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The Biology of Clara Cells -Review Paper

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INTRODUCTION

Clara cells, the nonciliated population in the epithelial lining of bronchioles, are one of the most heterogeneous and multifunctional cell types in the mammalian lung. The best-defined markers for Clara cell differentiation in adult mammals are the ultrastructural features (Table 1).

Clara cells are characterised as low cuboidal calls with minimal apical projections, bound to each other by junctional complexes on the luminal aspects of the basolateral membrane[1,2]. The distribution of organelles within the cytoplasm shows little polarization and includes small amounts (<10%) of rough (RER) and smooth (SER) endoplasmic reticulum, mitochondria and Golgi apparatus. The nucleus is approximately one-third of the total volume of the cells and is centrally placed. The apical portions of the cytoplasm generally contain a small number of ovoid, electron-dense membrane-bound secretory granules. This structural composition has been defined for humans and in three species of macacque monkeys[1].

The ultrastructure of the most of the mammalian species is slightly different from each other and is characterised by:

- Extensive apical projections into the airways
- A polarized organisation of organelles

In most species there is an abundance of apical smooth endoplasmic reticulum. In mouse, hamster, rat, guinea pig rabbit, pig, sheep and horse more than 40% of the cytoplasmic volume consists of smooth endoplasmic reticulum[3,7].

Clara cell functions: Clara cells have at least four roles in the normal lung function:

- Contribute a secretion to the extracellular lining fluid
- Progenitor cells for both themselves and for ciliated cells
- Contain a variety of cytochrome p-450 monooxygenases that have an active role in metabolism of xenobiotics.
- Regulate fluid balance in the distal conducting airways

Table 1: Comparison of volumes (μm^3) of cellular components in Clara cells adapted from[1]

Component	Rabbit	Cat	Bonnet monkey
Cell	471.8±51.8	496.1±40.8	418.9±36.1
Nucleus	83.0±7.0	132.5±45.6	119.9±18.4
SER	213.4±51.0	38.9±24.4	15.6±9.9
Glycogen	37.5±13.1	222.9±36.4	0
RER	25.8±19.4	1.56±3.38	38.9±1.2
Mitochondria	75.4±17.7	70.9±34.9	42.0±8.3
Granules	12.5±6.7	0	6.3±1.4
Golgi apparatus	5.7±5.7	14.6±13.1	24.1±2.8

Secretion products: Clara cells secrete a number of proteins including surfactants SP-A, B and D, Clara cell 10-kDa protein/uteroglobin, leukocyte protease inhibitors, β-galactoside binding lectin and a trypsin-like protease[2,8,9]. It was proposed that Clara cell section is both aprocrine and merocrine, the former prodominating[8].

Clara cells 10-kDa protein (CC10) is the predominant product of Clara cells and is distributed mainly in the bronchiole[10,11]. The protein has also been referred to as Clara cell 16-kDa protein according to results using electrospray/mass spectrometry. This protein was first identified in urine of patients with renal failure and purified later from lung lavage[12,14]. The entire human CC16 gene has been sequenced and localised to chromosome 11, p12-q13, a region occupied by several genes involved in the regulation of allergy and inflammation[12]. Human CC10 is identical to human Urinary Protein 1 (UP1) and Human Uteroglobin (UG)[11,15].

Changes in CC10 levels in sera and bronchoalveolar lavage (BAL) fluid have been reported in various lung diseases and in patients exposed to different toxins including cigarette smoking[15,19]. Although the function of CC10 is unclear, it may play a role in regulation of inflammation.

Surfactant proteins and the mRNA for SP-B have been localised in the Clara cells[20]. The detection of SP-B

in Clara cells is more consistent than that of SP-A. SP-A and SP-D are believed to be involved in host defence against micro-organisms by their lectin like activity[21]. The role of the surfactant proteins secreted by Clara cells is still unclear[2,19].

The 29-kDa β-galactoside binding lectin may be the same as the 30-kDa Clara cell tryptase, but their function and physiological role is still not clear. Clara cell tryptase has been shown to cleave haemagglutinin and activate infectivity of influenza A virus[22,24].

