Hearing Research 303 (2013) 20–29

Contents lists available at SciVerse ScienceDirect

Hearing Research

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

Review

# Lead roles for supporting actors: Critical functions of inner ear supporting cells

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ARTICLE INFO

*Article history:*
Received 26 November 2012
Received in revised form
20 December 2012
Accepted 10 January 2013
Available online 21 January 2013

ABSTRACT

Many studies that aim to investigate the underlying mechanisms of hearing loss or balance disorders focus on the hair cells and spiral ganglion neurons of the inner ear. Fewer studies have examined the supporting cells that contact both of these cell types in the cochlea and vestibular end organs. While the roles of supporting cells are still being elucidated, emerging evidence indicates that they serve many functions vital to maintaining healthy populations of hair cells and spiral ganglion neurons. Here we review recent studies that highlight the critical roles supporting cells play in the development, function, survival, death, phagocytosis, and regeneration of other cell types within the inner ear. Many of these roles have also been described for glial cells in other parts of the nervous system, and lessons from these other systems continue to inform our understanding of supporting cell functions.

*This article is part of a Special Issue entitled "Annual Reviews 2013".*

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## 1. Introduction

Hearing loss affects nearly 4 million American children and 36 million adults (NIDCD, 2010; NIDCD, 2006). Aging, noise trauma, ototoxic drugs, and hereditary mutations are all causes of hearing loss (Li-Korotky, 2012; Seixas et al., 2012; Cheng et al., 2009; Friedman and Griffith, 2003), a condition that has limited treatments and no known cure. In addition, in the United States, balance disorders affect over 600,000 individuals and similarly have few treatment options (NIDCD, 2010). Many studies aimed at understanding the mechanisms underlying hearing loss and balance disorders have focused on mechanosensory hair cells, the sensory receptor cells of the auditory and vestibular systems (Phillips et al., 2008). Fewer studies have examined the biology and functions of the supporting cells that surround hair cells. This review will discuss the emerging evidence indicating that auditory and vestibular supporting cells serve many critical functions, some of which are similar to functions carried out by glial cells (astrocytes, microglia, Schwann cells and oligodendrocytes), suggesting that supporting cells may represent a type of specialized glia.

The mammalian cochlea contains several types of supporting cells, each with a distinct morphology and a specific anatomical location within the organ of Corti (reviewed in Raphael and Altschuler, 2003). Deiters' cells provide structural support for the outer hair cells, which are positioned atop and in direct contact with the Deiters' cell layer (reviewed in Raphael and Altschuler, 2003). Pillar cells form the tunnel of Corti, which lies between the inner and outer hair cells. Hensen's and Claudius cells both lie lateral to the outer hair cells in the outer sulcus. Supporting cells are less well-characterized than hair cells and in striving for better characterization, analogies have been drawn between supporting cells of the inner ear and those of other sensory systems, including the olfactory sustentacular cells and the retinal Müller glia (Rubel et al., 1991). Some similarities and significant differences between auditory supporting cells and these other sensory supporting cell types will be discussed in this review.

Emerging evidence suggests that auditory and vestibular supporting cells serve important functions as mediators of hair cell development, function, death and phagocytosis (Tritsch et al., 2007; Jagger and Forge, 2006; Bird et al., 2010; Lahne and Gale, 2008). Recent reports also indicate that supporting cells may mediate the survival and function of SGNs (Zilberstein et al., 2012). Many of these supporting cell functions are paralleled by glia in their relationship with neurons. Glial cells support neuronal function and survival in many ways. For example, both astrocytes and oligodendrocytes provide trophic support for neurons (Wilkins et al.,

*Abbreviations:* SGN, spiral ganglion neuron; IHC, inner hair cell; ISC, inner supporting cell; BDNF, brain-derived neurotrophic factor; NT3, neurotrophin-3; NRG, neuregulin; GLAST, glutamate aspartate transporter; ERK 1/2, extracellularly regulated kinases 1 and 2; HSP70, heat shock protein 70; TEM, transmission electron microscopy; TIAR, T-cell restricted intracellular antigen-related protein; PS, phosphatidylserine; FGF, fibroblast growth factor.

