Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Role of the planar cell polarity pathway in regulating ectopic hair cell-like cells induced by Math1 and testosterone treatment

Xiao-yu Yang[a,b,c,1], Kai Jin[a,b,c,1], Rui Ma[a,b,c,1], Juan-mei Yang[a,b,c], Wen-wei Luo[a,b,c], Zhao Han[a,b,c], Ning Cong[a,b,c], Dong-dong Ren[a,b,c,*], Fang-lu Chi[a,b,c,*]

[a]Department of Otology and Skull Base Surgery, Eye & Ent Hospital of Fudan University, 83 Fenyang Road, Shanghai 200031, China
[b]Shanghai Auditory Medical Center, Shanghai, China
[c]Key laboratory of Hearing Science, Ministry of Health, Shanghai, China

ARTICLE INFO

Article history:
Accepted 10 April 2015
Available online 7 May 2015

Keywords:
Planar cell polarity
Cochlea
Hair cell
Testosterone
Mouse

ABSTRACT

Planar cell polarity (PCP) signaling regulates cochlear extension and coordinates orientation of sensory hair cells in the inner ear. Retroviral-mediated introduction of the Math1 transcription factor leads to the transdifferentiation of some mature supporting cells into hair cells. Testosterone, a gonadal sex steroid hormone, is associated with neuroprotection and regeneration in Central Nervous System (CNS) development. Experiments were performed *in vitro* using Ad5-EGFP-Math1/Ad5-Math1 in neonatal mouse cochleas. Establishment of ectopic hair-cell like cell(HCLC) polarity in the lesser epithelial ridge (LER) with or without testosterone-3-(O-carboxymethyl) oxime bovine serum albumin (testosterone-BSA) treatment was investigated to determine the role of the PCP pathway in regulating ectopic regenerated (HCLCs) through induction by Math1 and testosterone treatment. After Math1 infection, new ectopic regenerated HCLCs were detected in the LER. After the HCLCs developed actin-rich stereocilia, the basal bodies moved from the center to the distal side. Moreover, the narrower, non-sensory LER region meant that the convergent extension (CE) was also established after transfection with Math1. After 9 days of *in vitro* testosterone-BSA treatment, more Edu(+), Sox2(+), and HCLC cells were observed in the LER with an accompanying downregulation of E-cadherin. Interestingly, the CE of the Ad5-EGFP-math1 treated LER is altered, but the intrinsic cellular polarity of the HCLCs is not obviously changed. In summary, our results indicate that PCP signaling is involved in the development of ectopic HCLCs and the CE of the ectopic sensory region is altered by testosterone-BSA through downregulation of cell–cell adhesion.

*Corresponding authors at: Department of Otology and Skull Base Surgery, Eye & Ent Hospital of Fudan University, 83 Fenyang Road, Shanghai 200031, China.
E-mail addresses: dongdongren@fudan.edu.cn (D.-d. Ren), chifanglu@126.com (F.-l. Chi).
[1]These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.brainres.2015.04.017

Testosterone-BSA and Math1 treatment could promote an increase in HCLCs in the LER through proliferation and transdifferentiation.

1. Introduction

Sensorineural hearing loss is a frequent hearing disorder. The inability of stereocilia to self-repair leads to hair cell death and permanent hearing loss (Pan et al., 2012; Yang et al., 2012). Mammalian supporting cells are mitotically quiescent and not able to replace lost hair cells (Sinkkonen et al., 2011).

Math1, a proneural basic helix-loop-helix (bHLH) transcription factor, is required for hair cell fate commitment and/or initial differentiation of immature hair cells (Liu et al., 2012; Pan et al., 2012). Math1-null mice fail to generate cochlear and vestibular hair cells, due to the absence of stereocilia and other hair cell-specific characters (Yang et al., 2012). Cochlear supporting cells and cells of the lesser epithelial ridge (LER) demonstrate great potential to dedifferentiate into prosensory cells that can proliferate and differentiate (Zheng and Gao, 2000). Without Math1 overexpression, LER cells can divide, but cannot undergo differentiation into hair cells (Yang et al., 2013a). We and others have reported that Math1 overexpression *in vitro* and/or *in vivo* on the cochlea results in the production of ectopic hair cells derived from non-sensory supporting cells (Gubbels et al., 2008; Izumikawa et al., 2005; Kawamoto et al., 2003; Pan et al., 2012; Zheng and Gao, 2000).

