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TITLE: The Role of Lecithin: Retinol Acyltransferase (LRAT)
Mediated Esterification of Vitamin A in Regulating Human Breast
Cancer Cell Proliferation and Differentiation

PRINCIPAL INVESTIGATOR: Dan Su

CONTRACTING ORGANIZATION: Weill Medical College of
Cornell University
New York, NY 10021

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Introduction

Mechanism of Action of Retinoids

Vitamin A (retinol) and its physiologically active metabolites (retinoids) play essential roles in physiological processes such as embryonic development, proliferation, differentiation, pattern formation, and apoptosis. Retinoids have major effects on the growth and differentiation of normal, premalignant, and malignant epithelial cells (Gudas et al. 1994). Vitamin A deficiency in animal models is associated with altered mammary gland differentiation and increased susceptibility to carcinogens (Metz et al. 2002). Retinoids have proven to be effective in suppressing breast cancer development in experimental models of carcinogenesis (Lotan 1980). In addition to breast cancer, retinoids have been shown to be effective in suppressing tumor development and/or treatment of cancers of the skin, oral cavity, lung, prostate, bladder, liver, and pancreas (Kelloff et al. 1996; Lotan 1996). The biological effects of retinoids are thought to be mainly mediated through interactions with retinoic acid receptors (RARs) or retinoic X receptors (RXRs), which function as dimeric transcription factors modulating gene expression by binding to RA-response elements (RAREs or RXREs) in the promoter regions of target genes (Chambon 1996).

Since retinoid receptor mediated transcription is governed at least in part by the relative abundance of receptor ligands, the ability of cells to generate bioactive intracellular retinoids may play a key role in controlling the growth and differentiation of cells.

Retinol (ROL), which must be taken up by epithelial cells from the blood, is esterified within cells by the enzyme lecithin:retinol acyltransferase (LRAT) (Barry et al. 1989)

(Fig.1). LRAT plays an essential role in vitamin A metabolism and is particularly important in the processing of retinoids in the vertebrate visual cycle (Rando 2001).

Although another enzyme, acetylCoA:retinol acyltransferase (ARAT), can esterify ROL, the enzyme responsible for the majority of ROL esterification in epithelial cells is LRAT (Herr et al. 1991). Retinyl esters (REs) are thought to be the storage form of ROL, and these esters can be gradually metabolized back into ROL by retinyl ester hydrolases (REH) within the epithelial cells.

Retinoid metabolism is disrupted in breast cancer cells. There are greatly reduced levels of REs in the carcinogen induced rat mammary carcinoma model (Bhat and Moudgal 1989). In contrast, normal breast epithelium contains extremely high levels of REs in this animal model. Our laboratory has demonstrated that the levels of REs in human carcinoma cell lines from the breast, oral cavity, kidney, and prostate were also very low (Chen et al. 1997; Guo and Gudas 1998; Guo et al. 2001; Guo et al. 2002). In addition, we demonstrated that this low level of ROL esterification was associated with the absence of LRAT enzymatic activity, the lack of detectable LRAT protein, and abnormal *LRAT* transcripts (Guo et al. 2000). Furthermore, we demonstrated that high levels of ROL and REs were detected in normal kidney epithelial tissue, but were barely detectable in kidney tumor from patients (Guo et al. 2001). More recently, we have shown that human kidney cancers which exhibited a high level of LRAT staining by immunocytochemistry were of low malignant potential, whereas those which did not stain for LRAT exhibited much higher malignant potential (Zhan 2003). These examples confirm the importance of LRAT in the maintenance of normal cell phenotype. When the cancer cells are retinoid deficient, then retinoid signaling will be compromised. For

example, the expression of RARs will be affected given that retinoids directly regulate *RARα* and *RARβ* expression (Giguere 1994). Reduction of RAR expression will further affect retinoid signaling (Boylan et al. 1995; Isogai et al. 1997). This is particularly important as the growth inhibitory effects of retinoids are mediated largely by *RARα* and *RARβ* (van der Burg et al. 1993). Thus, a deficiency in esterification activity would lead to improper retinoid signaling and could contribute to carcinoma formation.