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0378-5955/$ – see front matter Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.heares.2013.01.008

2003; Banker, 1980). Astrocytes support neuronal function and survival by clearing glutamate from neuronal synapses (Rothstein et al., 1996) and buffering potassium through a system of gap junctions (reviewed in Leis et al., 2005). Microglia play critical roles in the response to neuronal injury, engulfing apoptotic neurons in the central nervous system (reviewed in Napoli and Neumann, 2009). Following neuronal death in the fish retina, Müller glia serve as neural precursors for regenerated retinal neurons and photoreceptors (Bermingham-McDonogh and Reh, 2011). Many of these functions of glial cells are similar to those that have been described for auditory and vestibular supporting cells.

## 2. Development and survival of hair cells and spiral ganglion neurons

In the developing mammalian cochlea, the onset of neuronal activity results from coordinated signaling from hair cells, supporting cells, and SGNs. Cochlear hair cells are depolarized upon deflection of their stereocilia (Flock, 1965; Russell et al., 1986), which triggers the release of glutamate from inner hair cells (IHCs) (Kataoka and Ohmori, 1994). Glutamate binds to synaptic receptors on adjacent SGNs, resulting in the generation of action potentials and transmission of the afferent signal to the auditory brainstem (Ruel et al., 1999; reviewed in Cunningham and Tan, 2011). While sound energy is the stimulus that ultimately results in the generation of action potentials after the developmental onset of hearing, hair cells depolarize and release glutamate, resulting in spontaneous action potentials prior to hearing onset (Lippe, 1994; Jones et al., 2007; Tritsch et al., 2007). This spontaneous activity may contribute to the guidance and refinement of synaptic connections, including the formation of the tonotopic map of the inner ear (reviewed in Walmsley et al., 2006).

Recent evidence indicates that cochlear supporting cells may mediate the initiation of spontaneous activity during cochlear development. Prior to the onset of hearing in rats, 'inner' supporting cells (ISCs), which are the columnar epithelial cells specific to Kolliker's organ, spontaneously release ATP (Tritsch et al., 2007; Tritsch and Bergles, 2010). As early as the day after birth (P1) this extracellular ATP release results in inward currents and depolarization of IHCs (Tritsch et al., 2007; Tritsch and Bergles, 2010). After P4, IHCs consistently exhibit spontaneous inward currents in response to ATP released by supporting cells. These events occur with increasing frequency and amplitude until the onset of hearing at P10-12 (Tritsch and Bergles, 2010). After hearing onset, the frequency and amplitude of the inward currents rapidly decline. Inward currents tend to occur simultaneously among neighboring IHCs ($<$100 μm from each other), suggesting that ATP released from supporting cells synchronizes patterns of IHC activity before hearing onset (Tritsch et al., 2007; Tritsch and Bergles, 2010). While not every IHC depolarization generates an action potential, SGN bursting is observed after P4 and appears to rely exclusively upon the ATP release from ISCs (Tritsch and Bergles, 2010). Taken together, these data indicate that supporting cells are important mediators of spontaneous neural activity in the developing organ of Corti. Additional recent data suggests that the resting mechanotransducer current of inner hair cells may also be an important factor in driving this spontaneous activity, although the interplay between the supporting cells and the mechanotransducer current during inner hair cell development remains unclear (Johnson et al., 2012).

