Developmental Biology 419 (2016) 348–356

Contents lists available at ScienceDirect

Developmental Biology

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

FGF and EDA pathways control initiation and branching of distinct subsets of developing nasal glands

Alison J. May [a], Denis Headon [b], David P. Rice [c,d], Alistair Noble [e], Abigail S. Tucker [a,*]

[a] Department of Craniofacial Development and Stem Cell Biology, Guy's Hospital, King's College London, United Kingdom
[b] The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, United Kingdom
[c] Orthodontics, Department of Oral and Maxillofacial Diseases, University of Helsinki, Helsinki 00014, Finland
[d] Orthodontics, Department of Oral and Maxillofacial Diseases, Helsinki University Hospital, Helsinki 00290, Finland
[e] MRC & Asthma UK Centre in Allergic Mechanisms of Asthma, King's College London, United Kingdom

ARTICLE INFO

Article history:
Received 6 July 2016
Received in revised form
27 August 2016
Accepted 29 August 2016
Available online 30 August 2016

Keywords:
Submucosal gland (SMGs)
FGF10
EDA
Branching morphogenesis

ABSTRACT

Hypertrophy, hyperplasia and altered mucus secretion from the respiratory submucosal glands (SMG) are characteristics of airway diseases such as cystic fibrosis, asthma and chronic bronchitis. More commonly, hyper-secretion of the nasal SMGs contributes to allergic rhinitis and upper airway infection. Considering the role of these glands in disease states, there is a significant dearth in understanding the molecular signals that regulate SMG development and patterning. Due to the imperative role of FGF signalling during the development of other branched structures, we investigated the role of *Fgf10* during initiation and branching morphogenesis of murine nasal SMGs. *Fgf10* is expressed in the mesenchyme around developing SMGs while expression of its receptor *Fgfr2* is seen within glandular epithelial cells. In the *Fgf10* null embryo, Steno's gland and the maxillary sinus gland were completely absent while other neighbouring nasal glands showed normal duct elongation but defective branching. Interestingly, the medial nasal glands were present in *Fgf10* homozygotes but missing in *Fgfr2b* mutants, with expression of *Fgf7* specifically expressed around these developing glands, indicating that *Fgf7* might compensate for loss of *Fgf10* in this group of glands. Intriguingly the lateral nasal glands were only mildly affected by loss of FGF signalling, while these glands were missing in *Eda* mutant mice, where the Steno's and maxillary sinus gland developed as normal. This analysis reveals that regulation of nasal gland development is complex with different subsets of glands being regulated by different signalling pathways. This analysis helps shed light on the nasal gland defects observed in patients with hypohidrotic ectodermal dysplasia (HED) (defect EDA pathway) and LADD syndrome (defect FGFR2b pathway).

1. Introduction

Impaired mucus clearance and pulmonary obstruction are common symptoms of a number of life-threatening respiratory diseases. Mucus hyper-secretion by the submucosal glands (SMG) is an important etiological factor in asthma, chronic bronchitis and cystic fibrosis with SMG hyperplasia and mucous metaplasia common to all (Reid, 1960; Oppenheimer and Esterly 1975; Aikawa et al., 1992). Hyper-secretion and abnormal mucociliary clearance leads to a build-up of mucus with a thick viscosity which can obstruct airways and increase bacterial lung infection, leading to premature death in severe cases (Oppenheimer and Esterly, 1975; Hoegger et al., 2014; Robinson and Bye, 2002). More commonly, altered mucus secretion of the nasal glands, particularly the sinus glands, gives rise to chronic rhinosinusitis and infection of the upper airway tract (Peña et al., 2007; Wu et al., 2011). Considering this significant involvement of SMGs in pulmonary diseases, research is lacking in the mechanisms modulating gland development and homeostasis. To understand the progression of airway disease, it is critical to elucidate the signalling factors and pathways required during SMG morphogenesis, and investigate if these mechanisms are defective in disease states.