The goal of this proposal is to determine the contribution of esterification of ROL to breast cancer growth inhibition and tumor inhibition. We will determine whether LRAT-mediated esterification of ROL can enhance the retinoid signaling in tumor cells and decrease breast cancer cell growth. The results from these studies could provide a rationale for targeting LRAT or the ROL esterification process in novel preventive and therapeutic treatments for breast cancer patients.

In this project, we stained for LRAT in normal vs malignant human breast tissues. Also, transgenic mice were generated in which human LRAT was ectopically expressed either in the suprabasal layer or in the basal layer of epithelial cells driven by the cytokeratin 10 promoter or the cytokeratin 14 promoter, respectively. The effects of ectopic LRAT expression on epithelial cell proliferation and differentiation were examined in these transgenic mice. We showed that with a normal level of retinol in the diet, the overexpression of human LRAT in the suprabasal layer or basal layer of the mouse epithelial cells affects mouse epithelial cell differentiation minimally. However, the epidermal hyperplasia induced by topical retinol treatment was greatly reduced in the human LRAT-expressing animals. These data suggest a role for LRAT in the maintenance of normal epithelial cells differentiation. Currently, studies are underway to assess the functions of LRAT in breast carcinoma prevention.

Body

Part 1 Measurement of LRAT in Normal Human Breast Tissue vs Tissue from Breast Cancer Patients

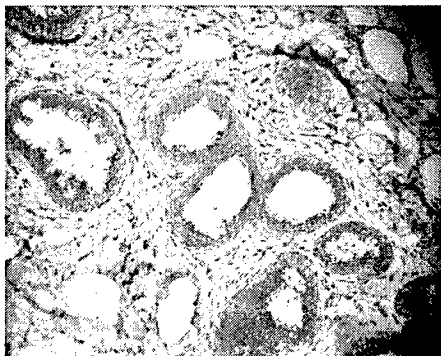


Figure 1. A sample from a human breast cancer patient (NY Hospital/Cornell) with normal ducts showing LRAT expression (brown stain) in the luminal cells. The surrounding stroma does not stain for LRAT. (Blue – nuclear stain in all cells). Tumor tissue from the same patient does not stain for LRAT (not shown).

Part 2: Generation of transgenic mice in which human LRAT was ectopically expressed either in the suprabasal layer or in the basal layer of epithelial cells driven by the cytokeratin 10 promoter or the cytokeratin 14 promoter, respectively.

