

Invited Review

In favour of an oncofoetal concept of bronchogenic carcinoma development

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Summary. Our recent studies in a heterotopic model of non-small cell lung cancer in dogs (subcutaneous bronchial autografts treated with 3-methylcholanthrene) have provided evidence that alveolar type II cells may newly arise during initial phases of bronchial carcinogenesis. In the light of these novel findings, which are in agreement with our observations in human non-small cell lung cancer, and in view of present insights into embryonic lung differentiation, we discuss evidence that favours a new, oncofoetal concept of bronchogenic carcinoma development. According to this concept, the primary cells of origin for these tumors are undifferentiated primordial-like cells that derive from bronchial epithelial cells present in major bronchi or their divisions by retrodifferentiation. Such primordial-like cells of origin undergo novel differentiation into the potential (alveolar, bronchial or primordial) tumor stem cells, which occupy the dividing cellular layers of the (pre)neoplastic lesions and constitute the actively dividing and invading part of the neoplasm. Examples of tumors that may originate from alveolar tumor stem cells are carcinomas of the bronchiolo-alveolar, papillary, acinar, and adenoid-cystic types. Squamous cell carcinomas could possibly belong to this group as well, but much more evidence is required to reach conclusions regarding this type of cancer.

We suggest that epithelial retrodifferentiation followed by novel differentiation (oncofoetal mechanism) is fundamental in bronchial carcinogenesis.

Key words: Alveolar type II cells, Bronchial carcinogenesis, Tumor stem (progenitor) cells, Oncofoetal mechanism, Non-small cell lung cancer

Introduction

Expanding knowledge about the cells of origin of bronchogenic carcinomas, and the changes in phenotypic and molecular properties during tumor progression, may contribute to earlier tumor detection and better insight into their clinical behaviour and response to therapy. More knowledge in this area may also provide the basis for insight as to mechanisms of chemoprevention.

At present, there are two different concepts concerning the developmental pathway of the two varieties of bronchogenic carcinomas, namely non-small and small cell lung cancers (NSCLC; SCLC). One of these concepts starts from the idea that bronchogenic carcinomas arise from specific bronchial epithelial cells that are capable of division (McDowell, 1987). In the adult bronchial epithelium, the cells which divide are mucous cells and basal cells (McDowell et al., 1979; Plopper et al., 1986; Inayama et al., 1988, 1989), and possibly also dense-core granulated 'endocrine' cells (McDowell, 1987). Mucous (McDowell, 1987) or basal (Nasiell et al., 1987) cells would therefore be the major progenitors for NSCLC in humans. The role of 'endocrine' cells in the development of SCLC in humans is less clear (McDowell, 1987). According to the same principle, carcinomas of the small peripheral airways would arise from the dividing cells in the distalmost airways, i.e., the columnar secretory (Clara) cells and cuboid alveolar type II cells (Reznik-Schüller and Reznik, 1979; Schüller, 1987).

The second concept, which is frequently put forward in discussions and sometimes also reported (Ten Have-Opbroek et al., 1990a, 1993b), attributes a primary and specific role in bronchogenic carcinoma development to undifferentiated cells that are capable of differentiating in multiple directions. The origin of such cells is not clear. In fact, there are two possibilities: (1) the undifferentiated multipotential cells are residual primitive endodermal cells of the lung anlage, which remain

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present in normal bronchial epithelium (Ten Have-Opbroek, 1981); or (2) the undifferentiated multipotential cells appear during conversion (via metaplasia) of normal bronchial epithelium to bronchogenic carcinoma (Ten Have-Opbroek et al., 1990a, 1993b).

The following is a consideration of altered concepts of bronchogenic carcinoma development in the light of studies that have provided new insights into embryonic lung differentiation (Ten Have-Opbroek, 1981, 1991; Ten Have-Opbroek and Plopper, 1992) and in view of observations in a canine model of NSCLC that have shown that alveolar type II cells may newly arise during initial phases of bronchial carcinogenesis (Ten Have-Opbroek et al., 1990a, 1992, 1993b). There is now reason to suspect that these cells differentiate from undifferentiated cells of origin (see Definitions, below) and function as potential stem cells (see Definitions, below) at least for some varieties of adenocarcinomas (Ten Have-Opbroek et al., 1990a, 1992, 1993b). In this context, we will also discuss the possible significance of observations by us and others regarding the occurrence of type II cells in adenocarcinomas and squamous cell carcinomas in humans and in other species (reviewed by Ten Have-Opbroek et al., 1993b; see also Broers et al., 1992; Linnoila et al., 1992a,b; Ten Have-Opbroek et al., 1993a; Tsutahara et al., 1993). We shall suggest the possibility of an oncofoetal mechanism in bronchial carcinogenesis, and we will present a few explanatory illustrations and a potential oncofoetal pathway of bronchogenic carcinoma development.

Definitions; canine model of NSCLC; cell markers

We reserve the term "cell of origin" to indicate the very first undifferentiated (primordial-like) tumor progenitor cell that appears during conversion (via metaplasia) of normal bronchial epithelium to bronchogenic carcinoma. We use the term "stem cell" to indicate the major epithelial cell type that occupies the dividing cellular layers of the (pre)neoplastic lesions and constitutes the actively dividing and invading part of the neoplasm.

To gain insight into the mechanisms that lead to bronchogenic carcinoma development, we developed a heterotopic model of NSCLC in dogs (Hammond et al., 1986; Derrick et al., 1988; Benfield and Hammond, 1992). In this model, varieties of NSCLC that resemble human NSCLC (Stünzi et al., 1974; The WHO, 1982) are induced in multiple subcutaneous bronchial autografts derived from major bronchi (SBAs) by exposure to 3-methylcholanthrene (MCA) contained in a silicone polymer sustained-release implant. This model allows serial sampling of epithelium during the sequential progression of carcinogenesis from normal bronchial epithelium to invasive NSCLC that metastasizes in autochthonous hosts and in xenotransplant recipients. The canine model was used to get information about a possible stem cell role of alveolar type II cells in bronchial carcinogenesis (Ten Have-

Opbroek et al., 1990a, 1992, 1993b). The materials studied were derived from control and treated SBAs, and included normal bronchial epithelium, preneoplastic lesions, early invasive carcinomas, and overt carcinomas of bronchiolo-alveolar, acinar, and adenoid-cystic types. We also studied transplants of these tumors in nude mice.