The SMGs are found in the submucosal connective tissue beneath the respiratory epithelium (RE) of the conductive airways (Fig. 1). The anterior nasal SMGs provide the first line of defence within the airway. The medial and lateral glands are found within the medial and lateral nasal walls respectively, while the sinus glands drain their secretions directly into the sinus cavity (Grüneberg, 1971; May and Tucker, 2015) (Fig. 1). In humans, SMGs are further found within the submucosa between the cartilaginous rings of the distal airways, stretching throughout the trachea and bronchi (Borthwick et al., 1999; Sturgess and Imrie, 1982). In mice,

* Corresponding author.
E-mail address: abigail.tucker@kcl.ac.uk (A.S. Tucker).

http://dx.doi.org/10.1016/j.ydbio.2016.08.030

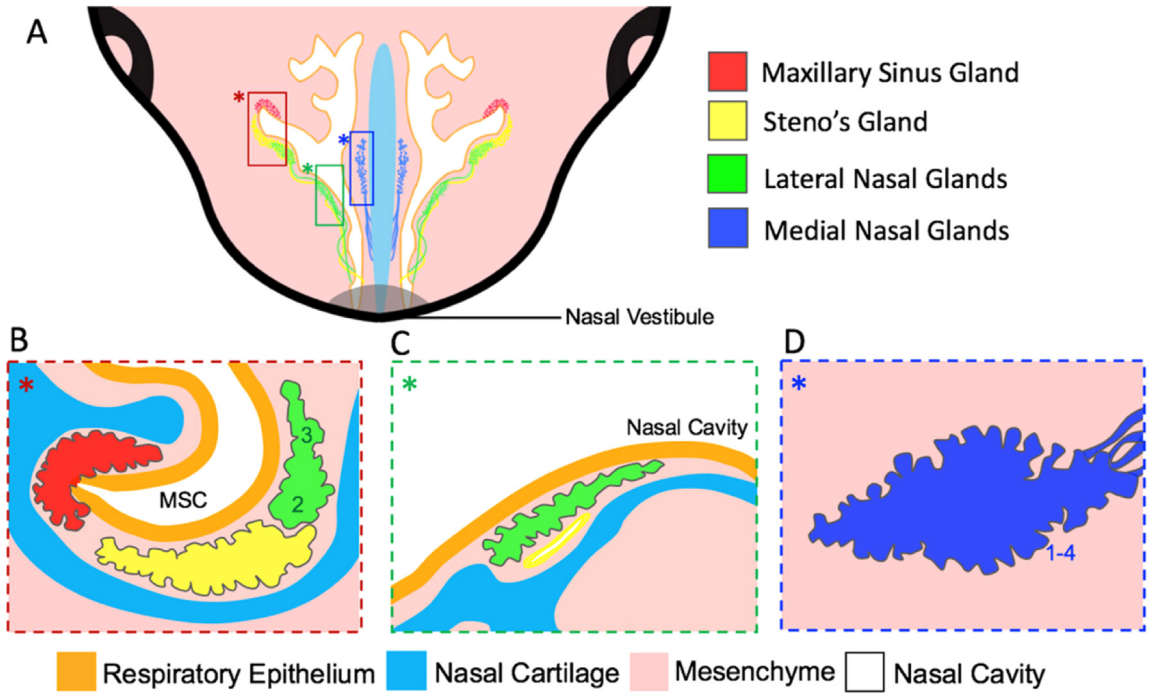

Fig. 1. Schematic summary of the morphology and location of the embryonic murine nasal SMGS. (A) Representation of an anterior view of a transverse section of the nasal SMGs surrounding the nasal cavity (white). The SMG ducts of the Steno's gland, Lateral Nasal Glands (LNGs) and Medial Nasal Glands (MNGs) open into the cavity at different locations close to the nasal vestibule, while the maxillary sinus gland (MSG) opens into the maxillary sinus cavity (MSC). Coloured box selections of the distal glands (A) are represented as sagittal sections (B–D). (B) Location of the MSG (red), Steno's gland (yellow) and LNG2 and 3 (green) below the MSC. (C) The LNG5 and an area of the Steno's duct below the respiratory epithelium. (D) MNGs 1–4 in the mesenchyme of the nasal septum. LNGs 6–13 develop adjacent to LNG2, 3 and 5, and undergo gland branching at different locations beneath the nasal cavity along the A/P body axis (not shown).