1. Construction of transgenes

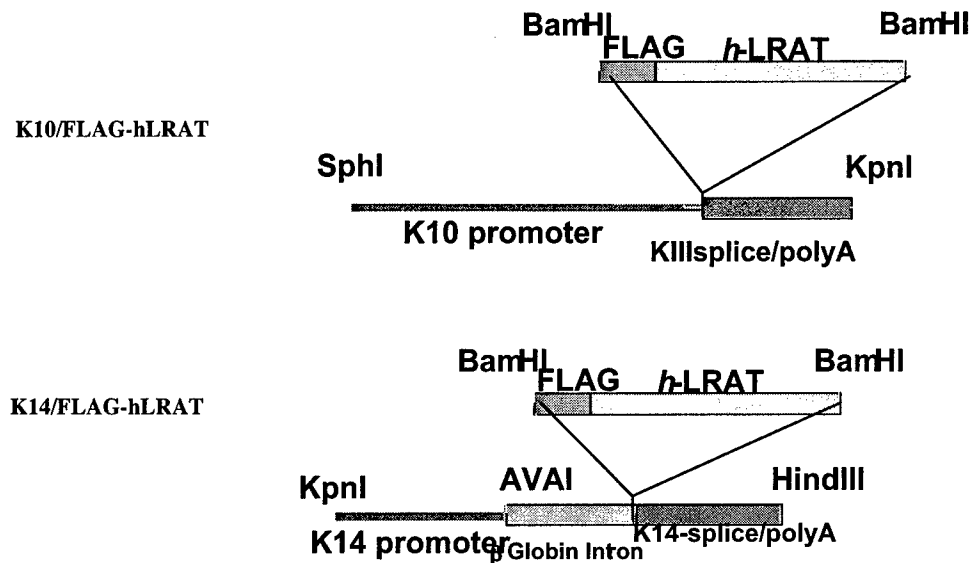


Fig 2. Constructions of transgenes

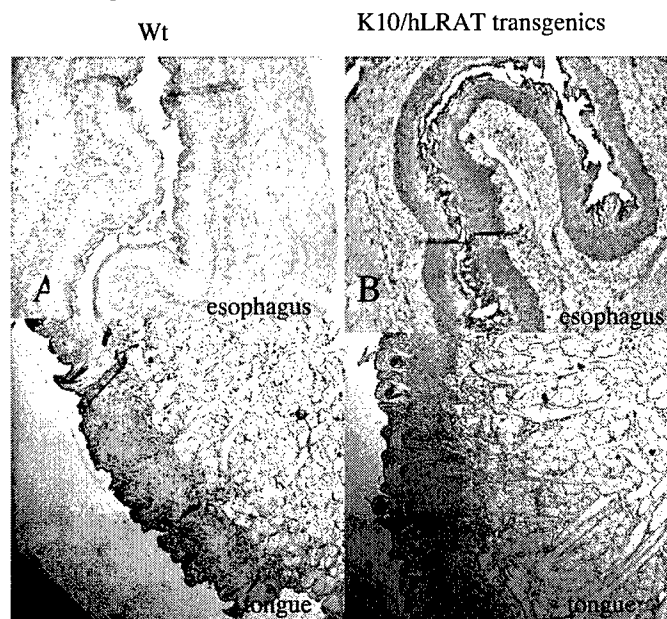


Fig 3. Expression of hLRAT in the suprabasal layer of keratinocytes. Sections from mouse tongue and esophagus were paraffin-embedded, immunostained with anti-hLRAT antibody, and the stain was developed by DAB. (A,C, wild-type mice; B,D, K10/hLRAT transgenics)

2. Topical retinol or RA treatment in wild type and hLRAT transgenic mice

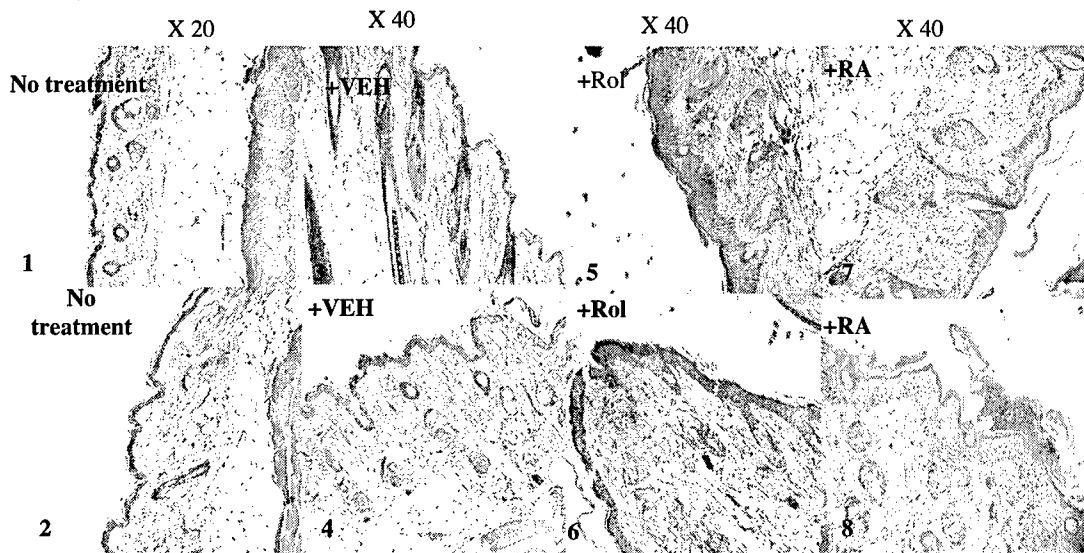


Fig 4a. Reduced epidermal response to *all-trans* retinol in *hLRAT* expressing mice. Micrographs of sections of mouse dorsal skin after topical treatment with retinoids. 400 μ l of retinoids (RA or Rol 0.1mM) in acetone (Vehicle), were applied to the dorsal skin (2 x 3 cm²) of transgenic mice or their wild-type littermates. After 4-day topical treatments, skin was paraffin- embedded and H&E stained. 1,3,5,7: Wild type ; 2,4,6,8: K10-31 hLRAT+ transgenics.

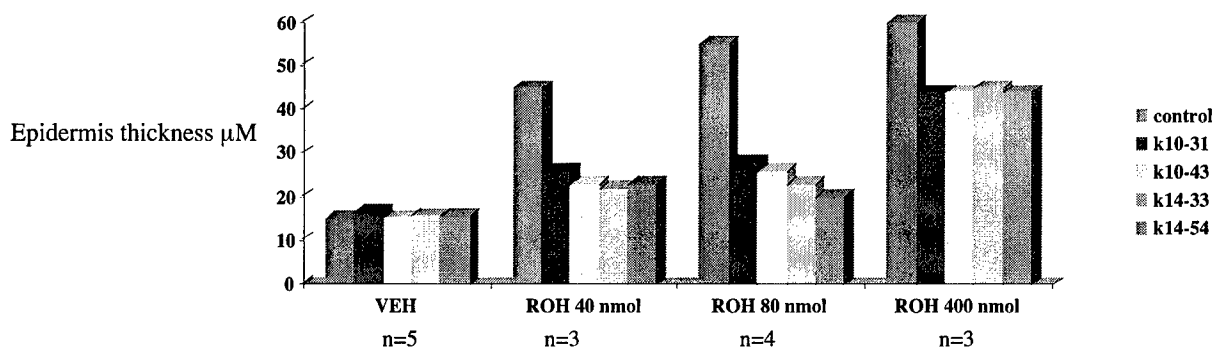


Fig 4b. Quantification of epidermal thickening in transgenic mice from 4 independent lines after topical retinol treatment. 5 different areas per section were randomly picked and measured under microscope.

3. Retinol metabolism after topical retinol or RA treatments in wild type and hLRAT transgenic mice

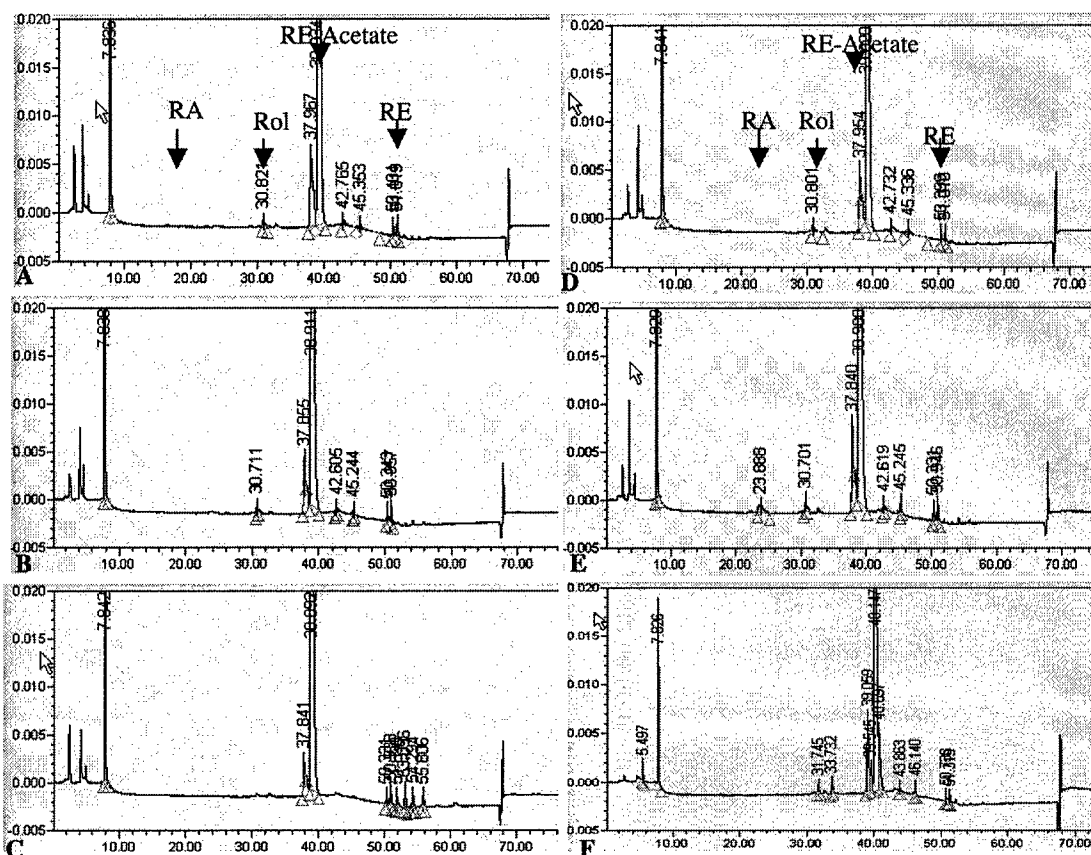


Fig 5. Higher retinyl-ester synthesis in K10-hLRAT transgenic mice than in wild-type mice. Epidermal retinoids in k10-hLRAT transgenic mice and wild-type littermates were detected by High Performance Liquid Chromatography (HPLC) as described by Guo et al., 2001. After a 4-day topical treatment with retinoids (40 nmol in 400 μ L daily) or vehicle (acetone) to the mouse back skin, 0.05g of back skin was processed. A, Wt mouse treated with acetone; B, Wt mouse treated with RA; C, Wt mouse treated Rol; D, K10-31 transgenic mouse treated with acetone; E, K10-31 transgenic mouse treated with RA; F, K10-31 transgenic mouse treated Rol. The retinoids corresponding to the respective retention times are indicated by arrows. Retinyl acetate, an internal control, was added to the skin tissue homogenates.

Key research accomplishments

1. There is reduced *all-trans* retinol (ROL) -induced epidermal hyperplasia in k10-flaghLRAT expressing mice.
2. Expression of hLRAT in suprabasal keratinocytes reduces ROL but not RA's stimulatory effect on keratinocyte proliferation.
3. HPLC studies showed that epidermis from suprabasal hLRAT expressing mice esterify more retinol after the retinol topical treatments than the wild-type mice.

4. Our results indicate that ectopic LRAT-mediated retinol metabolism controls aspects of epithelial cell growth and differentiation.

Reportable Outcomes

Su D and Gudas LJ. Ectopic expression of human lecithin:retinol acyltransferase in mouse suprabasal epithelial cells results in resistance of the skin to topical all-*trans* retinol induced epidermal hyperplasia (Manuscript in preparation)

Shren, M., Su, D., Bok D.,Gallagher,L.and Gudas LJ. LRATstaining in Normal and Tumor Tissue from Human Breast Cancer Patients (Manuscript in preparation)

Conclusions

Transgenic mice were generated in which human LRAT was ectopically expressed either in the suprabasal layer or in the basal layer of epithelial cells driven by the cytokeratin 10 promoter or the cytokeratin 14 promoter, respectively. The effects of ectopic LRAT expression on epithelial cell proliferation and differentiation were examined in these transgenic mice. We showed that with normal level of retinol in the diet, the overexpression of human LRAT in the suprabasal layer or basal layer of the mouse epithelial cells affects mouse epithelial cells differentiation minimally. However, the epidermal hyperplasia induced by topical retinol treatment was greatly reduced in the human LRAT-expressing animals. These data suggest a role for LRAT in the maintenance of normal epithelial cells differentiation. Currently, studies are underway to assess the functions of LRAT in breast carcinoma prevention.

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