Genomic Organization and Developmental Expression of Aquaporin-5 in Lung

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Studies in Human Lung Epithelial Cells

Human alveolar carcinoma cells (A549) were plated for 24 h prior to infection with MOIs of 1 to 50 of AdMRCMVα_1, AdMRCMVβ_1, and AdMRCMVβ-Gal. Cells infected with MOIs of 5 to 50 of AdMRCMVα_1 demonstrated twofold to threefold increases in ouabain-inhibitable $^{86}Rb^+$ uptake 24 h after infection. No changes in Na^+/K^+-ATPase function were noted following infection with AdMRCMVβ_1 or AdMRCMVβ-Gal. Northern blot analysis of total RNA using a rat-specific cDNA probe revealed the presence of α_1 mRNA only in cells infected with AdMRCMVα_1; no change in β_1 message was noted. Western blot analysis showed significantly increased α_1 protein in cells infected with AdMRCMVα_1. These findings suggest that α_1 subunit may be the rate-limiting subunit in A549 cells and that Na^+/K^+-ATPase function can be increased in these cells using gene transfer.

The human α_1 isoform is two to three logs more sensitive to ouabain than is the rat α_1 isoform. To demonstrate transgene expression, ouabain sensitivity was determined in A549 cells by ascertaining the concentration of ouabain that produced a 50% reduction in $^{86}Rb^+$ uptake (IC_{50}). A549 cells were infected with AdMRCMVα_1 at an MOI of 25, and $^{86}Rb^+$ uptake was measured using 15 different concentrations of ouabain (1×10^{-11} to 1×10^{-3}). IC_{50} values were determined using a computerized nonlinear least squares regression function designed to test for the presence of one or two isozymes of a receptor with different affinities for a ligand (ouabain). AdMRCMVα_1-infected cells demonstrated two distinct IC_{50} values ($IC_{50(1)}=3.5\times10^{-7}$, $IC_{50(2)}=3.2\times10^{-5}$). Sham and AdMRCMV$\beta$-Gal infected cells each demonstrated one IC_{50} that was not different from the first α_1 IC_{50}. The identification of a second α_1 IC_{50} two logs greater than that found in the sham or AdMRCMVβ-Gal infected cells suggests that presence of two functional α_1 isozymes: the endogenous ouabain-sensitive human α_1 and the ouabain-resistant transgenic rat α_1.

These results demonstrate that adenoviruses can efficiently transfer genes to lung alveolar epithelial cells and augment Na^+/K^+-ATPase abundance and function. The response to infection with individual subunits differs between cell types such that the β_1 subunit could be rate-limiting for rat ATII cells and α_1 may be the rate-limiting subunit for human A549 cells *in vitro*. Conceivably, gene transfer to the alveolar epithelium may be applicable to the prevention and treatment of pulmonary edema.

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Discovery of the aquaporin family of water transporters provided a molecular explanation for osmotically driven water transport across cell membranes of mammalian and plant tissues. At present, there are six known mammalian aquaporins distributed in a wide variety of tissues and cell types. In general, the distribution of each aquaporin is unique, though there are some scattered sites of overlap (reviewed by King and Agre[1]). Recent identification and characterization of the genes encoding these aquaporins have enabled investigators to begin establishing a role for aquaporins in the pathogenesis of several disease states.[2,3] Despite the wealth of evidence in recent years to explain the physiology of salt transport in the lung, the molecular mechanisms for transcellular movement of water in the lower respiratory tract remain poorly understood.

Four aquaporins have been identified in the lung by Northern blot analysis or immunoblotting[4-6] (Table 1).

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Table 1—*Aquaporins in Lung*

Protein	Human Gene Location	Expression in Lung
Aquaporin-1	7p14	Peribronchial endothelium, visceral pleura, rare alveolar pneumocytes
Aquaporin-3	9p21 → 12	Basolateral surface of bronchial epithelium
Aquaporin-4	18q11.2-12.1	Basolateral surface of bronchial and tracheal epithelium
Aquaporin-5	12q13	Type I pneumocytes, trachea, bronchial submucosa

Aquaporin-1 (AQP1) is expressed in the lung in vascular endothelium, visceral pleura, and a subset of pneumocytes.[4] AQP4, the major brain aquaporin,[7] localizes to the basolateral membrane of bronchial and tracheal epthelium in lung.[5] AQP3 is expressed in the lung exclusively on the basolateral surface of tracheal epithelium.[5] The absence of AQPs 1, 3, and 4 in type I pneumocytes or on the apical surface of airway epithelial cells suggests the presence of another aquaporin in those locations. Recently, the complementary DNA (cDNA) for the fifth mammalian aquaporin (AQP5) was isolated from rat; Northern blot analysis demonstrated AQP5 in rat salivary and lacrimal glands, cornea, and lung.[6] Herein we discuss the isolation and characterization of the human *AQP5* cDNA and gene, as well as the ontogeny and distribution of AQP5 in rat lung.

Using the rat *AQP5* cDNA as a molecular probe, an *AQP5* cDNA clone was isolated from a human submaxillary gland library. The 1,348-base pairs (bp) insert contained a 795-bp open reading frame encoding a 265 amino acid protein with 91% homology to rat *AQP5*.[8] Expression of human *AQP5* in *Xenopus laevis* oocytes conferred mercurial-sensitive osmotic water permeability equivalent to other aquaporins. A 7.4-kilobase *Sal* I-*Eco*RI fragment from a human genomic library was found to contain the human *AQP5* structural gene. Nucleotide sequencing with primers corresponding to the human *AQP5* cDNA identified four exons separated by introns of 1.2, 0.5, and 0.9 kilobases. Analysis of the 5′-flanking region determined *AQP5* to be a TATA-less gene with a transcription initiation site 518 bp upstream of the initiating methionine. Genomic Southern blot analysis confirmed *AQP5* to be a single copy gene that localized to human chromosome 12q13; this coincides with the chromosomal locations of the homologous human genes *MIP*[9] and *AQP2*,[10] thus demonstrating the site of an aquaporin gene cluster.