The leukocyte protease inhibitor has been immuno-histochemically localised to Clara cells. This protein inhibits leukocyte proteases, especially elastase[25].

Progenitor cells: Clara cells are self replicative but also terminally differentiate into ciliated cells in the bronchioles[1,26,28]. Clara cells isolated from the lungs of rabbit were shown to be able to re-populate denuded tracheas[29]. Clara cells were shown not to be necessary for pulmonary neuroendocrine cell hyperplasia[19].

Metabolism of xenobiotics: Among the other epithelial cells found in the lung epithelial bronchiolar region, Clara cells are distinguished for their ability to metabolise xenobiotics via the cytochrome P-450 (CYP) monooxygenase system and flavin-containing monooxygenases[30,32]. Bronchiolar Clara cells are considered to be one of the principal targets in the mammalian lung pulmonary toxicants. These include a wide variety of compounds including furans, chlorinated hydrocarbons and aromatic hydrocarbons. Many compounds injure Clara cells in most of the species but Clara cells in different species do not have the same level of sensitivity to any one compound[6,26,33].

Changes in Clara cells are manifested as early as 1 hour after exposure and involve clumping and margination of nuclear chromatin, mitochondrial swelling, dilation of endoplasmic reticulum membrane and disruption of cell junctions. Within 24 h of exposure the injury there is cellular enlargement and formation of large numbers of membrane-bound vacuoles[31,33,34].

There are a large number of studies showing the ability of Clara cells to metabolise a number of xenobiotics including naphthalene and its metabolites[35,40], ozone[18], styrene and its compounds[41,42], benzo (α) pyrene and its derivative compounds[43,44,45], cigarette smoke[46,48], coumarin[49,50], methylene chloride[51,52], trichloroethylene[53,54], amines[55] and nitrogen dioxide[56]. Dially sulfone, a derivative of garlic, was found to protect against Clara cell injury caused by 1,1-dichloroethylene by conjugating a glutathione with its reactive metabolites[57].

Regulation of fluid balances: Due to the fact that bronchiolar epithelium has lateral projections, it has been suggested that the airways epithelium of distal conducting airways is involved in re-absorption and clearance of airway-lining fluid[3,58]. It was shown that there is a net movement of sodium ions (Na^+) from the mucosal to the serosal side in cultured monolayers of rabbit Clara cells. This movement is amiloride-sensitive and occurs under short- or open-circuit conditions, thus most likely Clara cells are involved in fluid re-absorption. Chloride ions (Cl^-) secretion was observed to be induced by amiloride, but no net Cl^- movement was observed under basal conditions[60]. Cl^- channels that can be stimulated by c-AMP-activating agents and extracellular ATP were described in Clara cells. These channels share many bio-physiological properties with the Cystic Fibrosis Transmembrane Regulator (CFTR)-related Cl^- channel (1997).

REFERENCES

1. Plopper, C.G., D.M. Hyde and A.R. Buckpitt, 1991. Clara cells. *In* The Lung: Scientific Foundations. R.G.Crystal and West J.B., Eds. Raven Press, New York, USA., 215-228.
2. Massaro, G.D., G. Singh, R. Mason, C.G. Plopper, A.M. Malkinson and D.B. Gail, 1994. Biology of the Clara cell. Am. J. Physiol., 266: L101-L106.
3. Plopper, C.G. and D.L. Dungworth, 1987. Structure, function, cell injury and cell renewal of bronchiolar and alveolar epithelium. In Lung Carcinoma. E.M.Dowell, Editor. Churchill Livingstone, London, UK., pp: 29-44.
4. Plopper, C.G., A.T. Mariassy and L.H. Hill, 1980b. Ultrastructure of the nonciliated bronchiolar epithelial (Clara) cell of mammalian lung: I. A comparison of rabbit, guinea pig, rat, hamster and mouse. Exp. Lung Res., 1: 139-154.
5. Plopper, C.G., A.T. Mariassy and L.H. Hill, 1980c. Ultrastructure of the nonciliated bronchiolar epithelial (Clara) cell of mammalian lung: II. A comparison of horse, steer, sheep, dog and cat. Exp. Lung Res., 1: 155-169.
6. Plopper, C.G., J. Macklin, S.J. Nishio, D.M. Hyde and A.R. Buckpitt, 1992a. Relationship of cytochrome P-450 activity to Clara cell cytotoxicity. III. Morphometric comparison of changes in the epithelial populations of terminal bronchioles and lobar bronchi in mice, hamsters and rats after parenteral administration of naphthalene. Laboratory Investigation, 67: 553-565.
7. Plopper, C.G., L.H. Hill and A.T. Mariassy, 1980a. Ultrastructure of the nonciliated bronchiolar epithelial (Clara) cell of mammalian lung III. A study of man with comparison of 15 mammalial species. Exp. Lung Res., 1: 171-180.

