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The Ups and Downs of Retinoic Acid Signaling in Early Inner Ear Development

Dorothy A Frenz*

Department of Cell Biology and Anatomy, New York Medical College, Valhalla, New York, USA

Deficiency in vitamin A during human development can perturb a number of developmental processes, including cell proliferation, migration, and differentiation, and lead to congenital malformations. There is no de novo synthesis of vitamin A in the human embryo, thus in utero developing tissues are reliant on maternal retinol, which is converted intracellularly into a biologically active derivative of vitamin A, Retinoic Acid (RA). Deficiency in RA affects a number of ectodermally-derived organs [1], including the inner ear. Much of what is known about the requirement for RA during inner ear development has been elucidated through vitamin A deficiency studies [2,3] and analyses of double mutant mice deficient in RA receptors (RARα/RARγ) [4], in which a pronounced inner ear phenotype is manifested by a marked hypoplasia of the otic vesicle and arrest of development at a rudimentary stage. The inner ear is thus an excellent model in which to investigate the developmental anomalies resulting from deviations in RA concentrations. However, it is not yet fully clear as to what local sources of RA affect inner ear development.

The inner ear develops from a simple otic placode into one of the vertebrate body's most elaborate organs. This entails a series of sequential inductive interactions. Initially, cephalic surface ectoderm interacts with signals emanating from the neuroepithelium of the hindbrain, forming the otic placode [5]. RA is present in hindbrain rhombomeres and instrumental in ensuring correct hindbrain patterning and segmentation [6]. Experimental conditions which lead to retinoid insufficiency [2,7,8] result in misspecification of hindbrain rhombomeres and perturbation in segmentation. However, proper segmentation and patterning of the hindbrain is critical to formation of the inner ear [9-13], and thus otic defects, such as hypoplasia of the otic vesicle, are a consequence of hindbrain dysmorphogenesis.

It is therefore not surprising that defects in inner ear development due to deficiency in RA have primarily been considered indirect effects of the hindbrain. However, there are other local sources of RA that are likely to influence inner ear development. Retinaldehyde Dehydrogenase2 (Raldh2), an enzyme involved in the oxidation reactions which convert retinol to RA, is expressed in somitic mesoderm. RA synthesized by Raldh2 diffuses from presomitic/early somitic mesoderm and regulates gene expression in the posterior hindbrain [14]. Thus RA of somite mesodermal origin can affect inner ear development via an indirect pathway. However, given its location just posterior to the otic vesicle, it is also possible that RA derived from somitic mesoderm may directly act on the developing inner ear [14].

Raldh2 is strongly expressed within the epithelium of the otic vesicle [15], raising the possibility that RA of otic epithelial origin may act directly within this tissue [14,16]. Blockade of RA signaling by pharmacologic reagents in otic vesicle explants, used to model RA deficiency, suggests this to be the case [16]. Treatment of otic explants with the RA receptor antagonist Ro 41-5253 or with citral or DEAB (4-diethylaminobenzaldehyde), which inhibit aldehyde dehydrogenases and block the conversion of retinol to RA, produces small, hypoplastic otic vesicles that recapitulate the vitamin A deficiency phenotype [3]. Studies in mutant mouse models of RA deficiency concur with these findings [16], but unlike explant studies, in which the otic vesicle is harvested subsequent to patterning of the hindbrain, cannot distinguish between direct and indirect effects of RA on the inner ear.

Exposure to excess RA during a defined window of development also causes severe inner ear malformations, some of which paradoxically reflect the RA deficiency phenotype. In the developing mouse inner ear, these defects include vestibular and cochlear anomalies and in severe cases, the formation of small, hypoplastic and often cystic otic vesicles [17]. Opposite otic phenotypes, i.e., reduced and enlarged otic vesicles, are produced in zebrafish by blocking or increasing RA [18], suggesting that the effects of RA on inner ear morphology may be species-specific. Of significance, maternal ingestion of Isotretinoin (13-cis-RA) during the first trimester of pregnancy, used in treating cystic acne and certain cancers, leads to multiple embryopathies, including the inner ear [19].

Phenotypic changes in response to deviations in RA levels are correlated with changes in expression of RA responsive genes. Fibroblast Growth Factor 3 (FGF3) and FGF10 play critical cooperative roles in otic induction [20-21], and are subject to control through RA signaling. It is particularly intriguing that in the developing mouse inner ear, certain aspects of FGF signaling within the otic epithelium reflect the paradoxical effects of RA excess and deficiency on inner ear phenotype. Deviations in RA concentrations during a defined window of development, either in excess or deficiency, each result in a dose-dependent down regulation of Fgf3 and Fgf10 mRNA within the otic epithelium [16,22] in what has been termed a "Goldilocks phenomenon" [16]. Reminiscent of the classic children's story "Goldilocks and the Three Bears", in which the temperature of the porridge could neither be too hot nor too cold, the concentration of RA to which the developing inner ear is exposed must be "just right" for proper signaling of inner ear development. Thus regulation of local retinoid concentrations within the developing inner ear must be tightly regulated, and reflects coordinate control between the activities of synthesizing and degradation enzymes within the inner ear and neighboring embryonic tissues which influence inner ear development [14].

The hindbrain is a critical signaling center for otic induction and does so by means of FGF. An issue is thus raised as to whether regulation of otic Fgf by RA may be direct or secondary to RA-induced changes in hindbrain pattern/specification. A more detailed understanding of the role of RA in regulating Fgf expression has been attained through

***Corresponding author:** Dorothy A Frenz, Department of Cell Biology and Anatomy, New York Medical College Valhalla, New York, USA, E-mail: Dorothy_frenz@nymc.edu

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analyses of reporter expression in mice containing *lacZ* either under the control of a minimal Fgf3 or Fgf10 enhancer [23,24]. Exposure of embryos to RA at a stage where hindbrain patterning is either unaffected by RA exposure [25] or subsequent to hindbrain patterning, revealed that Fgf3 reporter activity is down regulated in the antero-ventral otic vesicle but unaltered in the developing posterior hindbrain [26]. These findings support direct regulation of otic Fgf3 expression by RA, and underscore the importance of determining whether the response to RA may be mediated via binding to RA responsive elements located at the Fgf3 locus [16].In common with Fgf3, Fgf10 reporter expression is down regulated in the antero-ventral otic vesicle by exogenous RA during hindbrain patterning, but to a lesser extent after hindbrain patterning [24]. It will thus be interesting to further investigate whether the response to RA post-hindbrain patterning is direct on the otic Fgf10 enhancer or a secondary consequence of alterations in hindbrain pattern that lead to different signals influencing the development of the inner ear. Of note, changes in localization of Fgf3 and Fgf10 by excess RA also occur within the otocyst epithelium during hindbrain patterning, implicating the ability of the inner ear to reprogram its spatial expression profiles during this developmental time point. However, the precise spatial changes differ between these related signaling molecules, and likely represent differences in the regulatory response of Fgf3 and Fgf10 to RA [24].

In summary, RA is indispensable to certain aspects of inner ear development. Otic defects can thus ensue in response to deviations in local RA concentrations, which may perturb downstream signaling molecules via direct /indirect RA pathways. FGFs [3,10] are but one molecular component comprising the intricate signaling interactions governing inner ear development. Thus further investigation into the molecular pathways linking RA activity and the program of inner ear development is necessary to more fully comprehend the complexities of RA signaling in inner ear development.

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