Recently the ontogeny, distribution, and steroid-induced expression of AQP1 in rat lung was described, implicating AQP1 in the clearance of water in both the perinatal and adult rat lung.[4] The absence of AQP1 in type I or type II pneumocytes suggested the existence of another water channel in that location. To determine the ontogeny of AQP5 in lung, membranes from fetal and postnatal rat lungs were immunoblotted with affinity-purified anti-AQP5 antibody. In contrast to the perinatal induction of AQP1, AQP5 was expressed in rat lung 1 to 2 days after birth, with protein levels steadily increasing into adulthood.[11] While AQP1 expression was induced in both perinatal and adult rat lung by corticosteroids,[4] AQP5 was not induced in rat lung at any age. Immunoelectron microscopic localization demonstrated AQP5 in the apical membrane of type I pneumocytes; AQP5 was not present in type II pneumocytes, airway epithelium, or vascular structures.

These studies have defined the gene structure for *AQP5* and localized the cell-specific expression of AQP5 protein in alveolar epithelium. Characterization of the *AQP5* gene structure should aid future studies directed at defining the promoter-specific elements required for *AQP5* gene regulation during normal development and in clinical disorders of respiratory tissue. The abundance of AQP5 on the apical surface of type I pneumocytes suggests a role in alveolar transmembrane water movement, though the lack of a subapical or basolateral location for AQP5 may indicate that other as yet undefined aquaporins may participate in the movement of water across the alveolar epithelium. Additional studies aimed at addressing this issue and determining the precise localization of AQP5 in upper airway epithelium are currently underway.

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Role of the Epithelial Sodium Channel in Lung Liquid Clearance*

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Fetal lung secretes into the future airspaces fluid that arises from a continuous active secretion of CI− by the lung's epithelium. At birth, the lungs rapidly transform into a fluid (Na^+)-absorbing organ to enable efficient gas exchange. Although involvement of an active transepithelial Na^+ transport in the lung during this transition to an air-filled organ has been demonstrated,[1,2] the importance and the role of the amiloride-sensitive epithelial sodium channel (ENaC) in these processes has not been established. In preterm rat[3] and human[4] lung, ENaC messenger RNA transcripts are barely detectable, but expression levels increase considerably in the perinatal period and remain high throughout adult life,[3] suggesting a role for ENaC in maintaining a "dry" alveolar state postnatally. An amiloride-sensitive electrogenic Na^+ reabsorption has been documented in upper and lower airways, but it was not established whether this sodium transport is mediated by ENaC *in vivo*.

The ENaC plays an important role in determining the final amount of sodium reabsorbed. ENaC is localized in the apical membrane of tight epithelia lining the distal part of the nephron, in the distal colon, in the ducts of several exocrine glands, and in the alveolar cells of the lung.[5] ENaC is a heteromultimeric protein made up of three homologous subunits (α, β, and γ).[6] Assembly and expression of functional active sodium channels *in vitro* are strictly dependent on the subunit αENaC, the β and γ subunits being unable, by themselves, to induce an amiloride-sensitive sodium current.

To elucidate the role of ENaC *in vivo*, we inactivated the mouse αENaC gene locus by gene targeting in mouse embryonic stem cells (ES).[7] At birth, the genotypic analysis of newborn animals from heterozygous mutant breeding pairs revealed that embryonic and fetal development in αENaC(−/−) animals was not significantly impaired. Whereas most newborns developed a normal breathing pattern after birth, αENaC(−/−) neonates developed respiratory distress and died within 40 h after birth. To evaluate the presence of αENaC-mediated electrogenic Na^+ transport in the neonatal lung, potential difference (PD) was measured across the epithelia in cultured tracheal cysts derived from freshly excised lungs. Mean baseline PD of αENaC−/− explants was lower than in cysts from +/+ or +/− airway epithelium, and in αENaC−/− animals, amiloride-sensitive electrogenic Na^+ transport was completely abolished.

Histologic analysis showed no differences in lung architecture or alveolar epithelial differentiation between αENaC−/− mice and control littermates, but whole lobes were filled with liquid and air was present in a few subpleural alveoli of αENaC−/− mice. This was confirmed by measuring whole lung wet-to-dry weight ratio (W/D), a parameter that reflects water content of the lung. The ratios of fetal lungs were similar in all three genotypes. Fifteen minutes after birth, the values of wild-type and heterozygous mutant mice were intermediate, reaching values similar to those of adult mouse lungs or 4 to 6 h after birth, indicating that the remaining fetal lung liquid had been removed. Contrarily, in αENaC knockout mice, the W/D values decreased slightly from fetal values, with no further clearance by 4 to 6 h and even higher W/D at 10 to 14 h, suggesting that liquid was reaccumulating in those lungs.

In this study, we present evidence of a direct relationship between the absence of ENaC function and neonatal death due to a defective lung liquid clearance. We propose that active Na^+ transport across the newborn alveolar epithelium, mediated by ENaC, is a critical factor in the successful adaptation of the newborn to air breathing, and immaturity of this pathway may contribute to the etiology of respiratory diseases associated with preterm delivery.

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