8. Peão, M.N.D., A.P. Águas, C.M. De Sá and N.R. Grande, 1993. Anatomy of Clara cell secretion: surface changes observed by scanning electron microscopy. J. Anatomy, 183: 377-388.
9. Cardoso, W.V., L.G. Stewart, K.E. Pinkerton, C. Ji, G.E.R. Hook, G. Singh, S.L. Katyal, W.M. Thurlbeck and C.G. Plopper, 1993. Secretory product expression during Clara cell differentiation in the rabbit and rat. Am. J. Physiol., 264: L543-L552.
10. Asabe, K., K. Tsuji, N. Handa, M. Kajiwara and S. Suita, 1998. Expression of clara cell 10-kDa protein (CC10) in congenital diaphragmatic hernia. Pediatric Surgery International, 14: 36-39.
11. Sagal, R.G. and A. Nieto, 1998. Molecular cloning of the cDNA and the promoter of the hamster Uteroglobin/Clara cell 10-kDa Gene (ug/cc10): Tissue-specific and horminal regulation. Archives of Biochem. Biophysics, 350: 214-222.
12. Hermans, C., O. Aly, B.I. Nyberg, C. Peterson and A. Bernard, 1998a. Determinants of Clara cell protein (CC16) concentration in serum: A reassessment with two different immunoassays. Clin. Chimica. Acta., 272: 101-110.
12. Broeckaert, F. and A. Bernard, 2000. Clara cell secretory protein (CC16): Characteristics and perspectives as lung peripheral biomarker. Clin. Exp. Allergy, 30: 469-475.
13. Hermans, C., O. Lesur, B. Weynand, T. Pieters, M. Lambert and A. Bernard, 1998b. Clara cell protein (CC16) in pleural fluids: A marker of leakage through the visceral pleura. Am. J. Respiratory and Critical Care Medicine, 157: 962-969.
14. Laing, I.A., C. Hermans, A. Bernard, P.R. Burton, J. Goldblatt and P.N. Le Souëf, 2000. Association between plasma CC16 levels, the A38G polymorphism and Asthma. Am. J. Respiratory and Critical Care Medicine, 161: 124-127.
15. Reynolds, S.D., G.W. Mango, R. Gelein, I.M. Boe, J. Lund and B.R. Stripp, 1999. Normal function and lack of fibronectin accumulation in kidneys of Clara cell secretory protein/uteroglobin deficient mice [see comments]. Am. J. Kidney Dis., 33: 541-551.
16. Lesur, O., A. Bernard, K. Arsalane, R. Lauwerys, R. Bégin, A. Cantin and D. Lane, 1995. Clara cell protein (CC-16) induces a phosphlipase A_2-mediated inhibition of fibroblast migration *in vitro*. Am. J. Respiratory andCare Medicine, 152: 290-297.
17. Johnston, C.J., B.R. Stripp, B. Piedbeouf, T.W. Wright, G.W. Mango, C.K. Reed and J.N. Finkelstein, 1998. Inflammatory and epithelial responses in mouse strains that differ in sensitivity to hyperoxic injury. Exp. Lung Res., 24: 189-202.
18. Mango, G.W., C.J. Johnston, S.D. Reynolds, J.N. Finkelstein, C.G. Plopper and B.R. Stripp, 1998. Clara cell secretory protein deficiency increases oxidant stress response in conducting airways. Am. J. Physiol. 275: L348-L356.
19. Margana, R.K. and V. Boggaram, 1997. Functional analysis of surfactant protein B (SP-B) promoter. Sp1, Sp3, TTF-1 and HNF-3alpha transcription factors are necessary for lung cell-specific activation of SP-B gene transcription. J. Biol. Chem., 272: 3083-3090.
19. Reynolds, S.D., K.U. Hong, A. Giangreco, G.W. Mango, C. Guron, Y. Morimoto and B.R. Stripp, 2000. Conditional Clara cell ablation reveals a self-renewing progenitor function of pulmonary neuroendocrine cells. Am. J. Physiol., 278: L1256-L1263.
20. Phelps, D.S. and J. Flores, 1991. Localisation of pulmonary surfactant proteins using immunohistochemistry and tissue in situ hybridization. Exp. Lung Res., 17: 985-995.
21. Kuan, S.F., K. Rust and E. Crouch, 1992. Interactions of surfactant protein D with bacterial lipopolysaccharides. Surfactant protein D in an *Escherichia coli*-binding protein in bronchoalveolar lavage. J. Clin. Investigation, 90: 97-106.
22. Tashiro, M., Y. Beppu, K. Sakai and H. Kido, 1996. Inhibitory effect of pulmonary surfactant on Sendai virus infection in rat lungs. Archives of Virology, 141: 1571-1577.
23. Kohri, T., K. Sakai, T. Mizunuma and Y. Kishino, 1996. Levels of pulmonary surfactant protein A in fetal lung and amniotic fluid from protein-malnourished pregnant rats. J. Nut. Sci. Vitaminol., 42: 209-218.
24. Kido, H., Y. Beppu, K. Sakai and T. Towatari, 1997. Molecular basis of proteolytic activation of Sendai virus infection and the defensive compounds for infection. Biol. Chem., 378: 255-263.
25. De Water, R., L.N.A. Willems, G.N.P. Van Muijen, C. Franken, J.A.M. Fransen, J.H. Dijkman and J.A. Kramps, 1986. Ultrastructural localization of bronchial antileukoprotease in central and peripheral human airways by a gold-labeling techique using monoclonal antibodies. Am. Rev. Respiratory Dis., 133: 882-890.
26. Plopper, C.G., S.J. Nishio, A.P. Kass and D.M. Hyde, 1992b. The role of the noncilated bronchiolar epithelial (Clara) cell as the progenitor cell during bronchiolar epithelial differentiation in the perinatal rabbit lung. Am. J. Cell and Molecular Biol., 7: 606-613.