For alveolar type II cell detection, use was made of our antibodies to SP-A (indirect immunofluorescence, using a fluorescein-conjugated secondary antibody) and H&E staining in adjacent, formalin-fixed 6 µm sections (Ten Have-Opbroek et al., 1990a, 1992, 1993b), and of transmission electron microscopy (Ten Have-Opbroek et al., 1993b). As will be further explained below, our antibodies to SP-A recognize both mature and immature type II cells in foetal and adult mammalian lungs, based on a highly characteristic staining pattern in phenotypically distinctive type II cells. In this context, it is important to emphasize that SP-A is a very good marker for type II cells. Confusion about its usefulness for type II cell identification has resulted from SP-A mRNA and protein studies in developing and adult lungs that have not sufficiently or not at all considered the morphology of the reactive cell types (Ten Have-Opbroek et al., 1991; Ten Have-Opbroek and Plopper, 1992; Ten Have-Opbroek and De Vries, 1993). These studies have assigned positive SP-A reactivity in bronchiolar epithelium to "Clara cells" without considering that this epithelium contains two categories of secretory cells (Clara, 1937), namely columnar Clara cells and cuboid alveolar type II cells (Ten Have-Opbroek, 1986, 1991; Ten Have-Opbroek et al., 1991; Ten Have-Opbroek and Plopper, 1992; Ten Have-Opbroek and De Vries, 1993; Plopper and Ten Have-Opbroek, 1994). In fact (see below), the presence of SP-A in true Clara cells is limited in terms of species, localization, and age (Ten Have-Opbroek et al., 1991; Ten Have-Opbroek and Plopper, 1992; Ten Have-Opbroek and De Vries, 1993).

Conclusions concerning the cell types of the various lesions were drawn based on the following data. *Type II cells*, irrespective of species, localization (bronchiolar; parenchymal), and stage of lung development (early or late foetal; postnatal; adult) are characterized by *an approximately cuboid shape, a large and roundish nucleus, and staining of the entire cytoplasm with antibodies to surfactant protein A (SP-A)* (Ten Have-Opbroek, 1975, 1979, 1981, 1991; Otto-Verberne and Ten Have-Opbroek, 1987; Otto-Verberne et al., 1988; Oomen et al., 1990; Ten Have-Opbroek et al., 1991; Ten Have-Opbroek and Plopper, 1992; Plopper and Ten Have-Opbroek, 1994). At the ultrastructural level, such cells also display *multilamellar bodies (MLB)* or their *precursory forms* (Otto-Verberne et al., 1988; Ten Have-Opbroek et al., 1988, 1990b; Oomen et al., 1990; Ten Have-Opbroek et al., 1991; Brandsma et al., 1993; Plopper and Ten Have-Opbroek, 1994). Type I cells, which derive from type II cells, lack SP-A when they exhibit their mature (squamous) form (Ten Have-Opbroek, 1981, 1991; Ten Have-Opbroek and Plopper,

1992). Bronchial epithelium is pseudostratified or simple columnar with ciliated and nonciliated secretory (mucous) cells, and basal cells (Ten Have-Opbroek, 1986; Ten Have-Opbroek et al., 1991; Mariassy, 1992). Columnar secretory (Clara) cells present in distal bronchioles, which are first described by Clara (1937), differentiate much more slowly than type II cells and acquire their specific phenotype and functions after birth (Plopper et al., 1983, 1992; Massaro et al., 1984; Ten

Have-Opbroek and De Vries, 1993). In rodents, postnatal and adult Clara cells stain for SP-A, although only apically; the basal cytoplasmic domain, where the rough endoplasmic reticulum is located, is negative (Ten Have-Opbroek, 1991; Ten Have-Opbroek and De Vries, 1993). This staining pattern suggests that SP-A may have been internalized from the air spaces (Ten Have-Opbroek, 1991; Ten Have-Opbroek and De Vries, 1993). In some larger mammals such as dogs, Rhesus monkeys, and

