Evidence for a partial epithelial-mesenchymal transition in rat auditory organ development

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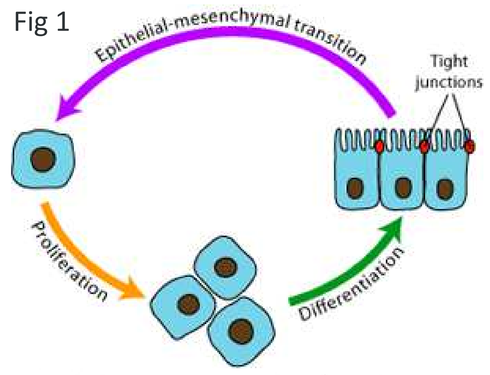
Introduction

An epithelial-mesenchymal transition (EMT) is a biological process that allows a polarized epithelial cell to undergo multiple biochemical changes that enable it to assume a mesenchymal cell phenotype (Kalluri and Weinberg 2009; Savagner 2010; Thiery et al. 2009). During this process, epithelial cells loosen cell-cell adhesion, module their polarity and rearrange their cytoskeleton: intermediate filaments typically switch from cytokeratin to vimentin. They also enhance their motility capacity. The EMT plays key roles in the formation of the body plan and in the differentiation of multiple tissues and organs but it is also involved in tissue repair, tissue homeostasis, fibrosis, and carcinoma progression (Thiery et al. 2009) (Fig 1).

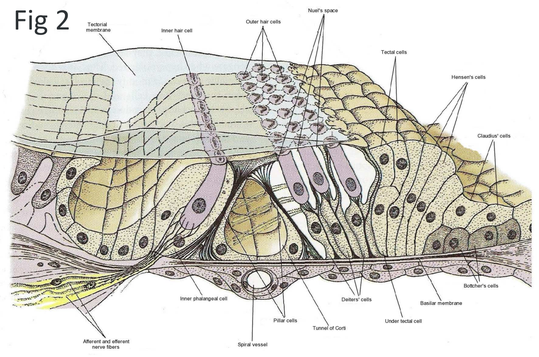
Fig 1



Until now, EMT has been rarely mentioned in the inner ear organogenesis. In chick, EMT has been reported as a possible mechanism of semicircular canal morphogenesis (Kobayashi et al. 2008). More recently, an *in vitro* study has also indicated that sensory epithelial cells from mouse utricle can undergo an EMT to become cells expressing features of prosensory cells (Zhang and Hu 2011). By contrast, EMT has never been observed during auditory organ morphogenesis.

The auditory organ, the organ of Corti (OC), is a highly specialized structure composed by specific cellular types. The sensory cells are characterized by stereocilia at their apex and are necessary for the sound perception. These cells are supported by supporting cells. Based on their morphology and physiology, at least four types of supporting cells can be identified in the OC (Fig 2): inner and outer pillar cells, phalangeal cell and Deiter's cells. The inner pillar cells and outer pillar cells combine to form the tunnel of Corti, a fluid filled triangular space that separates the single row of inner hair cells from the first row of outer hair cells. The Nuel spaces are another interval in the OC that is situated between the outer pillar cells and the different rows of outer hair cells and Deiters cells.

Fig 2



To determine whether an epithelial-mesenchymal transition may play a role in the morphogenesis of the auditory organ, we studied the spatial localization of several EMT markers, the cell-cell adhesion molecules and intermediate filament cytoskeletal proteins, in epithelium of the dorsal cochlea during development of the rat OC from 18th embryonic day (E18) until 25th postnatal day (P25).