SMGs extend to the anterior trachea, where they are found at high density adjacent to the cricoid cartilage (CC) and develop no further than the sixth cartilaginous tracheal ring (Borthwick et al., 1999; Rawlins and Hogan, 2005).

The SMGs develop through a process of branching morphogenesis. This process, common to other mammalian epithelial organs such as the mammary gland, salivary gland and lung, involves the formation of a single tube from an epithelial sheath that undergoes continual elongation and clefting to create a complex network of branched tubes and terminal buds. Cellular differentiation occurs within these structures to form ductal units within the branches, while end buds differentiate into functional units that transport liquid or gas. In SMGs, these distal functional units are composed of serous and mucous cells that produce airway mucus rich in mucins and bactericidal enzymes (Meyrick et al., 1969; Meyrick and Reid, 1970).

Members of the fibroblast growth factor (FGFs) family of polypeptide proteins have been shown to be involved in branching morphogenesis of other organs such as the lung, salivary and lacrimal glands. The FGF family consists of 22 ligands (FGF1–FGF22) and four cell membrane-bound tyrosine kinase FGF receptors (FGFR1–FGFR4) (Ornitz and Itoh, 2001). The essential requirement for FGF10 and its receptor FGFR2b during lung morphogenesis is emphasised by the shared defects of both *Fgf10* homozygous (−/−) and *Fgfr2b*−/− mice, who die at birth due to agenesis of the lungs (Sekine et al., 1999; Min et al., 1998; De Moerlooze et al., 2000). FGF10 and FGFR2b are also critical for salivary gland duct elongation and branching (Jaskoll et al., 2005; Steinberg et al., 2005). The submandibular salivary gland fails to develop past the initial bud stage at embryonic day E12.5 in both *Fgf10*−/− and *Fgfr2b*−/− mice, and salivary glands are hypoplastic and secrete a reduced volume of saliva in *Fgf10* heterozygous (+/−) adults (May et al., 2015). Mutations in *FGF10* or its receptor *FGFR2b*, lead to Lacrimo Auriculo Dento Digital (LADD) syndrome (OMIM 149730) in humans. This anomaly is characterized by hypoplasia, atresia or aplasia of the salivary glands and the lacrimal glands of the eyes, and obstruction of the nasolacrimal duct (Shiang and Holmes, 1977; Inan et al., 2006). A milder form of this disease, known as Aplasia of Lacrimal and Salivary Glands (ALSG) (OMIM 180920), gives rise to the same symptoms as LADD, most often including xerothalmia (dryness of the eye) and xerostomia (dry mouth) (Wiedemann, 1997; Milunsky et al., 1990).

The Ectodysplasin A (EDA) pathway is also required for morphogenesis of many glandular structures (Mikkola, 2009). A naturally occurring mutation in the *EDA* gene arises in the *Tabby* mouse, leading to defective hair, tooth and salivary gland development (Srivastava et al., 1997). Investigation of the nasal SMGs in the *Tabby* mouse revealed absence of some of the nasal glands, while others, such as Steno's gland, the largest of the nasal glands, also known as the lateral nasal gland 1 (LNG1) (Fig. 1), developed normally in the *Tabby* mouse (Grüneberg 1971). mRNA expression of *Edar*, the receptor for EDA, was found in the tracheal respiratory epithelium during postnatal SMG development (Rawlins and Hogan, 2005). The absence of tracheal SMGs were observed in both the adult *Tabby* mouse and postnatally in the *Edaradd* knockout mouse, which lacks an EDAR signalling adapter molecule, highlighting the requirement of the EDA signalling pathway in successful tracheal SMG morphogenesis (Rawlins and Hogan, 2005). Human patients with hypohidrotic ectodermal dysplasia (HED) have developmental defects in teeth, hair and salivary glands caused by mutations in EDA, EDAR or EDARADD (Mikkola, 2009). Respiratory difficulties and nasal gland defects have also been reported in HED patients with nasal dryness of the nasal mucosa, nasal crusting and abnormal nasal discharge all being symptoms of the disease (Al-Jassim and Swift, 1996; Dietz et al., 2013).

In this study, we have investigated the requirement for FGF10/FGFR2b and EDA signalling during the crucial stages of SMG development among the different nasal gland populations. We