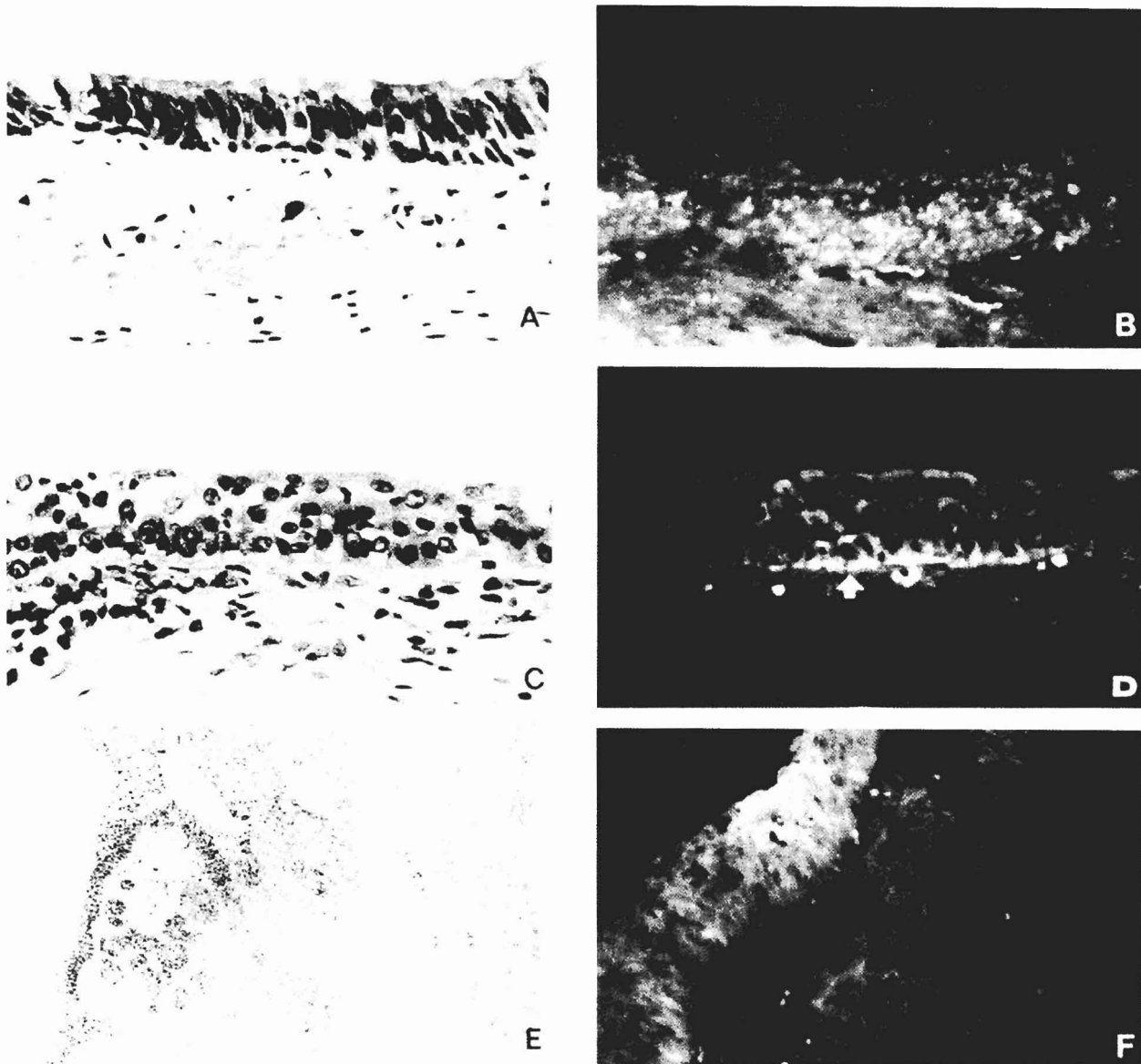


Fig. 1. Immunodetection of alveolar type II cells in apparently normal (untreated, A,B) bronchial epithelium and in abnormal (carcinogen-exposed, C,D; E,F) surface epithelium with early invasive carcinomatous lesions (E,F). The lesions arose from segmental bronchi treated with 3-methylcholanthrene in a heterotopic bronchogenic carcinoma model in dogs. H&E (left) and antibodies to surfactant protein A, SP-A (right; indirect immunofluorescence) in adjacent sections. Formalin fixation. Cells with type II cell characteristics (i.e., cuboid shape; large, roundish nucleus; cytoplasmic staining for SP-A) are never present in normal bronchial epithelium (A,B). After carcinogen exposure, such cells occur in the transformed surface epithelium, i.e., in the hyperplastic basal layer of atypical squamous metaplastic epithelium (C,D arrow), and its early invasive glandular outgrowths (E,F). Nonspecific fluorescence is present in blood vessels (B,D,F) and (adluminal) squamous metaplastic cells (D,F). A,B, x 280; C, x 320; D, x 340; E, x 50; F, x 160

humans, the corresponding columnar cells do not contain SP-A at all (Ten Have-Opbroek et al., 1991, 1993a; Ten Have-Opbroek and Plopper, 1992; Plopper and Ten Have-Opbroek, 1994) but appear to be mucous cells from their ultrastructure (Tyler and Plopper, 1985; Ten Have-Opbroek et al., 1991) and immunoreactivity to anti-mucins (Ten Have-Opbroek et al., 1991; Plopper and Ten Have-Opbroek, 1994).

A representative selection of light microscopic and immunofluorescence findings during bronchial carcinogenesis in the canine NSCLC model is shown below.

Bronchogenic carcinoma development and Alveolar type II cells

As illustrated (Figs. 1A,B), bronchial epithelium from control SBAs never contained cells with morphological and immunocytochemical type II cell characteristics (see above), either when it looked completely normal or displayed some basal hyperplasia. Such cells were also absent from normal bronchial glands (not shown). The fluorescence in capillaries and larger blood vessels in underlying connective tissue was nonspecific (formalin fixation).

Cells with type II cell characteristics (Figs. 1C,D) did, however, occur in the abnormal surface epithelium that had differentiated from normal bronchial epithelium after exposure to MCA in the major bronchi of SBAs. They were found locally in the hyperplastic basal layer of epithelium that was otherwise mildly or moderately atypical squamous metaplastic. These basally located cells (Fig. 1D) showed specific staining for SP-A (apple-green), which was delicate and included the relatively thin cytoplasmic rim. Their nuclei (Fig. 1C) were relatively large, round to ovoid, lightly to moderately basophilic, and contained one or sometimes more nucleoli. Squamous metaplastic cells (Figs. 1C,D) frequently showed a nonspecific (yellowish) fluorescence, which was solid cytoplasmic, superficially localized, and also present in negative immunohistochemical controls. Cells with type II cell characteristics (Figs. 1E,F) could also be observed in early invasive lesions. They occurred locally in the basal epithelial layers and were the only cells to constitute the invasive glandular lesions. The cytoplasmic staining for SP-A was more intense in lesions that were located further down in the connective tissue (not shown), which suggests cellular maturation. Cells with type II cell characteristics were also found in overt carcinomas arising from SBAs (Fig. 2 A-D). They constituted the lesions of bronchiolo-alveolar carcinomas (Figs. 2A,B). They were also present in acinar adenocarcinomas (Figs. 2C,D) and adenoid-cystic carcinomas (not shown), where they occupied the basal (possibly dividing) epithelial layers of all the tumor lesions in the sections and frequently also upper layers as far as the lumen, singly or in clusters. These findings are consistent with the concept that these kinds of carcinomas are type II

cell carcinomas of varying glandular (bronchiolo-alveolar, acinar or adenoid-cystic) growth patterns. This view is supported by ultra-structural findings (MLB or their precursors) concerning the major cell types in the latter two tumors (Ten Have-Opbroek et al., 1993b).

In humans (Ten Have-Opbroek et al., 1993a), our immunocytochemical and ultrastructural studies also detected type II cells in bronchogenic carcinomas. The type II cell distribution pattern in bronchiolo-alveolar, papillary, and acinar adenocarcinomas in humans corresponded to that seen in their canine counterparts (Ten Have-Opbroek et al., 1990a, 1993b). Other investigators also mention the presence of type II cells in some adenocarcinoma varieties in humans and other species, although usually only locally (review, Ten Have-Opbroek et al., 1993b; see also Broers et al., 1992; Linnoila et al., 1992a,b; Tsutahara et al., 1993). That we detect type II cells in larger numbers and at earlier stages of NSCLC development (Ten Have-Opbroek et al., 1990a, 1992, 1993b) and normal mammalian-lung development (reviewed in Ten Have-Opbroek and Plopper, 1992) suggests that our antibodies identify tumor and embryonic type II cells based on a higher affinity to SP-A. Another possibility is that such immature type II cells possess a foetal type II cell-specific antigen that may be related or not be related to SP-A, but is specifically recognized by our antibodies based on cross-reactivity (Ten Have-Opbroek et al., 1993b). In addition, we use very sensitive methods for SP-A detection, namely immunofluorescence and selective pre-absorption of sera with early-embryonic homogenate (reviewed in Ten Have-Opbroek and Plopper, 1992).

Studies in humans by us (Ten Have-Opbroek et al., 1993a) and others (Linnoila et al., 1992a,b; McDowell et al., 1978b) detected type II cells also in squamous cell carcinomas, although less frequently. The cells occurred in tumor areas that were immediately contiguous with fields of keratinizing cells as well as in lymph node metastases of these tumors (Ten Have-Opbroek et al., 1993a). These surprising findings open new perspectives to study the relationship between adenocarcinomas and squamous cell carcinomas, especially from a developmental standpoint (see below).

