Gene 554 (2015) 87–95

Contents lists available at ScienceDirect

Gene

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

Shh regulates chick *Ebf1* gene expression in somite development

Mohammed Abu El-Magd [a,b,*], Steve Allen [b], Imelda McGonnell [b], Ali A. Mansour [a], Anthony Otto [c], Ketan Patel [c]

[a] Department of Anatomy Embryology, Faculty of Veterinary Medicine, Kafrelsheikh University, El-Geish Street, Kafrelsheikh Post Box 33516, Egypt
[b] Department of Veterinary Basic Sciences, The Royal Veterinary College, Royal College Street, Camden, London NW1 0TU, UK
[c] School of Biological Sciences, University of Reading, Whiteknights, PO Box 228, Reading, Berkshire RG6 6AJ, UK

ARTICLE INFO

Article history:
Received 5 April 2014
Received in revised form 9 October 2014
Accepted 13 October 2014
Available online 23 October 2014

Keywords:
Chick embryo
Ebf1
Microsurgery
Gain and loss function
Shh
Somite

ABSTRACT

The chick early B-cell factor 1 (*cEbf1*) is a member of EBF family of helix loop helix transcription factors. Recently, we have proved that *cEbf1* expression in feather is regulated by Shh. It is therefore possible that the somitic expression of *cEbf1* is controlled by *Shh* signals from the notochord. To assess this hypothesis, the expression profile of *cEbf1* was first detailed in somites of chick embryos (from HH8 to HH28). *cEbf1* expression was mainly localised in the medial sclerotome and later around the vertebral cartilage anlagen of body and pedicles. Tissue manipulations (notochord ablation) and Shh gain and loss of function experiments were then performed to analyse whether the notochord and/or Shh regulate *cEbf1* expression. Results from these experiments confirmed our hypothesis that the medial somitic expression of *cEbf1* is regulated by Shh from the notochord. In conclusion, *cEbf1* gene is considered as a medial sclerotome marker, downstream to and regulated by the notochord derived Shh, which may be functionally involved in somitogenesis.

1. Introduction

The chick *cEbf1* is a member of a novel highly conserved EBF family of atypical HLH transcription factors (Dubois and Vincent, 2001; Liberg et al., 2002). *Ebf1* was originally discovered in rodents as regulators of B-lymphocyte and olfactory neuron differentiation (Hagman et al., 1993; Wang and Reed, 1993). Since then, the expression pattern and role of *Ebf1* have been studied extensively in the immune (Fields et al., 2008; Lukin et al., 2008) and nervous tissues (Davis and Reed, 1996; Garel et al., 1997). These studies have demonstrated the importance of this molecule for specification, differentiation and cell movements during development of these tissues.

Somites are segmented, paired blocks of mesodermal cells originated from the cranial end of the presomitic mesoderm in an anterior to posterior direction. According to Takahashi (2005), this cranial end is called somite 0 (S0) and its separation border (between S0 and the remaining crPSM) is called the segmenter. Somite segmentation has a defined time course with each cycle producing a somite every 90 min in chick embryo (Goldbeter and Pourquie, 2008). The furthest posterior somite is the most newly formed somite, somite I (SI), the next cranial somite is SII, and so on (nomenclature according to, Pourquie and Tam, 2001). The immature somites (from SI to SIII) are spherical and composed of an outer columnar epithelial layer and a mesenchymal core in the centre, the somitocoele. In response to ventral signals (*Shh* and *Noggin*) from the notochord and floor plate of the neural tube, the ventro-medial portion of somite IV which is epithelial undergoes epithelial–mesenchymal transition (EMT) to form the sclerotome giving rise to the first mature somite. Once formed, the sclerotome is subdivided along the anterior posterior axis into anterior and posterior halves by von Ebner's (intra-somitic) fissure (Christ and Wilting, 1992). The anterior half is invaded by neural crest cells and their derivatives, dorsal root ganglia and nerve axons (Bronner-Fraser and Stern, 1991). The sclerotome is the primordium for the entire vertebral column and also gives rise to the proximal ribs (Dietrich et al., 1997). Functionally, the sclerotome is subdivided into: medial (precursor of vertebral bodies and intervertebral discs), central (precursor of pedicle part of the neural arches, proximal ribs and transverse processes), dorsal (precursor of dorsal portion of the neural arches and the spinous processes) and lateral (precursor of distal ribs) domains (Christ et al., 2004, 2007; Monsoro-Burq and Le Douarin, 2000).

Recently, *Ebf2* was shown to be important during the late stage of skeletogenesis in mice (Kieslinger et al., 2005, 2010), however, to date there is scarce available data on the expression of *Ebf* genes during the early stages of skeletogenesis, particularly during somite formation and differentiation. Some members of vertebrate *Ebfs* were expressed in different domains of somites. For example, chick *cEbf2,3* (El-Magd et al., 2013) and mouse *mEbf1-3* genes (Garel et al., 1997; Kieslinger et al., 2005) were expressed in the sclerotome. In *Xenopus*, *xEbf2* was

Abbreviations: Ci, cubitus interruptus; *Col*, collier; crPSM, cranial presomitic mesoderm; DRG, dorsal root ganglia; Ebf1, early b-cell factor 1; EMT, epithelial–mesenchymal transition; HBC, hydroxypropyl-β-cyclodextrin; HLH, helix loop helix; NT, neural tube; PSM, presomitic mesoderm; SI-X, somite 1–10; Shh, sonic hedgehog.

* Corresponding author at: Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt.
E-mail address: mohrizk73@yahoo.com (M.A. El-Magd).

http://dx.doi.org/10.1016/j.gene.2014.10.028
0378-1119/