Supporting cells are also critical to SGN survival in the mature cochlea. Multiple groups have demonstrated degeneration of SGNs following aminoglycoside-induced hair cell death (Dupont et al., 1993; McFadden et al., 2004). One interpretation of these data is that the survival of SGNs is dependent upon being coupled to viable hair cells (Koitchev et al., 1982), which provide trophic support necessary for SGN survival (reviewed in Gillespie and Shepherd, 2005). However, recent evidence suggests that, at least in the short- to mid-term, SGNs may instead be dependent upon supporting cells for their survival. Using a mutant mouse model with a targeted deletion of a high-affinity thiamine transporter (*Slc19a2*), in which extensive IHC death can be induced without loss of supporting cells, Zilberstein et al. (2012) showed that SGNs survive for at least three months after the death of adjacent IHCs. These data indicate that viable IHCs may not be necessary for SGN survival, and they are in agreement with other data indicating that *Brn3c* (now called *Pou4f3*) null mutant mice, in which hair cells degenerate in the neonatal period, continue to exhibit some surviving SGNs, even in 6-month-old mutant mice (Xiang et al., 2003). Given the above, it is possible that the SGN degeneration that follows aminoglycoside treatment is at least in part a result of toxic effects of aminoglycosides on either supporting cells or SGNs (Sugawara et al., 2005).

Supporting cells provide trophic factors that promote the survival of SGNs. Brain-derived neurotrophic factor (BDNF), a critical trophic factor in neural development and survival, is expressed in vestibular supporting cells of postnatal mammals (Montcouquiol et al., 1998; Gomez-Casati et al., 2010). BDNF conditional knockout mice (in which the *Pax2* promoter drives Cre expression to eliminate BDNF expression in the entire inner ear) exhibit a reduction in IHC synaptic ribbons and afferent SGN fibers (Zuccotti et al., 2012). In addition, neuregulins (NRGs) are critical trophic factors for SGNs and are expressed by SGNs (Fig. 1). NRGs bind complementary erbB receptors expressed by multiple cochlear supporting cell types, including inner border cells, inner phalangeal cells, Deiters' cells, pillar cells, Boettcher cells and inner sulcus cells. When erbB-NRG signaling between supporting cells and SGNs is disrupted in transgenic mice expressing a dominant-negative erbB4 receptor, type I SGNs degenerate (Stankovic et al., 2004). The likely cause of the SGN degeneration in these mice is a reduction in neurotrophin-3 (NT3) expression (Stankovic et al., 2004), which is critical for SGN survival during development

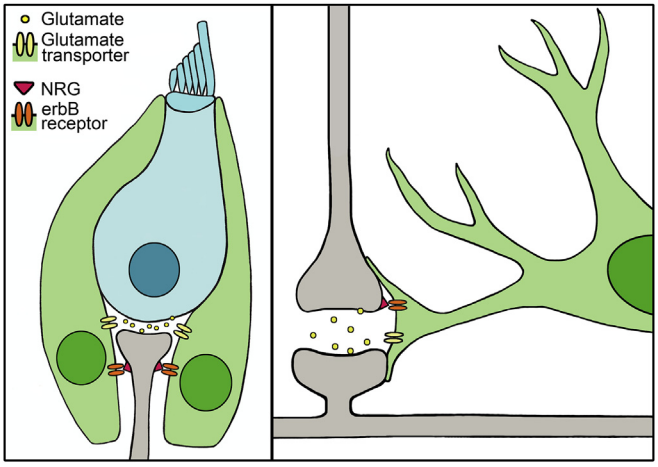


**Fig. 1.** Supporting cells and glia provide trophic factors to neurons and clear glutamate from the synapse. Left panel, hair cells (blue) synapse with spiral ganglion neurons (gray), and are surrounded by supporting cells (green). Hair cells release glutamate, which is cleared from the synapse by glutamate transporters expressed by supporting cells. Spiral ganglion neurons express NRG, which binds to erbB receptors located on the supporting cells, thereby promoting SGN survival. Right panel, illustration of a tripartite synapse between two neurons (gray) and an astrocyte (green). The presynaptic neuron (top) releases glutamate into the synapse, which is cleared from the synapse by glutamate transporters on the astrocyte. Neurons also express NRG, which binds to erbB receptors located on the astrocyte, an interaction necessary for normal astroglial morphology and neuronal migration.