Discussion

a. First appearance of alveolar type II cells during bronchial carcinogenesis

That the gene activation for type II cell expression can already take place at an early time-point during bronchial carcinogenesis is supported by the finding of SP-A-reactive cuboid cells in the basal layer of slightly abnormal surface epithelium of major bronchi, as described above (see also Ten Have-Opbroek et al., 1992, 1993b). It is evident that these SP-A-reactive cuboid cells are not identical to basal cells as found in normal bronchial epithelium; these have a different

morphology and never contain SP-A. Neither are they identical to or derived from pre-existing alveolar type II cells, because there are no alveolar epithelial cells in the adult segmental bronchus from which the lesions arose. Furthermore, they were first seen within the transformed surface epithelium near the bronchial lumen and not in the surrounding connective tissue, which is more adjacent to the pulmonary parenchyma. Unquestionably, they are *newly-formed type II cells* that differentiated locally in the preneoplastic surface epithelium of the segmental bronchus and were capable of invading the underlying connective tissue.

It is clear that the carcinogen used (MCA) affects bronchial epithelial cells very effectively. Exposure to this carcinogen leads not only to tumor development but even to tumors of a different cell type (i.e., type II cells). The fact that second to seventh generation nude mouse transplants have patterns of type II cells similar to those found in the original adenocarcinomas from canine

SBA suggests that the tumor type II cells represent an active but yet stable type II cell population (Ten Have-Opbroek et al., 1990a, 1993b). Very likely, our observations in the canine SBA model may be relevant for further insight in bronchogenic carcinoma development in humans, because tobacco smoke contains numerous polycyclic aromatic hydrocarbon compounds, of which MCA is an example.

b. First appearance and origin of alveolar type II cells during normal lung morphogenesis

In the embryo, alveolar type II cells first appear during the pseudoglandular period of lung development (Ten Have-Opbroek, 1979, 1981, 1991; Ten Have-Opbroek and Plopper, 1992). They differentiate from the undifferentiated columnar epithelium of the pulmonary primordial system. Type II cells *never* arise from bronchial epithelial cells (Ten Have-Opbroek, 1979).

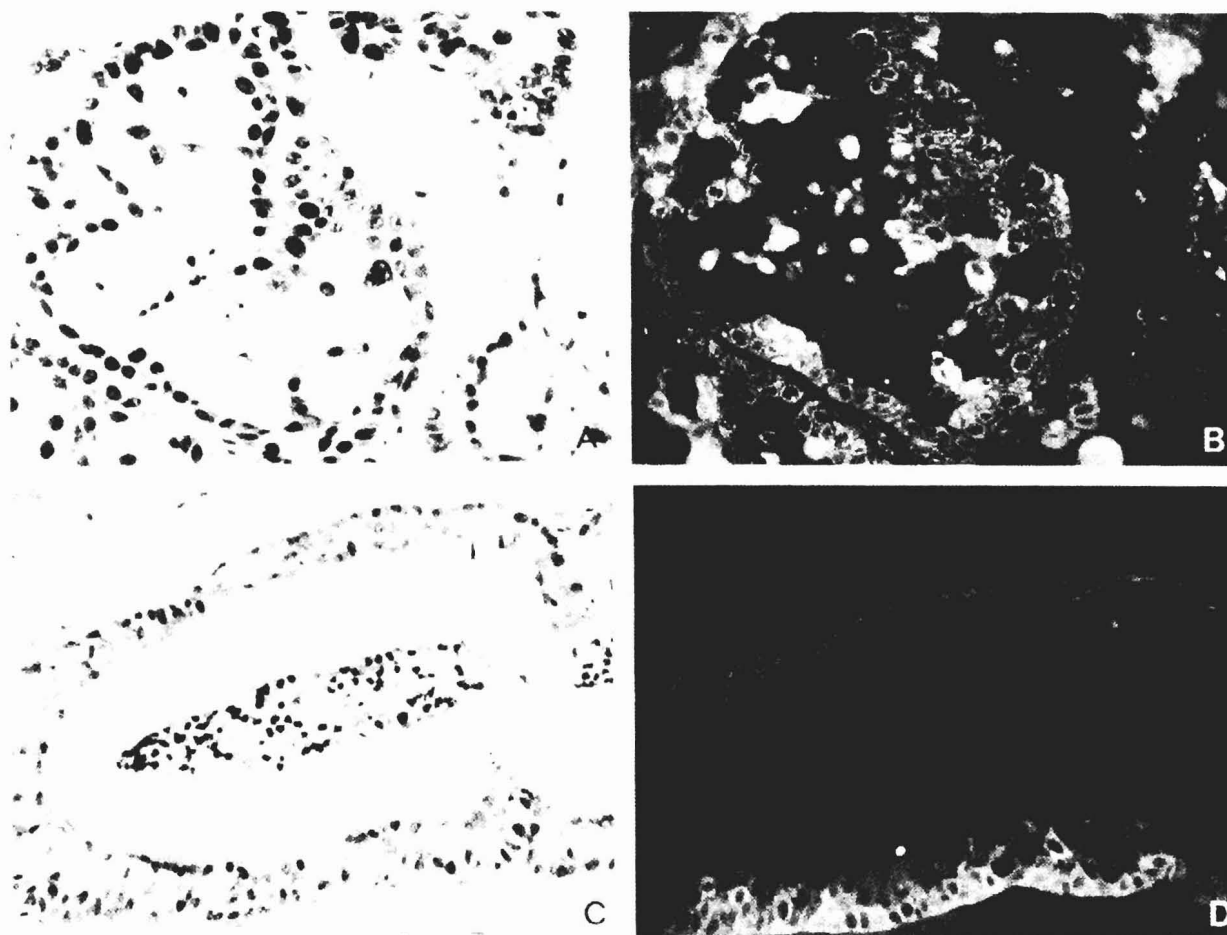


Fig. 2. Immunodetection of alveolar type II cells in overt carcinomas. The carcinomas arose from segmental bronchi in the same canine bronchogenic carcinoma model. H&E (left) and antibodies to surfactant protein A, SP-A (right; indirect immunofluorescence) in adjacent sections. Formalin fixation. Type II cells are present in all the lesions of bronchiolo-alveolar carcinomas (A,B) and acinar adenocarcinomas (C,D), where these cells occupy the basal (dividing) epithelial layers and frequently also upper layers. This supports the concept that such carcinomas are type II cell carcinomas of varying glandular growth patterns. A, x 240; B, x 340; C, x 185; D, x 255

The primordial lung epithelium plays a basic role in the generation of different cell lineages (Ten Have-Opbroek, 1981) and it does so over a remarkably long period of prenatal lung development (primates: 22-25%; rodents: 48-49%; Ten Have-Opbroek and Plopper, 1992). Depending on the localization in the prospective respiratory tract (i.e., proximal or distal), the primordial epithelium differentiates in an either bronchial or alveolar direction, the choice depending largely on unknown factors. Initially, the alveolar epithelium consists only of approximately cuboid type II cells, which contain SP-A; at later stages, particular type II cells transform to squamous type I cells, which eventually lack SP-A. In fact, type II cells are actively dividing *pluripotential* cells in normal mammalian lung morphogenesis; they generate the entire pulmonary acinus and also maintain it in adulthood (Ten Have-Opbroek, 1981, 1991; Ten Have-Opbroek and Plopper, 1992; Ten Have-Opbroek et al., 1991).

