and claws that serve diverse functions and help animals adapt to their environment. Interestingly, changes in integument organs such as the evolution of the mammary glands help define a whole vertebrate class, the Mammals.

According to shape and size, the appendages at the tip of the digits are classified as nails, claws or hoofs. Among them, the claw is considered the most primitive, the nail and the hoof arising as modifications produced by differential growth probably driven by selection pressures for functional requirements. The evolution of the hooves associates with the cursorial adaption typical of ungulates and was probably necessary for their major innovation in running lifestyle in association with long legs, fused bones and reduced side digits (Hamrick 2003, Cooper et al. 2014; Lopez-Rios et al., 2014). In contrast, the evolution of nails was closely associated with the use of hands and feet as manipulatory organs.

The nail (also referred to as the nail unit or nail apparatus) consists of the nail matrix, the nail bed, and the hyponychium, all covered by the nail plate (de Berker and Baran, 2012; Fleckman et al., 2013; Fig. 2). The proximal nail fold limits the nail proximally. The nail plate continuously grows throughout life, normally reaching equilibrium between growth and wear-and-tear. The **nail stem cells** reside in the proximal nail matrix, the origin of the nail plate (Leung et al., 2014; black dots in Fig. 2A).

![Figure A) Hematoxylin-eosine stained longitudinal section through the digit tip of a new born mouse and B) schematic representation of the nail structure. The black arrows show the origin and direction of growth of the nail plate. Red: nail matrix; Orange: nail bed; Yellow: nail plate; Pink: hyponychium](image)

As epidermal appendages, nails, claws and hooves form through epithelial-mesenchymal interactions similar to those observed in patterning other vertebrate integuments such as feathers, hair, teeth, and scales (e.g., Dhouailly 2009; Naveau et al., 2014, Dhouailly et al., 2017). Three phases can be distinguished in the formation of all ectodermal organs including the nail/claw (Hamrick 2001). The first phase, induction, is characterized by the formation of the placode or primary nail field, anectodermal origin adapted for a variety of purposes...
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plate. During the third phase, differentiation/growth of the nail/claw plate occurs in association with the distal phalanx. Differential regulation of the proliferation of the germinal matrix affects the length of the appendage (nail, claw, or hoof).

It is now well established that the same toolkit of signaling molecules is used during the development of all integumentary structures (reviewed in Saito et al., 2015) and that relatively minor changes in epithelial mesenchymal interactions can explain the diversity in epithelial appendages (Lu et al., 2016). The induction of the nail placode depends on signaling from the underlying mesenchyme, most likely Wnt signaling. Bmp signaling is also involved in this process but the details of the signaling circuitry remains largely unknown. Once the placode forms, signaling events downstream of Wnts/BMPs drive further maturation in most epidermal organs. It should be noted here that Sp6 and Sp8, necessary mediators of both Wnt and Bmp signaling at earlier stages, are also expressed (unpublished) at the stages of nail formation and their possible implications will also be investigated here.

Evidence gathered from mice mutant and human pathology has also yielded important insights into the molecular regulation of nail/claw morphogenesis and homeostasis. Deficiencies in Msx2, Msx1, R-spondin4 (Rspo4), Fzd6, Wnt10a, Hoxc13 and Lmx1b have been shown to bear a nail phenotype (reviewed in Bergqvist et al., 2017). These observations further confirm the implication of the Wnt and Bmp pathways but with some unexpected effects. For example, while early removal of Wnt or Bmp signaling leads to double dorsal phenotypes (double or conical nails; Wang et al., 2004; Shosnikova et al., 2003), late removals leads to absence of nails possible interfering with the local specification of the nail field or with proliferation (Plikus et al., 2014; Takeo et al., 2013).

In summary, the nail field is in need of elucidating the basis of the molecular interplay leading to its induction and adult homeostasis including maintenance and renewal of germinal cells. We consider this as a necessary previous step for our objective of understanding Hoxc function in the development of this ectodermal appendage.

Regeneration of mammalian digit tips

It is remarkable that several rodents and primates, including human children, are capable of re-growing the tips of amputated digits (Borgens 1982; Han et al., 2008). Most interestingly, the regenerative response requires the presence of the nailorgan; amputations proximal to the insertion of the nail does not regenerate (Leung et al., 2014). The bastema that forms includes heterogenous progenitors that are activated under the nail dependent Wnt signaling environment (Lehoczky et al. 2011; Rinkevich et al. 2011; Lehoczky and Tabin, 2015). These observations show that mammals have the capacity for epimorphic regeneration and have important implications for novel therapeutic approaches to treat amputees. They also place the nail as an important organ to understand regeneration. Gathering information on the molecular mechanisms controlling nail formation may increase the understanding of this regeneration potential.
Adaptive variation in the mammalian limb: the ungulates

The fossil record of early mammals during the evolutionary diversification in the Cenozoic era is characterized by an adaptive radiation of distal limb structures. The form and size of the integumentary organ (nail, claw or hoof) correlates with that of the distal phalanx, both adapted to the functional use of the limb (Hamrick 2003). The hoof is just a modified nail/claw, the most diverged of the digital organs, and serves to define, in a descriptive manner, the ungulates (literally meaning: hooved animals). The development of hooves was a major innovation in the evolution of a cursorial lifestyle. Hooves perform crucial functions, among them to protect the terminal limb structures and to support the whole body weight while moving. The hoof permits the unguligrade stance characterized by only the extreme distal most part of the limb (the distal phalanx) contacting the ground typical of Artiodactyla (even-toed) and Perissodactyla (odd-toed) ungulates (Prothero, 2009). Given our observation of Hoxc function in nail development together with the wide evidence supporting the role of Hox genes in the evolution of novel body morphologies, it seems reasonable to explore the implication of Hoxc genes in the nail/claw/hoof transition (Duboule 2005).

References

Developmental Biology, group of Dr. Marian Ros  
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(http://www.biodes.unican.es)

Regulation of gene expression during vertebrate limb development

For developing a PhD Thesis investigating the function of HoxC genes in the development of ectodermal derivatives. The research is highly multidisciplinary and integrative and uses transcriptomics and epigenomics analysis, Chip-seq, CRISPR and classical developmental genetics approaches.

We work towards understanding the mechanisms responsible for morphogenesis at cellular, molecular and genetic levels in both health and disease. We use several model organisms, including chick and mice, and concentrate in the study of limb development.

The IBBTEC (http://web.unican.es/ibbtec/Paginas/default.aspx) is a new research institute of the CSIC, University of Cantabria and SODERCAN located in the Scientific and Technologic Park of Santander. It provides an enthusiastic and supportive environment with state-of-the-art research facilities.

If interested, please contact, Marian Ros (rosm@unican.es)

Selected publications: