Lung Retinoid Metabolism and Signaling in Chronic Obstructive Pulmonary Disease

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There are a number of sites that are required for the production and/or action of all-trans retinoic acid (ATRA). In particular, interruption of different components of the chain of trafficking and metabolism has been associated with cancers arising in numerous organs of the body. Preliminary work suggests that such interruptions may be a factor in lung disorders induced by the smoke exposure. The active metabolite of retinoid, ATRA offers a therapeutic strategy to protect against functional abnormality in the lung, including chronic obstructive pulmonary disease (COPD). This review deals with the lung retinoid metabolism and mediators of retinoid trafficking and signaling with special emphasis on their roles in health and disease.

Keywords: Retinoic acid, Nuclear receptors, Retinoid intra cellular binding proteins, Enzymes of retinoid metabolism, Chronic obstructive pulmonary disease, Signalling

Introduction

Examination of known disorders of all-trans retinoic acid (ATRA) production or its nuclear action provides a map for evaluation of a role of similar disorders in lung disease. This is considered here as possible contributors to the pathophysiology of the lung associated with smoke, particularly second hand smoke. Such considerations suggest different therapeutic strategies for use of retinoids in reversal or prevention of smoke-induced disorders, such as chronic obstructive pulmonary disease (COPD).

Normal retinoid metabolism and signaling

Enzymes in retinoid metabolism (LRAT, RDH, RALDH and CYP26)

Pulmonary cells store retinol in the form of retinyl esters. A model of retinyl ester storage and metabolism [predominantly all-trans retinyl palmitate (RP)] has been supported with experimental evidence. This metabolic pathway is strictly controlled by the enzymes and binding proteins that provide a highly regulated production of retinoids and shelter these retinoids from non-specific reactions (Fig. 1). Esterification of retinol is mediated by two enzymes — lecithin: retinol acyltransferase (LRAT) and acyl-CoA: retinol acyl transferase (ARAT). However, only LRAT is physiologically important in the lung. Retinyl ester hydrolase (REH) is the major enzyme that converts retinyl ester to retinol in most tissues, including lung. Retinol is then metabolized and bound to retinol binding protein (apo-CRBP). Two successive reactions produce all-trans retinoic acid (ATRA) from all-trans retinol. The rate-limiting step is a reversible dehydrogenation into all-trans retinal catalyzed by the retinol dehydrogenase (RDH) and the irreversible (may be rate-limiting) dehydrogenation of all-trans retinal catalyzed by retinal dehydrogenase (RALDH). There are several forms of RDH that belong to the super family of short chain dehydrogenase/reductase (SDR) that are regulated by estrogens, androgens etc. The physiologically important RDH in epithelium is likely epithelial retinol dehydrogenase (eRolDH), which exists at a site of demonstrated retinoic acid (RA) synthesis. Four different RALDHs have been
identified that convert all-trans- or 9-cis retinal into RA. They belong to the ALDH super family, which consists of at least 86 eukaryotic members. The important RALDHs are RALDH 2 and 3.

The gene regulatory activities of retinoids are mediated primarily by the all-trans and 9-cis isomers of RA. Although 13-cis RA does not have the potent gene regulatory activity of the other two isomers, it is an effective pharmacologic agent for treating a variety of dermatologic conditions. It is well-accepted that 13-cis RA is a naturally occurring form of RA that is normally present in blood and tissues of humans and higher animals. Of the naturally occurring RA, all-trans RA is the predominant form. RA is able to induce its own metabolism catalyzed by members of the cytochrome P450 family, the CYP26 isoforms. There are 3 types of CYP26 — CYP26A1, CYP26B1 and CYP26C1. CYP26A1 is expressed in number of tissues, including lung, CYP26B1 is expressed in brain and skin, whereas CYP26C1 is expressed in hind brain and is not widely expressed in adult tissues. All isoforms convert RA, particularly ATRA to less active metabolites (4-hydroxy-RA, 18-hydroxy RA and predominantly 4-oxo-RA) that can then exit. To recharge RP stores, ROH can be sequestered by esterification with fatty acids in reactions catalyzed by LRAT21,22, which transfers the sn-1 fatty acid from lecithin to retinol and is induced by ROH itself, holo-CRBP and high concentrations of RA23-25.

Auto-regulation of RA biosynthesis through regulation of retinol esterification by LRAT has been demonstrated in human keratinocytes26. Induction of LRAT by RA reduces conversion of retinol to RA by 50%. This type of LRAT induction may be triggered by acute smoke exposure prior to the development of lung cancer in which the RP level is increased and the RA level is decreased. However, LRAT expression may be differently regulated in chronic smoke exposure that can ultimately result in lung cancer.
including squamous metaplastic alterations in the bronchial epithelium. It has been demonstrated that retinol metabolism and LRAT levels are reduced in human renal, prostate and bladder cancers. Thus, it is important that we learn whether LRAT activity in lung cells is also influenced by second hand smoking.

**Mediators of vitamin A action in lung**

**STRA6**

STRA6, a multi-transmembrane domain protein has been identified as a specific membrane receptor for retinol binding protein (RBP) and is responsible for cellular internalization of retinol. Interestingly, STRA6 activity is found to be most evident in the presence of LRAT. It is expressed in a variety of adult tissues and during embryonic development in tissues for which a vitamin A supply is essential. STRA6 is reported to be a RA induced gene in P19 embryonic carcinoma cells. Furthermore, patients carrying mutations in the STRA6 gene display a broad spectrum of malformations, including lung hypoplasia.

**CRBP and CRABP**

Pulmonary cells contain distinct cellular retinol-binding proteins (CRBP-I and -II) and cellular RA-binding proteins (CRABP-I and -II). CRBP-I and CRABP-I are involved in channeling their ligands to particular sites for metabolism or function. CRBP-I is the predominant cellular retinol binding protein in lung. Furthermore, CRABP-I is suggested to be involved in RA degradation, while CRABP-II is involved in directing RA to retinoid receptors. In most biological systems studied in whole animals or cells in culture, RA, which is metabolically-derived from retinol is more active than retinol in affecting the CRBP gene.

**Nuclear RARs**

RARs function as ligand-dependent transcription factors. Their actual down-regulation (loss or low expression of RARs, specifically RARβ with tumor suppressor activity) or “functional” down-regulation due to lack of RA could interfere with retinoid signal transduction, resulting in enhanced cell proliferation and potentially in malignant transformation. In fact, a progressive loss of RARβ has been shown in certain human cancers, including lung cancer and RARβ gene silencing by DNA methylation is found in many cases. Besides expression of RARβ, expression of other retinoid receptors may play a role in lung carcinogenesis. RA is a potent inducer of cell differentiation by a molecular mechanism that apparently involves simultaneous activation and repression of specific genes. The action of RA is mediated by nuclear retinoid receptors — RARs and RXRs, the two gene families within the gene super family of steroid hormone receptors. Each is encoded by three genes (RARα, RARβ, RARγ and RXRα, RXRβ, RXRγ) having different isoforms. In mice, there are four isoforms for RARβ described thus far (β1, β2, β3, β4) and three (β1, β2, β4) in humans. In addition, RARβ1 and RAR-β5 have recently been described in human breast cancer cells. The latter cannot bind cis-acting DNA elements and thus might act through stoichiometric competition.

Of the naturally occurring retinoids, ATRA binds to RARs, while 9-cis RA binds to both RARs and RXRs. RARs and RXRs can form homodimers or heterodimers in vitro and in vivo and can bind to specific DNA sequences called RA response elements (RAREs) or retinoid X response elements (RXREs). As a consequence of such an interaction, the expression of specific genes is activated or repressed by some unknown mechanisms. In addition, a metabolic interaction between nuclear RARs and cellular oncoproteins c-jun/c-fos has been suggested, and this interaction should lead to inhibition of c-jun/c-fos activities. The products of two proto-oncogenes — c-jun and c-Fos form a complex in the nucleus [activator protein 1 (AP-1)] that binds to a DNA sequence motif referred to as the AP-1 response element (AP-1 RE). Components of AP-1 are important in modulating carcinogenesis and the transactivation of AP-1-dependent genes is required for tumor promotion.

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Several RARβ isoforms exist and are deregulated in lung carcinogenesis, but only some involve epigenetic changes or altered retinoid metabolism. The reduced expression of RARβ2 in the development of different human cancers results from epigenetic silencing by methylation of cytosine-phospho-guanosine (CpG) islands in the promoter region of the gene. Its expression also depends on the cellular levels of retinoids because this receptor is itself an RA-inducible gene\textsuperscript{54}. In fact, RARβ2 expression is reduced in several organs during vitamin A-deficient states and is enhanced by RA\textsuperscript{55}. Another isoform RARβ4 is generated by alternative splicing from the same primary transcripts as RARβ2. The RARβ4 protein, although shorter can still heterodimerize with RXRα and transcription cofactors, but cannot bind DNA to regulate gene expression\textsuperscript{56}. For example, it is not able to inhibit AP-1 activity, unlike RARβ2\textsuperscript{57}. Therefore, RARβ4 may act as a dominant-negative form of RARβ2. The methylation of RARβ P2 promoter is reported as one mechanism that silences RARβ2 and RARβ4 expression in many lung cancers, particularly small cell lung cancer (SCLC)\textsuperscript{58}. Thus, chemical demethylation is a potential approach to the lung cancer therapy.

**CYP26 Family**

Both the formation of active retinoids as well as the catabolism to inactive retinoids is important. The enzymes that metabolize RA may be involved not only in the regulation of the concentration of active retinoids, but also in the determination of the location and time of retinoid action. A number of different enzyme families that are able to catalyze retinoid metabolism have been identified and include various alcohol/aldehyde dehydrogenases and members of the cytochrome P450 family CYP26\textsuperscript{59}. CYP26 is induced by RA in a number of cell types and several functions for this enzyme in the regulation of development and differentiation have been proposed\textsuperscript{15,60,61}.

**Functional role of retinoids in COPD**

Smoking has been linked in the pathogenesis of various types of pulmonary diseases\textsuperscript{62}. Non-smokers who are exposed to second hand tobacco smoke suffer from alterations in lung functions that characterize COPD\textsuperscript{63}. Presently, COPD is the fourth leading killer of adults in US and likely to be third leading cause of death by 2020\textsuperscript{64}. Approximately 12 million adults in US are diagnosed with COPD and 120,000 die from it each year and it is estimated that an additional 12 million adults in US may have undiagnosed COPD\textsuperscript{65}. Death rates from COPD are clearly higher among cigarette smokers and smokers with COPD have a higher risk of lung cancer\textsuperscript{66}. Oxidants present in cigarette smoke may play a role in COPD\textsuperscript{66}.

Chronic bronchitis and emphysema are two types of COPD. In COPD, the airways are partially blocked making it difficult for air to get in and out of the lungs\textsuperscript{67,68}. These conditions develop as a result of certain lung conditions such as: (a) the airways and sacs lose their elasticity, (b) the wall between the sacs is destroyed, (c) the walls and airways become thick and inflamed, and/or (d) the cells in the airway produce more mucus than usual which tends to clog the airways.

Vitamin A and its active metabolites play an essential role in the respiratory tract by influencing differentiation and the integrity of epithelial cells\textsuperscript{69}. The following studies have been carried out in order to understand the relationship between vitamin A deficiency, lung function and lung diseases\textsuperscript{70,78}.

- A deficiency of vitamin A reduces antioxidant activity, leading to type I brittle asthma\textsuperscript{70}.
- There is a relationship between a deficiency of vitamin A and the degree of bronchopulmonary dysplasia in neonates\textsuperscript{71}.
- Stansfield et al.\textsuperscript{22} have claimed that vitamin A supplements improve acute respiratory infections in children, while in another study\textsuperscript{72}, high oral doses of vitamin A have not been found to improve confirmed cases of acute lower respiratory tract infection in children. It is also reported that children who experience frequent respiratory episodes may benefit from vitamin A supplementation\textsuperscript{74}.
- One study has revealed a lower serum retinol concentration in patients with moderate to severe COPD\textsuperscript{74}. Treatment with retinol improves the FEV1 in these individuals\textsuperscript{75,76}. These authors have concluded that their findings “suggest an association between COPD and vitamin A status” and may represent “one possible link between vitamin A metabolic pathways and lung function”.
- An advanced case of vitamin A deficiency results in replacement of mucus-secreting ciliated epithelium by squamous epithelium in the respiratory tract\textsuperscript{77}. The cellular defense against lung infection is impaired in vitamin A deficiency by a widespread reduction in the number of ciliated cells throughout the trachea, bronchi and bronchiolar epithelium\textsuperscript{78}. 


Our laboratory has previously reported that vitamin A deficiency decreases superoxide dismutase, glutathione peroxidase and glutathione levels in guinea pig lung. Simultaneously, it causes a marked increase in microsomal oxidation. This suggests that vitamin A plays an essential role in a defense mechanism associated with the cellular removal of these toxic radicals.

Other studies have shown that risk for COPD increases with decreasing vitamin A serum levels and daily oral doses (25,000 IE) of vitamin A for 30 days attenuate symptoms. Although smokers with COPD do not have a systemic vitamin A deficiency, a local lung deficiency does occur as a result of exposure to cigarette smoke. An immune response subsequent to smoke exposure is thought to be one reason for this deficiency. The same deficiency may eventually lead to lung cancer via a COPD-associated metaplasia and dysplasia. NHANES II data support this association as the relative risk of smokers developing COPD is strongly and dose-dependently associated with the intake of vitamin A. Thus, low vitamin A intake in smokers not only enhances the risk of developing COPD, but can also increase their risk for ultimately developing lung cancer. In addition, benzo[a]pyrene (a component of cigarette smoke) is known to decrease the intake of vitamin A into lung cells and leads to a local vitamin A deficiency of the lung tissues. The decisive process that induces serious pathophysiologic consequences is the development of a local vitamin A deficiency in the lung tissue.

ATRA is known to activate genes involved in lung development and to promote alveolarization and growth in both pre- and post-natal periods. It has shown promise in promoting alveolar repair in elastase-induced emphysema in rats and in papain-induced emphysema in dogs. Thus, ATRA is considered as one of the most promising therapeutic agents for the treatment of emphysema associated with COPD. Furthermore, it is reported to suppress the growth of tumor cells by transcriptional regulation of the epidermal growth factor (EGFR) promoter. However, a feasibility study (FORTE) on the use of systemic ATRA or 13-cis RA for treatment of emphysema associated with moderate to severe COPD has not identified any dramatic or lasting improvements in lung function, CT density mask score, or health-related quality of life (QOL) at the end of 6 months. There is also a problem associated with using oral ATRA per se, in that circulating levels vary, depending upon the individuals and the cellular responses decrease over time with repeated oral dosing due to the induction of cytochrome P450 enzymes. However, it is suggested that RA may protect against emphysema by increasing elastin synthesis and by preventing its degradation, a process that appears to be mediated through nuclear retinoic acid receptors, particularly RARα.

We have reported earlier that guinea pigs exposed to direct and second hand cigarette smoke exhibit lung injury accompanied with an accumulation of retinol and a decrease in RA in the lung, suggesting an occurrence of abnormality in retinoid metabolism and signaling. We used guinea pigs as our in vivo model as the lungs of these animals best mimic those of human. Unfortunately, almost nothing is known about whether the gene expression of certain mediators of retinoid action is altered or not in COPD and in particular among individuals exposed to smoke, in particular second hand smoke. If we can establish a relationship between expression of some of these genes and COPD, it will help us to design therapeutic drugs targeting these genes.

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