Figure 3 gives a survey of the major steps in normal

mammalian lung morphogenesis.

c. Oncofoetal mechanism in bronchial carcinogenesis; Impact upon concepts of bronchogenic carcinoma development

It seems reasonable to assume that general embryologic principles of lung differentiation may apply to bronchial carcinogenesis, whether the latter process is induced by exogenous or endogenous factors, and whether or not it eventually leads to NSCLC or SCLC. This oncofoetal standpoint has the following consequences for our insight into the process by which alveolar type II cells with malignant potentials can arise in preneoplastic bronchial epithelium, as in (a) above. Firstly, such alveolar type II tumor cells cannot differentiate from bronchial epithelial cells of larger or smaller conducting airways. Secondly, they can arise only from primordial-like epithelial cells. It is evident that these conclusions are in conflict with the first concept of bronchogenic carcinoma development, that assigns a progenitor role to specific bronchial epithelial cells. On the contrary, however, they clearly favour the second concept, which postulates that undifferentiated epithelial cells are the cells of origin for bronchogenic carcinomas.

The origin of the primordial-like cells in pre-neoplastic bronchial epithelium is not clear. As mentioned in the Introduction, there are two possibilities. The cells may be identical to or originate from residual primitive endodermal cells of the lung anlage, which in principle may still occur locally in normal bronchial epithelium (Ten Have-Opbroek, 1981). However, to date, there is no evidence for the existence of such undifferentiated cells. The conclusion that they are present in normal adult human bronchial epithelium (McDowell et al., 1978a) is disputable; the material was derived from patients who were heavy smokers and was not usually completely normal (McDowell et al., 1978a). In some animal species, including dogs (Pavelka et al., 1976; Breeze and Wheeldon, 1977; Becci et al., 1978), cells otherwise resembling undifferentiated cells were found to display early signs of ciliary or mucous differentiation. In contrast, however, there is some support for the second possibility, i.e., primordial-like cells of origin appear during conversion (via metaplasia) of normal bronchial epithelium to bronchogenic carcinoma (Ten Have-Opbroek et al., 1990a, 1993b), although this support comes from non-cancer studies (Lane and Gordon, 1974; McDowell et al., 1979). After mechanical injury of tracheal epithelium in rats (Lane and Gordon, 1974) and hamsters (McDowell et al., 1979), undifferentiated cells (also termed indifferent, or immature preciliated or presecretory, cells; see McDowell et al., 1979, 1985) were found to occur in the surface layer of the regenerating squamous metaplastic epithelium before this epithelium was fully differentiated into mucous and ciliated cells and indistinguishable from normal epithelium. The data presented (Lane and

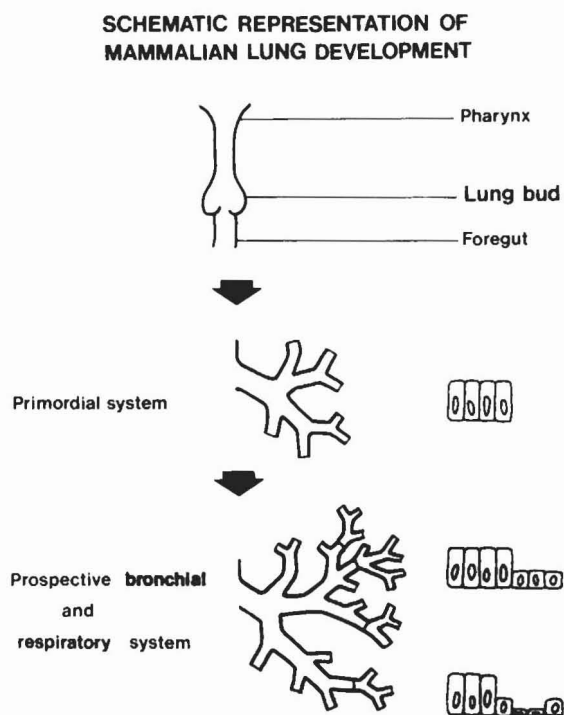


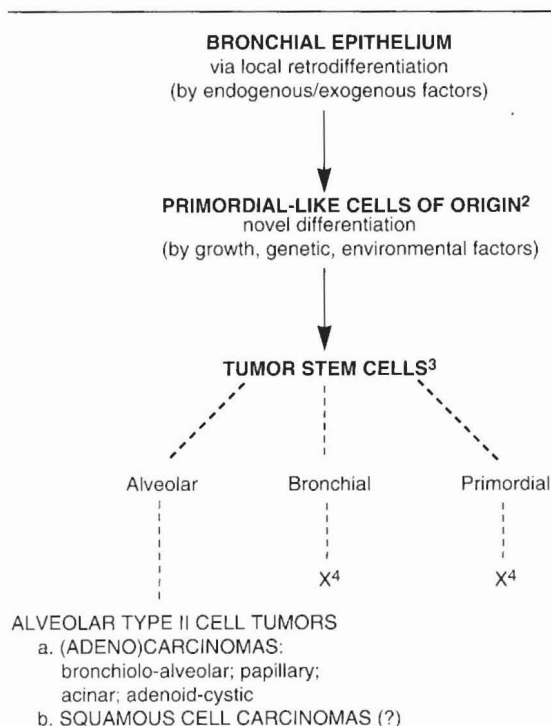
Fig. 3. Diagram of mammalian lung development proposed by Ten Have-Opbroek (1981; reviewed in Ten Have-Opbroek, 1991; Ten Have-Opbroek and Plopper, 1992). In the embryo, the two lung buds arising from the primitive foregut develop into the primordial systems of the right and left lungs. The undifferentiated columnar epithelium of these systems, termed primordial epithelium (Ten Have-Opbroek, 1981), gives rise to the epithelia of the bronchial and respiratory systems. The prospective bronchial epithelium is columnar with oblong (cigar-shaped) nuclei and is always devoid of SP-A. The prospective alveolar epithelium consists initially of type II cells (cuboid shape; large, roundish nucleus; cytoplasmic staining for SP-A; precursory forms of MLB). At later stages, there are also type I cells, which originate from type II cells but become squamous and devoid of SP-A and MLB.

