水管道蛋白於人體肺組織與細胞株之表現及調控

Expression And Regulation of Water Channel Proteins In Human Lung Tissues and Cell Lines

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一、中文摘要

關鍵詞：水管道蛋白，人體肺組織與細胞株，基因表現及調控

近年來細胞膜水管道(water channel)**的發現可說對細胞膜水滲透之分子機制提供了新的解釋與研究方向。水管道是一膜蛋白族群，又稱為** Aquaporins (AQP)**，目前在老鼠已發現有八種水管道蛋白**(AQP1**至**AQP8)**，人類水管道基因在人體肺部組織之分部及表現仍未完全清楚。吾人推測水管道蛋白或許與肺癌惡性胸水之產生及某些** cytokines, **如** Interleukin-2 (IL-2)**等導致肺水腫之機制有關。此研究之目的在於應用分子生物學之技術檢視水管道蛋白在人體肺組織及各種呼吸道細胞株之表現。並進一步探討類固醇及**IL-2**是否可誘導其表現。我們選擇** AQP1~5 **基因設計** PCR primer**。在細胞株方面，抽取**RNA**再經** RT-PCR**，再經**Northern blot**。在正常呼吸道上皮細胞株**(BSF-2)**及肺腺癌細胞株**(A549**，**CRL-5806**，**CRL-5807)**以及扁平癌細胞株**(HTB58)**中只發現**A549**細胞有明顯**AQP3**之表現。**AQP3**基因可受類毋醇**(dexamethasone 10^{-5} M 24 hr) **而調控其表現，但在**IL-2 (1 nM 8 hr)**刺激下則無明顯反應。在組織分部方面，我們收集肺切除手術或胸水病患所得之肺部及肋膜切片組織，運用水管道蛋白之多株抗體用於組織染色。發現** AQP1**在肺部各部位都有表現，**AQP3 **在遠端係支氣管無表現，**AQP5**則存於遠端係支氣管以下之肺組織。

二 、英文摘要

Keywords: Aquaporin, Water channels, Human lung tissues and cell line, Gene expression, Tissue distribution

Recent discovery of water channel proteins (aquaporins, AQP) has provided insight into the molecular mechanism of water transport across the cell membrane. Nevertheless, tissue distribution and the pathophysiology of these proteins in lung disorders remain unclear. This study applied techniques in molecular biology to screen human lung cell lines for the expression of known aquaporin genes, and also investigated tissue distribution of aquaporins in adult human lung and pleura by immunohistochemistry. Lung tissue sections, obtained during lobectomy, were analyzed by immunohistochemistry and immunoblot with aquaporin antibodies. Expression of AQP3 mRNA and protein is identified in A549 cells. No other cell lines of human airway epithelium and pleura in our laboratory were positive for other AQP genes. AQP3 mRNA expression in the dexamethasone-treated group (10^{-5} M for 8 h) increased to approximately five times the level of that in the control group but did not change significantly after incubation with IL-2 (1

nM) for 8 hrs.
Immunohistochemistry and Western blotting revealed that AQP1 is expressed in all portions of the lung, AQP3 is less strongly expressed in trachea and not detectable in distal lung. AQP5 is expressed in distal lung. Data obtained from his study might provide the basis for future study regarding the regulation and pathophysiology of aquaporins in human lung water homeostasis.

三、緣由與目的

Certain biological membranes of the erythrocyte and some epithelial cells have a high osmotic water permeability (Reizer, 1993), and such membranes have long been suspected of harboring water channels. These channels are small hydrophobic membrane proteins (called “aquaporins”) which were only recently discovered (Verkman, 1993; Agre, 1993). Up to now, eight water channels (aquaporins) have been identified and cloned in rat. Expression patterns of aquaporins in organs or tissues are quite different from one another. The distribution of AQPs in human lung tissues is not completely known (King, 1996), and the pathways and molecular mechanisms for water transport across lung epithelia or pleura have not been identified. In situ hybridization and quantitative immunoblot have demonstrated high abundance of AQP1 and AQP5 in rat alveolar epithelium (Nasegawa, 1993). The purposes of this study was to screen human airway and pleural epithelial cell lines for the expression of known aquaporin genes, and to investigate their distribution in adult human lung and pleura tissues.

