Developmental Biology 410 (2016) 98–107

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

DB Letters

Cell autonomous roles of Nedd4 in craniofacial bone formation

Sophie Wiszniak, Natasha Harvey, Quenten Schwarz *

Centre for Cancer Biology and University of South Australia, Frome Road, Adelaide, SA 5000, Australia

ARTICLE INFO

Article history:
Received 17 July 2015
Received in revised form
2 November 2015
Accepted 1 December 2015
Available online 8 December 2015

Keywords:
Craniofacial development
Neural crest cell
Osteoblast
Ossification
Nedd4

ABSTRACT

Nedd4 is an E3 ubiquitin ligase that has an essential role in craniofacial development. However, how and when Nedd4 controls skull formation is ill defined. Here we have used a collection of complementary genetic mouse models to dissect the cell-autonomous roles of Nedd4 in the formation of neural crest cell derived cranial bone. Removal of Nedd4 specifically from neural crest cells leads to profound craniofacial defects with marked reduction of cranial bone that was preceded by hypoplasia of bone forming osteoblasts. Removal of Nedd4 after differentiation of neural crest cells into progenitors of chondrocytes and osteoblasts also led to profound deficiency of craniofacial bone in the absence of cartilage defects. Notably, these skull malformations were conserved when Nedd4 was specifically removed from the osteoblast lineage after specification of osteoblast precursors from mesenchymal skeletal progenitors. We further show that absence of Nedd4 in pre-osteoblasts results in decreased cell proliferation and altered osteogenic differentiation. Taken together our data demonstrate a novel cell-autonomous role for Nedd4 in promoting expansion of the osteoblast progenitor pool to control craniofacial development. Nedd4 mutant mice therefore represent a unique mouse model of craniofacial anomalies that provide an ideal resource to explore the cell-intrinsic mechanisms of neural crest cells in craniofacial morphogenesis.

1. Introduction

Craniofacial malformations represent a significant clinical issue, with many cases requiring highly invasive and recurring surgical interventions throughout life. Unfortunately, the vast majority of these disorders have unknown genetic or pathological origin. These highly prevalent disorders manifest as a result of the complex array of cellular interactions and morphogenetic events required for correct development of the craniofacial skeleton. In contrast to the mesodermal origin of the axial and appendicular skeleton, the mammalian skull requires the unique contribution of cells from the cranial neural crest (Trainor, 2013). In addition, the calvaria and the bones of the face form primarily through the process of intramembranous ossification in which mesenchymal progenitors differentiate directly into bone-forming osteoblasts without a cartilage precursor (Percival and Richtsmeier, 2013). Identifying the molecular mechanisms by which neural crest cells differentiate into osteoblasts and bone is critical to our understanding of craniofacial development and the origins and potential treatments for craniofacial birth defects.

Cranial neural crest cells are multipotent cells that arise from the dorsal neural folds during early embryonic development (Trainor, 2013). Following delamination from the neural tube, cranial neural crest cells migrate vast distances into the facial primordia where they receive instructive signals to initiate a transcriptional programme that ultimately leads to their differentiation into bone and cartilage (Jeong et al., 2004). In the first of a series of differentiation steps, neural crest cells entering the facial primordia form mesenchymal progenitors of bone and cartilage. Cell-type specificity is later initiated through the expression of the HMG box transcription factor *Sox9* in cartilage precursors (Bi et al., 2001) or the sequential activity of the runt family member *Runx2* (Komori et al., 1997) and the zinc finger transcription factor *Osterix* (*Osx*) (Nakashima et al., 2002) in osteoblast precursors. The outcome of these transcriptional programmes is the progression of precursor cell differentiation into bonafide chondroctyes or osteoblasts that in turn secrete extracellular matrix components and mineral specific to cartilage or bone.

While our knowledge of neural crest cell and cranial bone formation is biased toward the roles of transcription factor networks acting downstream of morphogenic gradients, intrinsic roles for post-translational modifications in neural crest cells are also coming to light (Wiszniak et al., 2013; Vermillion et al., 2014). Ubiquitination is a post-translational modification that instructs functional changes in target proteins by controlling degradation, localisation or biochemical properties (Rotin and Kumar, 2009). We recently identified an essential role for ubiquitination in neural crest cell development by demonstrating that the E3 ubiquitin

* Corresponding author.
E-mail address: quenten.schwarz@health.sa.gov.au (Q. Schwarz).

http://dx.doi.org/10.1016/j.ydbio.2015.12.001

ligase Nedd4 plays an important role in cranial neural crest cells (Wiszniak et al., 2013). Nedd4 is expressed in neural crest cells as they delaminate from the neural tube and *Nedd4*$^{-/-}$ mice have profound deficiency of cranial ganglia and neural crest cell-derived cranial bones, as well as milder reductions in bone in the axial skeleton. As Nedd4 is essential for maintaining neural crest cell stem-cell identity and survival, our results suggested that the craniofacial defects may arise from a lack of neural crest cells at earlier developmental time points. However, whether Nedd4 is cell-autonomously required by neural crest cells or whether early neural crest cell deficiency underpins craniofacial defects remained unresolved. Here we have removed Nedd4 at various stages of cranial neural crest cell and bone development using Cre/LoxP technologies to answer these questions.