Gordon, 1974; McDowell et al., 1979) suggest that such undifferentiated cells are derived from division of metaplastic mucous and basal cells, and subsequently differentiate into mucous or ciliated cells. These observations support our view (Ten Have-Opbroek et al., 1990a, 1993b) that existing normal bronchial epithelial cells such as mucous or basal cells may undergo *retro-differentiation* during metaplastic stages of bronchial carcinogenesis.

d. Oncofoetal concept of bronchogenic carcinoma development

In view of the above considerations, we hypothesize (Table 1) (Ten Have-Opbroek et al., 1990a) that neoplastic progression for bronchogenic carcinoma development implicates local retrodifferentiation in existing bronchial epithelium, which results in the appearance of undifferentiated primordial-like cells.

Table 1. Oncofoetal concept of bronchogenic carcinoma development¹.



¹: based on studies of non-small cell lung cancer in a canine model (subcutaneous bronchial autografts treated with 3-methylcholanthrene) (Ten Have-Opbroek et al., 1990a, 1992, 1993b) and in humans (Ten Have-Opbroek et al., 1993a), and on new insights into embryonic lung differentiation (Ten Have-Opbroek, 1981, 1991; Ten Have-Opbroek and Plopper, 1992); ²: undifferentiated omnipotential cells first appearing in preneoplastic surface epithelium of major bronchi or their divisions; ³: major epithelial cell types that occupy the dividing cellular layers of (pre)neoplastic lesions and constitute the actively dividing and invading part of the neoplasm; ⁴: X, data about tumor types derived from these newly-appearing bronchial or primordial tumor stem cells are not yet available.

These cells, which may occur in any layer of the transforming bronchial epithelium, are the cells of origin for three tumor stem cell lineages. These are in principle either of the alveolar, bronchial or primordial kind; the choice may depend on multiple (growth, genetic, environmental) factors. However, it is possible that retrodifferentiation may sometimes affect existing bronchial epithelial cells only incompletely or occur simultaneously with novel differentiation in those cells. If that is the case, the primordial-like cells of origin and their tumor stem cell lineages may display either predominantly bronchial or mixed (primordial, bronchial, and/or alveolar) cell properties. We expect that our further analysis of this process will show whether or not such progenitor cell varieties exist.

Examples of tumors (Table 1) that may originate from alveolar tumor stem cells are carcinomas of the bronchiolo-alveolar, papillary, acinar, and adenoid-cystic types. These tumors may be better considered as members of a family of embryonic type II cell carcinomas of varying glandular growth patterns, rather than as unrelated entities.

As shown in Table 1, squamous cell carcinomas have also been assigned to the group of embryonic type II cell carcinomas. We fully recognize that much more evidence is required to confirm this conclusion, but it is our view that there is a common cell-biological pathway for adenocarcinoma and squamous cell carcinoma development. This viewpoint is based on the following arguments. Firstly, in humans, we and others have detected type II cells in squamous cell carcinomas (McDowell et al., 1978b; Linnoila et al., 1992a,b; Ten Have-Opbroek et al., 1993a) and lymph node metastases of these tumors (Ten Have-Opbroek et al., 1993a). Secondly, time studies in foetal monkey lungs (Chi et al., 1985), using electron microscopy and cytokeratin immunocytochemistry, demonstrate that differentiation and maturation of type II cells are related to intermediate filament expression. In older type II cells, the filaments may even form bundles or aggregates. Thirdly, when cultured (Oomen et al., 1990), and during embryonic lung morphogenesis (Ten Have-Opbroek, 1979, 1981, 1991; Ten Have-Opbroek and Plopper, 1992), cuboid type II cells are capable of phenotypic squamous differentiation. Finally, studies in a lung-tumor transplantation system in the mouse (Williams and Nettesheim, 1973) show that squamous cell carcinomas may transform to tumors composed of large, undifferentiated cells with little or no evidence of keratinization (=retrodifferentiation to primordial-like epithelial cells?). At 3 weeks and beyond, the centre of these tumors may differentiate to "acinar" structures, many of which show squamous cell differentiation and signs of keratinization. Based on these arguments, we speculate that the influence of aging or other factors on tumor type II cells (e.g., change of nutrients by poor vascularization) may lead to the development of type II cell-derived squamous cell carcinomas instead of type II cell-derived adenocarcinomas. On the contrary, any

evidence that large or small cell varieties of bronchogenic carcinomas or other pulmonary carcinomas may originate from the newly arisen alveolar, bronchial or primordial tumor stem cells (Table 1) is not yet available.

At this moment, the available experimental evidence supports only the type II cell route in our postulated pathway of bronchogenic carcinoma development (Table 1). The activity of this cell type in bronchial carcinogenesis is new and impressive but not really surprising, in view of its major role in embryonic lung morphogenesis. That squamous cell carcinomas also frequently display mucous differentiation (McDowell et al., 1978b) suggests that individual primordial-like cells of origin may follow a different (either alveolar or bronchial) route, which results in the occurrence of a mixed type of tumor. If in the latter case such cells of origin also display mixed cell properties (see above), there may even be a broad mix of partially differentiated epithelial cells within a single tumor. Our future studies may show whether or not, and to what extent, the bronchial and primordial routes for bronchogenic carcinoma development (i.e., via *newly* arisen bronchial or primordial tumor stem cells) actually exist.

We believe that the mechanism of carcinogenesis proposed in our oncofoetal concept of bronchogenic carcinoma development, namely "epithelial retro-differentiation followed by novel differentiation", may be a more general principle in the development of carcinomas than unidirectional transformation. More insight into the oncofoetal mechanism postulated here may come from studies of the regulation of epithelial expression (Jetten, 1991), especially under conditions which produce tumors.

Prospects

Our studies of bronchogenic carcinomas have opened exciting perspectives for study of the mechanisms that control the phenotypic expression of one potential tumor stem cell, the alveolar type II cell, at *preneoplastic stages*. This pluripotential cell, which may give rise to several varieties of bronchogenic carcinomas, first appears in mildly to moderately preneoplastic epithelium. Nothing is yet known about the differentiation of type II cells from their progenitors, the "primordial-like" cells of origin. Expanding knowledge of phenotypic and molecular aspects of this differentiation process may contribute to early detection of bronchogenic carcinomas. Furthermore, this knowledge can be used to seek links between the phenotypic expression of these tumors and their clinical behaviour and response to therapy. This approach may also have bearing on tumor classification. Finally, the combined phenotypic and molecular data may possibly contribute to chemoprevention of bronchogenic carcinomas.