27. Plopper, C.G., C. Suverkropp, D. Morin, S. Nishio and A. Buckpitt, 1992c. Relationship of cytochrome P-450 activity to Clara cell cytotoxicity. I. Histopathologic comparison of the respiratory tract of mice, rats and hamsters after parenteral administration of naphthalene. The J. Pharmacol. Exp. Therapeutics, 261: 353.
28. Ji, C.M., C.G. Plopper and K.E. Pinkerton, 1995. Clara cell heterogeneity in differentiation: Correlation with proliferation, ultrastructural composition and cell position in the rat bronchiole. Am. J. Respiratory Cell and Molecular Biol., 13: 144-151.
29. Hook, G.E., A.R. Brody, G.S. Cameron, A.M. Jetten, L.B. Gilmore and P. Nettesheim, 1987. Repopulation of demuded tracheas by Clara cells isolated from the lungs of rabbits. Exp. Lung Res., 12: 311-329.
30. Buckpitt, A., M. Buonarati, L.B. Avey, A.M. Chang, D. Morin and C.G. Plopper, 1992. Relationship of cytochrome P450 activity to Clara cell cytotoxicity. II. Comparison of stereoselectivity of naphthalene epoxidation in lung and nasal mucosa of mouse, hamster, rat and rhesus monkey. J. Pharmacol. Exp. Therapeutics, 261: 364-372.
31. Lakritz, J., A. Chang, A. Weir, S. Nishio, D. Hyde, R. Philpot, A. Buckpitt and C. Plopper, 1996. Cellular and metabolic basis of Clara cell tolerance to multiple doses of cytochrome P450-activated cytotoxicants. I: Bronchiolar epithelial reorganization and expression of cytochrome P450 monooxygenases in mice exposed to multiple doses of naphthalene. The J. Pharmacol. Exp. Therapeutics, 278: 1408-1418.
32. Watt, K.C., C.G. Plopper, A.J. Weir, B. Tarkington and A.R. Buckpitt, 1998. Cytochrome P450 2E1 in rat tracheobronchial airways: Response to ozone exposure. Toxicol. Applied Pharmacol., 149: 195-202.
33. Buckpitt, A., A.M. Chang, A. Weir, L. Van Winkle, X. Daun, R. Philpot and C. Plopper, 1995. Relationship of cytochrome P450 activity to Clara cell cytotoxicity. IV Metabolism of naphthalene and napthalene oxide in microdissected airways from mice, rats and hamsters. Molecular Pharmacol., 47: 74-81.
34. Van Winkle, L.S., A.R. Buckpitt and C.G. Plopper, 1996. Maintenance of differentiated murine Clara cells in microdissected airway cultures. Am. J. Cell and Molecular Biol., 14: 586-598.
35. Sauer, J.M., R.R. Eversole, C.L. Lehmann, D.E. Johnson and L.J. Beuving, 1997. An ultrastructural evaluation of acute 1-nitronaphthalene induced hepatic and pulmonary toxicity in the rat. Toxicol. Lett., 90: 19-27.