Consistent with our analyses in *Nedd4*$^{-/-}$ embryos we found that mice lacking Nedd4 specifically in neural crest cells had pronounced bone deficiency in the absence of notable cartilage defects. Our analyses at earlier embryonic stages further suggest

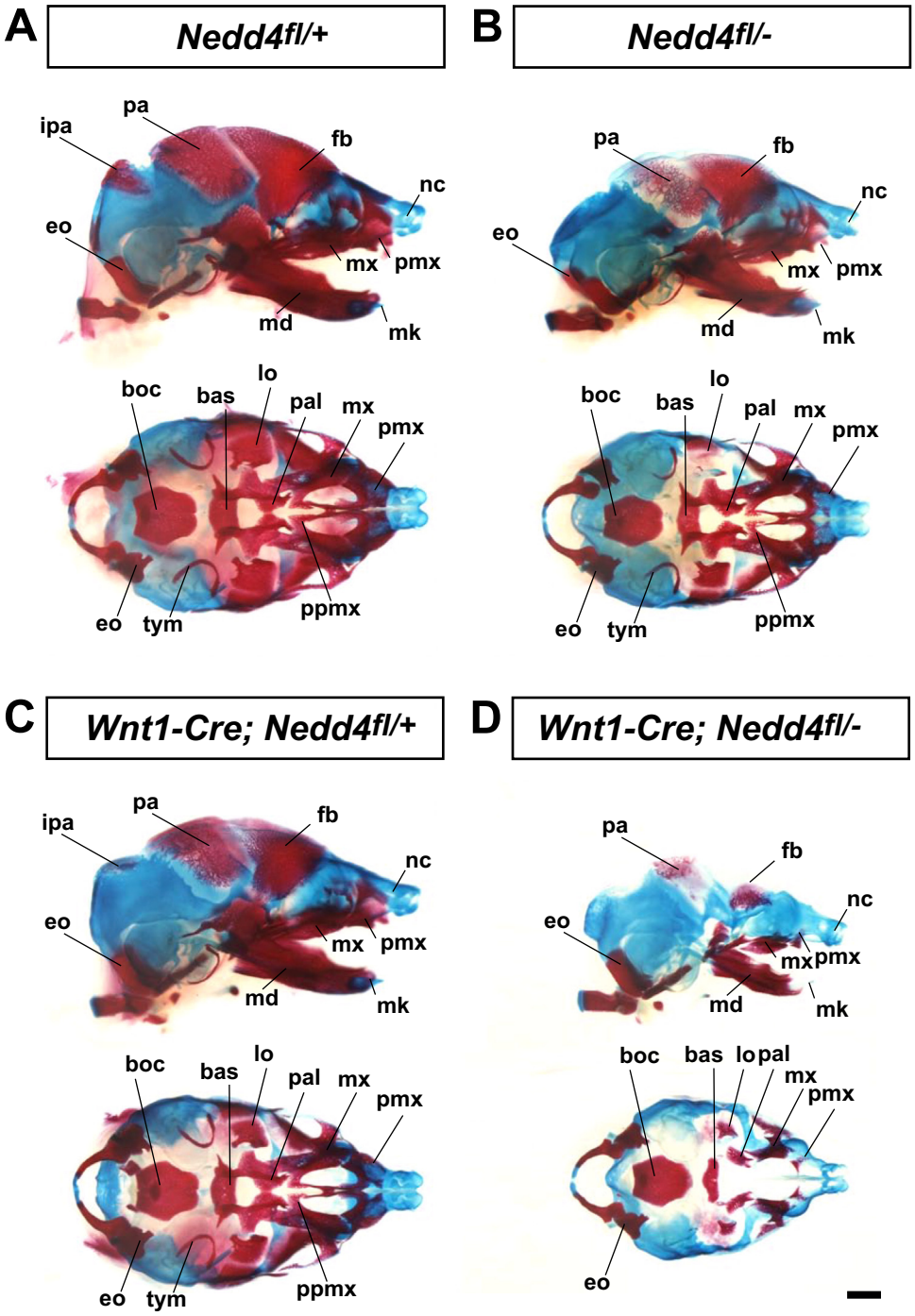

Fig. 1. Loss of *Nedd4* in neural crest cells and derivatives causes craniofacial defects. E17.5 littermate skulls stained with Alizarin red (bone) and Alcian blue (cartilage). A: *Nedd4*$^{fl/+}$ wildtype embryo. B: Loss of a single allele of *Nedd4* in *Nedd4*$^{fl/-}$ embryos causes a mild reduction in bone. C: Loss of a single allele of *Nedd4* specifically in neural crest-derived tissue in *Wnt1-Cre; Nedd4*$^{fl/+}$ embryos similarly causes a mild reduction in neural crest derived bone. D: Complete lack of *Nedd4* in neural crest-derived tissue in *Wnt1-Cre; Nedd4*$^{fl/-}$ embryos causes severe hypoplasia of craniofacial bone. bas, basisphenoid; boc, basioccipital; eo, exoccipital; fb, frontal bone; ipa, interparietal; lo, lamina obturans; md, mandible; mk, Meckel's cartilage; mx, maxillary; nc, nasal capsule; pa, parietal; pal, palatal; pmx, premaxillary; ppmx, palatal process maxillary; tym, tympanic. Scale bar = 1 mm.