四、材料及方法(Subjects and Methods)

Aquaporin expression in human airway epithelial and pleural cell lines1.

1. Cell line derived from normal bronchial epithelial cells: BSF-2
2. Cell lines derived from adenocarcinoma: A549, CRL-5806, CRL-5807, PC-9, PC13, PC14, H2981
3. Cell line derived from squamous cell carcinoma: HTB58
4. Cell line derived from pleural mesothelial cells: MET-5A

The cells were plated at a density of 10^5 to 10^6 cells/ml in culture dishes and then incubated at 37^0C in 5% CO_2 in appropriate medium (DMEM or RPMI) with fetal bovine serum (FBS) and gentamycin. Cells were used when they reached a near-confluent state. Total RNA was isolated from the cultured cells using RNAzol B solution. RNA was precipitated from the aqueous phase with 1 vol isopropanol at -70^0C, and washed with 75% ethanol and dissolved in ribonuclease-free water. One μg of total RNA was reverse transcribed and amplified by polymerase chain reaction (PCR) with five sets of AQP gene primers. The sequences of each primer were:
AQP1:
Sense 5’-ATCATCGCCCAGTGCGTG-3’,
Antisense: 5’-GTAGATGAGTACAGCCAG-3
AQP2
Sense: 5’-ATGAGATCACGCCAGCAG-3’
Antisense: 5’-AGTGACGACAGCTGGAGC-3’;
AQP3
Sense: 5’-TGCCTGGGGACCCTCATC-3’
Antisense: 5’-GATCATATCCAAGTGTCC-3’
AQP4
Sense: 5’-GCAGGAATCCTCTATCTG-

3’
Antisense 5’-TTCAACATCTGGACAGAA-3’
AQP5
Sense: 5’-GGTGTGGCACCGCTCAAT-3’
Antisense 5’-ACTCAGGCTCAGGGAGTT-3’
The PCR was carried out in the following profile: 94^{0}C for 1 min, 56^{0}C for 1 min, and 72^{0}C for 1 min (30 cycles) and adjusted for each AQP for the optimal condition. PCR products were resolved on a 2% agarose gel and then sequenced using an automated fluorescence DNA sequencer (Applied Biosystems).

Northern blot analysis. Cells were cultured in DMEM without FBS in 90-mm culture dish. After 24 h, cells were treated with dexamethasone (10^{-5} M) for 24 h. or IL-2 (1 nM) for 8 hr. At the end of incubation, total RNA was isolated. Ten μg of total RNA was separated by electrophoresis on a 1% agarose gel containing formaldehyde and 3-(N-morpholino) propanesulfonic acid. The RNA was transferred onto nylon membranes with 10 x SSC and was cross-linked by ultraviolet light. The specific cDNA probe and that for GADPH was labeled by random priming using [^{32}P]dCTP (Amersham). After prehybridization for 4 h, the membrane was hybridized for 24 h at 42^{0}C in 50% formamide, 5 x sodium chloride-sodium phosphate-EDTA, 5x Denhardt's solution, 0.16% SDS, and 20 μg/ml salmon sperm DNA. The membrane was washed two times for 20 min at 65^{0}C in 0.2x SSC and 0.1% SDS and then exposed to a film for 24-72 h at -70^{0}C. Autoradiograms was analyzed by densitometric scanning. AQP mRNA expression was shown relative to the GAPDH band measured in the same lane by quantitative densitometry.

Tissue procurement: Lung and pleura specimens from pediatric and adult patients undergoing lobectomy. Tissues were isolated from several regions; distal lung, right mainstem bronchus and perihilar lung tissue, and trachea. Membranes from each of these sections were electrophoresed into SDS-PAGE gels and analyzed by immunoblot with aquaporin antibodies. Immunoblot procedures were similar as those listed as in Part A.

