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Expression of the integrin genes in the developing cochlea of rats ☆

Katsuhiro Toyama, Masashi Ozeki, Yuki Hamajima, Jizhen Lin *

Auditory Molecular Biology Laboratory, Department of Otolaryngology, University of Minnesota School of Medicine, Minneapolis, MN, USA

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Abstract

Integrins play an important role in the development of the cochlea. However, little is known about the expression pattern of integrins in the developing cochlear tissue. In this study, we investigated the dynamic expression profile of the integrin genes in the developing cochlear tissue of rats by Affymetrix microarrays and explored the role of the integrin genes in vitro by using antisense oligonucleotides. It was demonstrated that the α1, α7, αv, β3, and β4 genes were expressed in the developing cochlear tissue of rats. Inhibition of the integrin expression with antisense oligonucleotides against αv, α7, β3, and β4, respectively, in cochlear sensorineural epithelial cells significantly decreased the [^{3}H]thymidine incorporation, suggesting that these integrins are involved in cell growth and proliferation. Inhibition of the αv and β4 integrins significantly decreased the transcription of nuclear factor-kappa B (NF-κB, a signal molecule involved in cell growth and proliferation) induced by epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), respectively. It suggests that EGF-induced cell growth is dependent upon the αv integrin whereas bFGF-induced cell growth is dependent upon the β4 integrin in the cochlear tissue during the development of the inner ear.

Keywords: Integrins; Cochlear development; Microarrays; NF-κB; Sprague–Dawley rats

1. Introduction

Integrins are important cell surface proteins. They are composed of two subunits, α and β, which form strict heterodimers in a non-covalent manner (Hynes, 1992). Due to the different mRNA splicing of integrins, many variants of the α and β chains exist which greatly increase the diversity of integrins. To date, at least 24 different heterodimers have been identified and they are derived from 18 α and 8 β combinations (van der Flier and Sonnenberg, 2001). These integrins reside on the cellular surface and interact with other cell surface proteins or extracellular matrix (ECM) proteins, mediating cell–cell and cell–matrix interactions. Well-known ECM molecules that interact with integrins or act as ligands include fibronectin, laminins, collagens, and vitronectin.

Each integrin recognizes specific ligands such as laminin, collagen, fibronectin, vitronectin, and intercellular adhesion molecule-1, etc. While some integrins bind only one specific ligand, the others bind several types of ligands. Conversely, some types of ECM bind only one type of integrin, whereas the others bind multiple integrins (Hynes, 1987; Ruoslahti and Pierschbacher,

Abbreviations: NF-κB, nuclear factor kappa B; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor; ECM, extracellular matrix; PDGF, platelet-derived growth factor; RT-PCR, reverse transcription-polymerase chain reaction; DLU, digitalized light unit; RLU, relative luciferase unit; DNA, deoxyribonucleid acid; cDNA, complementary deoxyribonucleid acid; RNA, ribonucleid acid; cRNA, complementary ribonucleid acid; mRNA, messenger ribonucleid acid

☆ This study was performed in accordance with the PHS Policy on Human Care and Use of Laboratory Animals, the NIH guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of University of Minnesota.

* Corresponding author. Tel.: +612 626 9872; fax: +612 626 9871.
E-mail address: linxx004@umn.edu (J. Lin).

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1987). Not only does this system provide diversity of signals, but also allows the production of extremely specific signals between a cell and its ECM.

Integrins are known to link to signal molecules with their intracellular domains. Through these molecules, integrins elicit a variety of signal transduction in cells, which acts through the NF-κB/cyclin D1 pathway that drives cell cycle progression (Giancotti and Ruoslahti, 1999). These cascade events may loosen cell-substrate contacts and rearrange cell skeleton proteins, allow cells to divide, move apart and migrate. Integrins are essential for cellular survival, growth, proliferation, and differentiation. Without integrins, cells may not respond to growth factors. Mutation of the integrins $\alpha_8\beta_1$ results in stereocilia defects in mice (Littlewood-Evans and Muller, 2000). Likewise, mutations of ECM components such as collagen IV, collagen II, α-tectorin, USH2A, and otogelin cause inherited hearing impairment (Petit et al., 1996; Cohen-Salmon et al., 1997; Eudy et al., 1998; McGuirt et al., 1999; Simmler et al., 2000). Interaction between integrins and ECM is fundamental to morphogenesis and functionality of the inner ear.