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References

- Becci P.J., McDowell E.M. and Trump B.F. (1978). The respiratory epithelium. II. Hamster trachea, bronchus, and bronchioles. *J. Natl. Cancer Inst.* 61, 551-561.
- Benfield J.R. and Hammond W.G. (1992). Bronchial and pulmonary carcinogenesis at focal sites in dogs and hamsters. *Cancer Res. (suppl)* 52, 2687s-2693s.
- Brandsma A.E., Tibboel D., Vulto I.M., Egberts J. and Ten Have-Opbroek A.A.W. (1993). Ultrastructural features of alveolar epithelial cells in the late fetal pulmonary acinus: A comparison between normal and hypoplastic lungs using a rat model of pulmonary hypoplasia and congenital diaphragmatic hernia. In: *Microscopic evaluation of respiratory tract function*. Ten Have-Opbroek A.A.W. and Plopper C.G. (eds). *Microsc. Res. Techn.* 26, 389-399.
- Breeze R.G. and Wheeldon E.B. (1977). The cells of the pulmonary airways. *Am. Rev. Respir. Dis.* 116, 705-777.
- Broers J.L.V., Jensen S.M., Travis W.D., Pass H., Whitsett J.A., Singh G., Katyal S.L., Gazdar A.F., Minna J.D. and Linnoila R.I. (1992). Expression of surfactant associated protein-A and Clara cell 10 kilodalton mRNA in neoplastic and non-neoplastic human lung tissue as detected by in situ hybridization. *Lab. Invest.* 66, 337-346.
- Chi E.Y., Gown A.M., Vogel A.M. and Teh E.C. (1985). Intermediate filaments in the developing type II cell of fetal monkey lung. *J. Histochem. Cytochem.* 33, 161-168.
- Clara M. (1937). Zur Histobiologie des Bronchalepithels. *Z. Mikrosk. Anat. Forsch.* 41, 321-347.
- Derrick M.J., Hammond W.G., Pak H.Y., Azumi N., Smith S.S. and Benfield J.R. (1988). Non-small cell lung cancer in autogenous subcutaneous bronchial grafts in dogs. *J. Thorac. Cardiovasc. Surg.* 95, 562-571.
- Hammond W.G., Benfield J.R., Paladugu R.R., Azumi N., Pak H.Y. and Teplitz R.L. (1986). Carcinogenesis in heterotopic respiratory epithelium in canine subcutaneous bronchial autografts. *Cancer Res.* 46, 2995-2999.
- Inayama Y., Hook G.E.R., Brody A.R., Cameron G.S., Jetten A.M., Gilmore L.B., Gray T. and Nettesheim P. (1988). The differentiation potential of tracheal basal cells. *Lab. Invest.* 58, 706-717.
- Inayama Y., Hook G.E.R., Brody A.R., Jetten A.M., Gray T., Mahler J. and Nettesheim P. (1989). In vitro and in vivo growth and differentiation of clones of tracheal basal cells. *Am. J. Pathol.* 134, 539-549.
- Jetten A.M. (1991). Growth and differentiation factors in tracheo-bronchial epithelium. *Am. J. Physiol.* 260 (Lung Cell Mol Physiol 4), L361-L373.
- Lane B.P. and Gordon R. (1974). Regeneration of rat tracheal epithelium after mechanical injury. I. The relationship between mitotic activity and cellular differentiation. *Proc. Soc. Exp. Biol. Med.* 145, 1139-1144.
- Linnoila R.I., Jensen S.M., Steinberg S.M., Mulshine J.L., Eggleston J.C. and Gazdar A.F. (1992a). Peripheral airway cell marker expression in non-small cell lung carcinoma. Association with distinct clinicopathologic features. *Am. J. Clin. Pathol.* 97, 233-243.