36. Fanucchi, M.V., A.R. Buckpitt, M.E. Murphy and C.G. Plopper, 1997. Naphthalene cytotoxicity of differentiating Clara cells in neonatal mice. Toxicol. Applied Pharmacol., 144: 96-104.
37. Van Winkle, L.S., J.M. Isaac and C.G. Plopper, 1997. Distribution of epidermal growth factor receptor and ligands during bronchiolar epithelial repair from naphthalene-induced Clara cell injury in the mouse. Am. J. Pathol. 151: 443-459.
39. Zheng, J., M. Cho, A.D. Jones and B.D. Hammock, 1997. Evidence of quinone metabolites of naphthalene covalently bound to sulfur nucleophiles of proteins of murine Clara cells after exposure to naphthalene. Chem. Res. Toxicol., 10: 1008-1014.
40. Paige, R., V. Wong and C. Plopper, 1997. Dose-related airway-selective epithelial toxicity of 1-nitronaphthalene in rats. Toxicol. Applied Pharmacol., 147: 224-233.
41. Male, R., J.R. Lillehaug, R. Djurhuus and I.F. Pryme, 1985. *In vitro* transformation and tumor promotion studies of styrene and styrene oxide. Carcinogenesis, 9: 1367-1370.
42. Cruzan, G., J.R. Cushman, L.S. Andrews, G.C. Granville, R.R. Miller, C.J. Hardy, D.W. Coombs and P.A. Mullins, 1997. Subchronic inhalation studies of styrene in CD rats and CD-1 mice. Fundamental and Applied Toxicology, 35: 152-165.
43. Boutin, A.C., P. Shirali, G. Garçon, P. Gosset, B. Leleu, T. Marez, A. Bernard and J.M. Haguenoer, 1998. Peripheral markers (Clara cell protein and α-glutathione S-transferase) and lipidoperoxidation (Malondialdehyde) assessment in Sprague-Dawley rats instilled with haematite and benzo(α)pyrene. J. Applied Toxicol., 18: 39-45.
44. Wu, X., J. Gu, C.I. Amos, H. Jiang, W.K. Hong and M.R. Spitz, 1998. A parallel study of *In Vitro* sensitivity to Benzo(a)pyrene diol expoxide and Bleomycin in lung carcinoma cases and controls. Cancer, 83: 1118-1127.
45. Jyonouchi, H., S. Sun, K. Iijima, M. Wang and S.S. Hecht, 1999. Effects of anti-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[α]pyrene on human small airway epithelial cells and the protective effects of *myo*-inositol. Carcinogenesis, 20: 139-145.
46. Ji, C.M., C.G. Plopper, H.P. Witschi and K.E. Pinkerton, 1994. Exposure to sidestream cigarette smoke alters bronchioles epithelial cell differentiation in the postnatal rat lung. Am. J. Respiratory Cell and Molecular Biol., 11: 312-320.
47. Gebremichael, A., A.M. Chang, A.R. Buckpitt, C.G. Plopper and K.E. Pinkerton, 1995. Postnatal development of cytochrome P4501A1 and 2B1 in rat lung and liver: Effect of aged and diluted sidestream cigarette smoke. Toxicol. Applied Pharmacol., 135: 246-253.