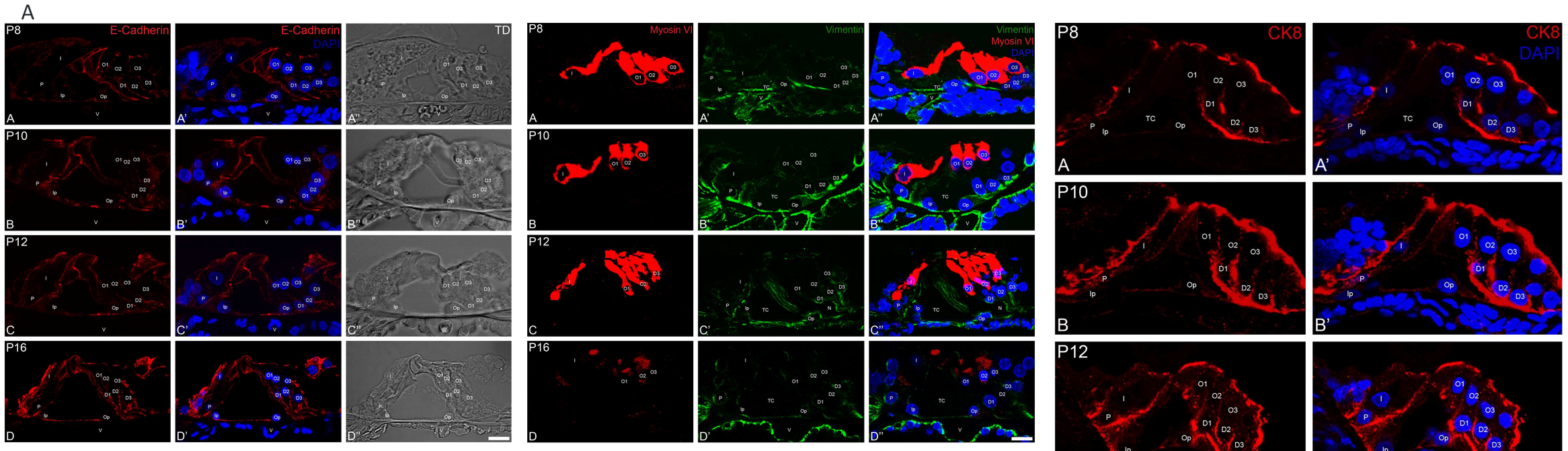
Results

Partial loss of E-cadherin between the pillar cells and between the Deiters' cells from P8

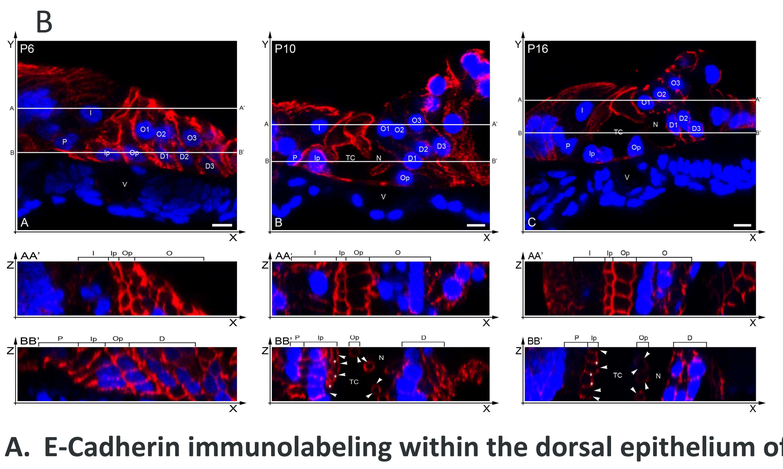
Temporary presence of vimentin in pillar and Deiters cells at P8-10

Intense expression of cytokeratin in supporting cells at P10-12

A



B



Vimentin immunolabeling within the dorsal epithelium of the cochlear duct from P8 to P16.
A-D. Localization of the hair cells by using Myosin VI (red). A'-D'. Immunolocalization of the vimentin (green). A''-D''. Merged image with cell nuclei stained with DAPI (blue). Bar = 10 µm

Annotations: D(1-3) Deiters' cell, I inner hair cell, Ip inner pillar cell, N Nuel's spaces, O(1-3) outer hair cell, Op outer pillar cell, P phalangeal cell, PX Post-natal day X, TC tunnel of Corti, and V spiral vessel.

Cytokeratin 8 immunolabeling within the dorsal epithelium of the cochlear duct from P8 to P12.
A-C Localization of the supporting cells by using p27kip1 (green). A'-C'. Immunolocalization of the cytokeratin 8 (red). A''-C''. Merged image with cell nuclei stained with DAPI (blue). Bar = 10 µm

A. E-Cadherin immunolabeling within the dorsal epithelium of the cochlear duct from P8 to P16. A-D. Immunolocalization of the E-Cadherin (red). A'-D'. Merged image with cell nuclei stained with DAPI (blue). A''-D''. Visualization of the OC by transmission detector. Bar = 10 µm

B. E-Cadherin immunolabeling of the organ of Corti at P6, P10 and P16 in transversal view projection on the XY axis. The lines indicate the starting level for the projection on the XZ level shown on XX' of the OC and they are signalled below by XX'. XX' projection of the organ of Corti on the XZ plane. Arrowheads/Asterisks represent loss/maintaining of E-cadherin labeling (red), respectively. Cell nuclei are stained with DAPI (blue). Bars = 15 µm

Conclusion

Our results show a local loss of adhesion between supporting cells of the OC from P8, an increase expression of cytokeratins in supporting cells around P10 and a temporary appearance of vimentin in supporting cells at P8-10. These observations suggest that a partial epithelial-mesenchymal transition might be involved in the remodeling of the Corti organ during the postnatal stages of development in rat.

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