Oncofoetal concept of bronchial carcinogenesis

- Linnoila R.I., Mulshine J.L., Steinberg S.M. and Gazdar A.F. (1992b). Expression of surfactant-associated protein in non-small-cell lung cancer: A discriminant between biologic subsets. *J. Natl. Cancer Inst. Monogr.* 13, 61-66.
- Mariassy A.T. (1992). Epithelial cells of trachea and bronchi. In: *Treatise on Pulmonary Toxicology. I. Comparative Biology of the Normal Lung*. Parent R.A. (ed). CRC Press. Boca Raton, FL. Chap 6, pp 63-76.
- Massaro G.D., Davis L. and Massaro D. (1984). Postnatal development of the bronchiolar Clara cell in rats. *Am. J. Physiol.* 247 (Cell Physiol 16), C197-C203.
- McDowell E.M. (1987). Bronchogenic carcinomas. In: *Lung carcinomas*. McDowell E.M. (ed). Churchill Livingstone. Edinburgh. Part IV, Neoplasms of the human lung, Chap 10, pp 255-285.
- McDowell E.M., Barrett L.A., Glavin F., Harris C.C. and Trump B.F. (1978a). The respiratory epithelium. I. Human bronchus. *J. Natl. Cancer Inst.* 61, 539-549.
- McDowell E.M., McLaughlin J.S., Merenyl D.K., Kieffer R.F., Harris C.C. and Trump B.F. (1978b). The respiratory epithelium. V. Histogenesis of lung carcinomas in the human. *J. Natl. Cancer Inst.* 61, 587-606.
- McDowell E.M., Becci P.J., Schürch W. and Trump B.F. (1979). The respiratory epithelium. VII. Epidermoid metaplasia of hamster tracheal epithelium during regeneration following mechanical injury. *J. Natl. Cancer Inst.* 62, 995-1008.
- McDowell E.M., Newkirk C. and Coleman B. (1985). Development of hamster tracheal epithelium: II. Cell proliferation in the fetus. *Anat. Rec.* 213, 448-456.
- Nasiell M., Auer G. and Kato H. (1987). Cytological studies in man and animals on development of bronchogenic carcinoma. In: *Lung carcinomas*. McDowell E.M. (ed). Churchill Livingstone. Edinburgh. Part III, Progression to neoplasia, Chap 8, pp 207-242.
- Oomen L.C.J.M., Ten Have-Opbroek A.A.W., Hageman Ph.C., Oudshoorn-Snoek M., Egberts J., Van der Valk M.A., Calafat J. and Demant P. (1990). Fetal mouse alveolar type II cells in culture express several type II cell characteristics found in vivo, together with Major Histocompatibility antigens. *Am. J. Respir. Cell Mol. Biol.* 3, 325-339.
- Otto-Verberne C.J.M. and Ten Have-Opbroek A.A.W. (1987). Development of the pulmonary acinus in fetal rat lung: a study based on an antiserum recognizing surfactant-associated proteins. *Anat. Embryol.* 175, 365-373.
- Otto-Verberne C.J.M., Ten Have-Opbroek A.A.W., Balkema J.J. and Franken C. (1988). Detection of the type II cell or its precursor before week 20 of human gestation, using antibodies against surfactant-associated proteins. *Anat. Embryol.* 178, 29-39.
- Pavelka M., Ronge H.R. and Stockinger G. (1976). Vergleichende Untersuchungen am Trachealepithel verschiedener Säugetiere. *Acta Anat.* 94, 262-282.
- Plopper C.G. and Ten Have-Opbroek A.A.W. (1994). Anatomical and Histological Classification of Bronchioles. In: *Diseases of the bronchioles*. Epler G.R. (ed). Raven Press. New York. Chap 2. (in press).
- Plopper C.G., Alley J.L., Serabjit-Singh C.J. and Philpot R.M. (1983). Cytodifferentiation of the nonciliated bronchiolar epithelial (Clara) cell during rabbit lung maturation: An ultrastructural and morphometric study. *Am. J. Anat.* 167, 329-357.
- Plopper C.G., Alley J.L. and Weir A.J. (1986). Differentiation of tracheal epithelium during fetal lung maturation in the Rhesus monkey *Macaca mulatta*. *Am. J. Anat.* 175, 59-71.
- Plopper C.G., St George J., Cardoso W., Wu R., Pinkerton K. and Buckpitt A. (1992). Development of airway epithelium. Patterns of expression for markers of differentiation. *Chest* 101, 2S-5S.
- Reznik-Schüller H. and Reznik G. (1979). Experimental pulmonary carcinogenesis. III. Chemically induced pulmonary carcinogenesis. *Internat. Rev. Exp. Pathol.* 20, 237-281.
- Schüller H.M. (1987). Experimental carcinogenesis in the peripheral lung. In: *Lung carcinomas*. McDowell E.M. (ed). Churchill Livingstone. Edinburgh. Part III, Progression to neoplasia, Chap 9, pp 243-254.
- Stünzi H., Head K.W. and Nielsen S.W. (1974). International histological classification of tumours of domestic animals. I. Tumors of the lung. *Bull. Wld. Hlth. Org.* 50, 9-19.
- Ten Have-Opbroek A.A.W. (1975). Immunological study of lung development in the mouse embryo. I. Appearance of a lung-specific antigen, localized in the great alveolar cell. *Dev. Biol.* 46, 390-403.
- Ten Have-Opbroek A.A.W. (1979). Immunological study of lung development in the mouse embryo. II. First appearance of the great alveolar cell, as shown by immunofluorescence microscopy. *Dev. Biol.* 69, 408-423.
- Ten Have-Opbroek A.A.W. (1981). The development of the lung in mammals: An analysis of concepts and findings. *Am. J. Anat.* 162, 201-219.
- Ten Have-Opbroek A.A.W. (1986). The structural composition of the pulmonary acinus in the mouse. A scanning electron microscopical and developmental-biological analysis. *Anat. Embryol.* 174, 49-57.
- Ten Have-Opbroek A.A.W. (1991). Lung development in the mouse embryo. *Exp. Lung Res.* 17, 111-130.
- Ten Have-Opbroek A.A.W. and De Vries E.C.P. (1993). Clara cell differentiation in the mouse: Ultrastructural morphology and cytochemistry for surfactant protein A and Clara cell 10 kD protein. In: *Microscopic evaluation of respiratory tract function*. Ten Have-Opbroek A.A.W. and Plopper C.G. (eds). *Microsc. Res. Techn.* 26, 400-411.
- Ten Have-Opbroek A.A.W. and Plopper C.G. (1992). Morphogenetic and functional activity of type II cells in early fetal Rhesus monkey lungs. A comparison between primates and rodents. *Anat. Rec.* 234, 93-104.
- Ten Have-Opbroek A.A.W., Dubbeldam J.A. and Otto-Verberne C.J.M. (1988). Ultrastructural features of type II alveolar epithelial cells in early embryonic mouse lung. *Anat. Rec.* 221, 846-853.
- Ten Have-Opbroek A.A.W., Hammond W.G. and Benfield J.R. (1990a). Bronchiolo-alveolar regions in adenocarcinoma arising from canine segmental bronchus. *Cancer Lett.* 55, 177-182.
- Ten Have-Opbroek A.A.W., Otto-Verberne C.J.M. and Dubbeldam J.A. (1990b). Ultrastructural characteristics of inclusion bodies of type II cells in late embryonic mouse lung. *Anat. Embryol.* 181, 317-323.
- Ten Have-Opbroek A.A.W., Otto-Verberne C.J.M., Dubbeldam J.A. and Dijkman J.H. (1991). The proximal border of the human respiratory unit, as shown by scanning and transmission electron microscopy and light microscopical cytochemistry. *Anat. Rec.* 229, 339-354.
- Ten Have-Opbroek A.A.W., Hammond W.G., Teplitz R.L., Benfield J.R. and Dijkman J.H. (1992). First expression of alveolar type II cell markers during bronchial carcinogenesis. *Proc. Am. Assoc. Cancer Res.* 33, 118.
- Ten Have-Opbroek A.A.W., Dijkman J.H., Van Krieken J.H.J.M., Boex J.J.M., Hammond W.G. and Benfield J.R. (1993a). The presence of alveolar type II cells in human bronchogenic carcinoma. *Am. Rev. Respir. Dis.* 147 (suppl.), A158.

Oncofoetal concept of bronchial carcinogenesis

- Ten Have-Opbroek A.A.W., Hammond W.G., Bentfield J.R., Teplitz R.L. and Dijkman J.H. (1993b). Expression of alveolar type II cell markers in acinar adenocarcinomas and adenoid-cystic carcinomas arising from segmental bronchi. A study in a heterotopic bronchogenic carcinoma model in dogs. *Am. J. Pathol.* 142, 1251-1264.
- The World Health Organization histological typing of lung tumours. (1982). 2nd ed. *Am. J. Clin. Pathol.* 77:123-136.
- Tsutahara S., Shijubo N., Hirasawa M., Honda Y., Satoh M., Kuroki Y. and Akino T. (1993). Lung adenocarcinoma with type II pneumocyte characteristics. *Eur. Respir. J.* 6, 135-137.
- Tyler N.K. and Plopper C.G. (1985). Morphology of the distal conducting airways in Rhesus monkey lungs. *Anat. Rec.* 211, 295-303.
- Williams M.L. and Nettesheim P. (1973). Lung colony assay with a squamous cell carcinoma derived from the respiratory tract of mice. *J. Natl. Cancer Inst.* 51, 1513-1520.