48. Ji, C.M., F.H. Royce, U. Truong, C.G. Plopper, G. Singh and K.E. Pinkerton, 1998. Maternal exposure to environmental tobacco smoke alters Clara cell secretory protein expression in fetal rat lung. Am. J. Physiol., 275: L870-L876.
49. Born, S.L., A.S. Fix, D. Caudill and L.D. Lehman-McKeeman, 1998. Selective Clara cell injury in mouse lung following acute administraction of Coumarin. Toxicol. Applied Pharmacol., 151: 45-56.
50. Born, S.L., A.S. Fix, D. Caudill and L.D. Lehman-McKeeman, 1999. Development of tolerance to Clara cell necrosis with repeat administration of coumarin. Toxicol. Sci., 51: 300-309.
51. Foster, J.R., T. Green, L.L. Smith, S. Tittenson and I. Wyatt, 1994. Methylene chloride: An inhalation study to investigate toxicity in the mouse lung using morphological, biochemical and Clara cell culture techniques. Toxicology, 91: 221-234.
52. Green, T., 1997. Methylene chloride induced mouse liver and lung tumours: an overview of the role of mechanistic studies in human safety assessment. [Review] [60 refs]. Human and Exp.Toxicol., 16: 3-13.
53. Green, T., G.W. Mainwaring and J.R. Foster, 1997. Trichloroethylene-induced mouse lung tumors: Studies of the mode of action and comparisons between species. Fundamental and Applied Toxicology, 37: 125-130.
54. Giovanetti, A., L. Rossi, M. Mancuso, C.C. Lombardi, M.R. Marasco, F. Manna, P. Altavista and E.M. Massa, 1998. Analysis of lung damage induced by trichloroethylene inhalation in mice fed diets with low, normal and high copper content. Toxicol. Pathol., 26: 628-635.
55. Masek, L. and R.J. Richards, 1990. Interactions between paraquat, endogenous lung amines' antioxidants and isolated mouse Clara cells. Toxicology, 63: 315-326.
56. Evans, M.J., S.G. Shami, L.J. Cabral-Anderson and N.P. Dekker, 1986. Role of nonciliated cells in renewal of the bronchial epithelium of rats exposed to NO_2. Am. J. Pathol., 123: 126-133.
57. Forkert, P.G., A.M. Malkinson, P. Rice and M. Moussa, 1999. Diminished CYP2E1 expression and formation of 2-S-glutathionyl acetate, a glutathione conjugate derived from 1,1-dichloroethylene epoxide, in murine lung tumors. Drug Metabolism and Disposition, 27: 68-73.
58. Van Scott, M.R., S. Hester and R.C. Boucher, 1987. Ion transport by rabbit nonciliated bronchiolar epithelial cells (Clara cells) in culture. Proceeding of the National Academy of Science, U.S.A., 84: 5496-5500.
59. Van Scott, M.R., C.W. Davis and R.C. Boucher, 1989. Na^+ and Cl^- transport across rabbit nonciliated bronchiolar epithelial (Clara) cells. Am. J. Physiol., 256: 893-901.