The development of the mammalian cochlea involves a complex series of cell–cell and cell–ECM interactions (Legan et al., 1997; Legan and Richardson, 1997) which include integrins and ECM molecules. Much remains to be learned about the dynamic expression profiles and functions of integrins in the developing cochlear tissues and sensorineural epithelial cells although the α2 α3, α6, α8, β1, and β4 integrins molecules have been reported (Davies and Holley, 2002). We know that integrins play an essential role in mediating the responses of growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF). However, we do not know which integrins mediate them.

In this study, we demonstrated the expression profiles of the integrin genes in the inner ear from embryonic day 12 (E12) to postnatal day 1 (P1) by Affymetrix cDNA microarrays. The expression of the integrin genes was confirmed by reverse transciption PCR (RT-PCR). The effects of integrins on cellular growth and proliferation were studied using antisense oligonucleotides for evaluation of DNA synthesis. Finally, the NF-κB activity that signals the growth and proliferation of cells was studied by luciferase assays.

2. Materials and methods

2.1. Procurement of embryonic inner ear tissues of rats

Five pregnant female rats were used in this study. Embryos on embryonic days 12, 14, 16, and 18 (E12, E14, E16, and E18) were removed surgically from the pregnant rats with anesthesia of ketamine hydrochloride (40 mg/kg) to obtain cochlear tissues (otocysts and otocapsules). Postnatal day 1 (P1) rats were also prepared to obtain cochlear tissues (otocapsules). Total RNA was isolated using the StrataPrep^R total RNA Miniprep kit (Stratagene) according to the manufacturer's instructions.

2.2. Affymetrix microarrays of embryonic inner ear tissues

Microarrays were performed as described previously (Lee et al., 2004). Briefly, cDNA was prepared from 20 μg total RNA using the double-strand DNA synthesis kit (Life Technologies, Rockville, MD). cRNA was synthesized from cDNA and biotinylated using the BioArray High Yield RNA Transcript Labeling Kit (Enzo Diagnostics, Farmingdale, NY). Fifteen μg of cRNA were fragmented and hybridized to the Rat U34 GeneChip. Signals were detected by anti-streptavidin antibody conjugated to fluorescence. Raw data were scanned with the Agilent GeneArray Laser Scanner and normalized across the Genechips with Affymetrix® Microarray Suite 5.0. Data sets (in triplicates, three independent sample pools) from each time point were scaled and relative mRNA expression levels were expressed as average ± SD (SD). Data presented in this study represent the integrin genes. Microarray results were verified by RT-PCR using total RNA from the E12 cochlear tissue of rats, as described previously (Lin et al., 1999, 2003), using 1 μg of total RNA and 0.2 μM sense and antisense primer pairs with 43 cycles (94 °C for 20 s, 60 °C for 1 min and final step, hold 72 °C for 7 min). The sequence information for the integrin genes was from the National Center of Biotechnology Information (fttp://www.ncbi.nlm.nih.gov) (Table 1). Negative controls for RT-PCR (omitting total RNA sample) were included in each RT-PCR experiment for evaluation of whether PCR products were from target total RNA. PCR products were analyzed on a 2% agarose gel.

2.3. Inhibition of the integrin expression with antisense oligonucleotides

To determine whether integrins were involved in the proliferation of cochlear epithelial cell lines, OT12 and OC1 were used which express multiple hair cell markers and have been identified as being progenitor hair cell lines (Ozeki et al., 2003). Cells were maintained in minimal essential medium (MEM, Sigma) supplemented with 20 mM Hepes, 2 mM L-glutamine, 10 mL/L nonessential amino acid, 0.4 μg/mL hydrocortisone, 5 μg/mL insulin, 2.5 μg/mL transferrin, 10 ng/mL epidermal growth factor (EGF), 10% fetal bovine serum (FBS), 50 μg/mL streptomycin, and 50 units/mL penicillin for 24 h. OT12 cells in the above medium were seeded on 24-well plates at an approximate density of 10^4 cells/well