Immunohistochemistry: Tissue were fixed by immersion in PBS containing 4% paraformaldehyde for 4 h, cryoprotected overnight with PBS containing 30% sucrose, embedded in OCT compound, and frozen in liquid N_2. Cryostat sections were mounted on microscope slides, which were incubated for 10 min with PBS containing 1% BSA and then with anti-AQP antibodies at 25^{0}C in PBS containing 1% BSA. Antibody-primed slides were incubated for 30 min with horseradish peroxidase-conjugated second antibodies. Peroxidase activity was visualized by reaction with diaminobenzidine (DAB) for 20 min. Slides were counterstained with hematoxylin and photographed.

四、結果與討論

Expression of AQP3 mRNA and protein was identified in A549 cells, with a 1.8 kb product after RT-PCR. AQP3 gene in A549 cells. cDNA and the promoter region of AQP3 in A549 cells were sequenced for comparison with those in the normal human genome. The sizes of the PCR products representing cDNA and the promoter region of AQP3 were identical in both A549 cells and the normal human genome. No other cell lines of human airway epithelium and pleura in our laboratory were positive for other AQP genes. AQP3 mRNA expression in

the dexamethasone-treated group (10^{-5} for 24 h) increased to about five times the level of the control group but did not not change significantly after incubation with IL-2 (1 nM) for 8 hrs.
Immunohistochemistry and Western blotting revealed that AQP1 is strongly expressed in all portions of the lung. AQP3 is weakly positive in the tracheal epithelium, and not detectable in distal lung. AQP5 is expressed in distal lung, perihilar region, but is only weakly expressed in trachea. AQP5 was not detected in bronchial surface epithelium.

Characterization of the relevant molecules is essential to understand the regulation of water transport in the respiratory tract. There is growing recognition of the role that lung water can play in modulating airway obstruction (Boucher, 1995). We did not identify any of the known water channels in airway epithelial cell lines except for the presence of AQP3 in A549 cells. This is somewhat unexpected, and might indicate that either a still unknown aquaporin resides or another mechanism may be responsible for water transport at this location. It is still speculative that AQP expression is altered in malignant pleural effusion. A549 cell line is originally derived from human adenocarcinoma, which is frequently associated with malignant pleural effusion. Our data are similar to those in a recent report demonstrating the ability of steroid to induce AQP3 expression in A549 cells. Failure of IL-2 to up-regulate the gene expression of AQP3 indicated that AQP3 expression might not be important in the pathogenesis of pleural effusion associated with capillary leak syndrome induced by IL-2 immunotherapy.

By immunohistochemistry, other studies also showed abundance of AQP1 in the peribronchial vasculature (King, 1995), providing insight into the location and regulation of lung fluid secretion. AQP1 in the visceral pleura of rat has also been suggested to mediate fluid transport from the lung into the pleural space (King, 1995). However, we did not observe significant AQP1 staining in the pleura. Whether the protein is lost during the procedure of processing remains unclear. The unique tissue distribution of each AQP in the lung indicates the need for cautious interpretation of the data from function studies in respiratory systems.

五、參考文獻 (References)

Folkesson HG. Matthay MA, Hasegawa H, et al. (1994). Transcellular water transport in lung alveolar epithelium through mercury-sensitive water channels. Proc. Natl. Acad. USA 91:4970-4974.

King LS and Agre P. (1996) Pathophysiology of the aquaporin water channels. Ann Rev Physiol 58:619-648.

King LS. Nielsen S. and Agre P. (1996) Aquaporin-1 water channel protein in lung: ontogeny, steroid-induced expression, and distribution in rat. Journal of Clinical Investigation. 97:2183-2191.
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Raina S. Preston GM. Guggino WB. Agre P.(1995) Molecular cloning and characterization of an aquaporin cDNA from salivary, lacrimal, and respiratory tissues. J Biol Chem 270:1908-1912