The PCP pathway, which orchestrates complex tissue movements and patterning events in different types of tissues during development in both invertebrates and vertebrates (Rida and Chen, 2009), is essential in both the uniform orientation of stereocilia and cochlear extension (Wang et al., 2005). The formation of the polar hair bundle involves the relocation of the centrally positioned microtubule-based kinocilium to the periphery and formation of a dense F-actin mesh on the apical surface, although kinocilia disappear postnatally in mouse and chicken cochleae (Jones and Chen, 2008; Jones et al., 2008; Tanimoto et al., 2011). The appearance of V-shaped actin-based stereociliary bundles of graded heights and the alignment of a pair of centrioles in the cell indicates the characteristic planar cell polarity, which is associated with hearing function (Jones and Chen, 2008). The cochlear convergent extension (CE) is defined as cellular intercalations along the mediolateral axis and extension along the perpendicular longitudinal axis, which is another character of PCP (Jones and Chen, 2008; Qian et al., 2007). This may be mediated by directly regulating cell adhesion (Chacon-Heszele et al., 2012; Rida and Chen, 2009). Expression of certain PCP pathway molecules was also observed during the development of the inner ear in mice (Jones et al., 2008; Wang et al., 2005). Nevertheless very little is known about the role of these PCP molecules during regeneration in the organ of Corti.

Testosterone is a gonadal sex steroid hormone that acts on cells through intracellular transcription-regulating androgen receptors (ARs) (Benten et al., 1999). Using an albumin-conjugated steroid, testosterone-BSA, studies have shown significant induction of actin polymerization and apoptosis, which can be reversed by actin-disrupting agents (Papadopoulou et al., 2008). Testosterone can also modulate the activity of regeneration components, such as fibroblasts and immune and myogenic precursor cells (Brown et al., 2013; Friedl et al., 2000; Zhang et al., 1998). In addition, testosterone administration has been shown to accelerate the functional recovery rate after laryngeal nerve injury and facial nerve impairment (Sharma et al., 2010). It is also able to induce skeletal muscle hypertrophy due to protein accumulation and myonuclear accretion (Sinha-Hikim et al., 2006) and plays a key role in CNS development and neuroprotection in injury and disease (Fargo et al., 2008). Testosterone plays a role in a variety of regenerative processes in different organs and while the importance and benefits of testosterone have been demonstrated in peripheral nerve injury models, no prior studies have explored its role in hair cell regeneration.

To further analyze the role of PCP signaling pathways in ectopic HCLCs in the LER, we constructed organ cultures of mouse utricles co-transfected with a human adenovirus serotype 5 (Ad5) vector encoding Math1 and/or the reporter gene EGFP and compared and analyzed in detail the cellular responses and pathways triggered by testosterone-BSA. We attempted to elucidate the role of T-BSA in hair cell regeneration and planar cell polarity formation. Our results indicate that PCP signaling is involved in the development of ectopic HCLCs, and the CE of the ectopic sensory region is regulated by testosterone-BSA through downregulation of cell–cell adhesion. Testosterone-BSA could accelerate HCLC formation in the LER through proliferation.

2. Results

2.1. *Establishment of planar cell polarity*

The basal body, which is nucleated and lies at the base of the kinocilium, located just below the apical plasma membrane in the fonticulus (an actin-free hole in the cuticular plate; Figs. 1K,L and 2E,F), can be used to observe cell polarity. The position of the basal body in regenerated HCLCs changed dynamically over the culture time when Math1 was overexpressed. At the beginning (Div4), the basal body, marked by γ-tubulin, appeared in the center of single ectopic MyoVIIa(+) cells (Fig. 1G, white arrows). One week later (Div10), it had moved to the lateral side of the cochlear spiral (Fig. 2C, white arrows). The ectopic, regenerated HCLCs presented with numerous stereocilia bundles (Fig. 2B and C). The polarized stereocilia bundles had "V,"