# Retinoid pathway alterations related to breast cancer

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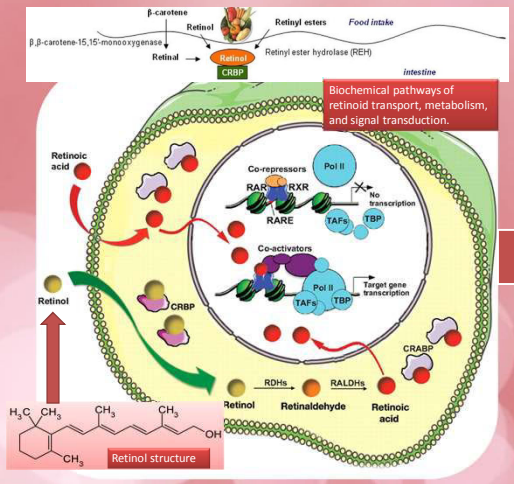
## Introduction

Retinoids, which include vitamin A and its natural and synthetic analogs, have been used as potential chemotherapeutic or chemopreventive agents because of their differentiative, anti-proliferative, pro-apoptotic and antioxidant properties. On the other hand, it has been shown that in many cancers these retinoid signaling pathways are altered.

## Objectives

The aim of this review is to make a brief introduction of retinoids, to show their connection with cancer, to study some of the dysfunctions in the signaling pathway that occur in breast cancer and the origin of these dysfunctions (binding proteins, enzymes, receptors…), and finally to explain how retinoids can be used as a cancer therapy or prevention currently and their expectations in the future.



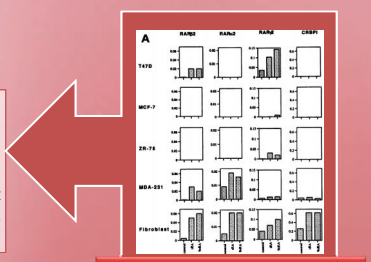

Biochemical pathways of retinoid transport, metabolism, and signal transduction.

## Mechanism of action of retinoids

- Vitamin A is obtained through diet in the form of retinol, retinyl ester, or β-carotene.
- Retinoids absorbed from food are converted to retinol and bound to CRBP in the intestine.
- The liver up takes retinyl esters, which are converted to retinol-RBP complex in the hepatocyte.
- The uptake of retinol by the target cell is mediated by a trans-membrane protein named "stimulated by retinoic acid 6" (STRA6), which is a RBP receptor.
- When retinol is taken up from the blood is bound to CRBP (cellular retinol-binding protein) in the cytoplasm
- The retinol dehydrogenase (RoDH) enzymes metabolize retinol to retinal.
- Then retinal is metabolized to RA by the retinaldehyde dehydrogenases (RALDHs).
- RA is bound in the cytoplasm by CRABP (cellular RA-binding protein).
- RA enters the nucleus and binds to the RA receptors (RARs) and the retinoid X receptors (RXRs), which themselves heterodimerize and bind to a sequence of DNA known as the RARE (RA-response element).
- This activates transcription of the target gene.

## Connection with cancer

Understanding the function of these binding proteins and nuclear receptors is essential for the development of compounds with specific effects. Retinoids have been used in the treatment of breast cancer because they have shown to have an action against malignancies. However, this treatment with retinoids may be ineffective if some of these metabolic or signaling pathways of the of retinoids are altered. Some of these alterations can cause additional effects and others can cause basic effects. For example, several studies have shown that epigenetic silencing of the receptor RARβ is a common occurrence in various human breast cancers. Another important example that has been found in many breast cancers is the loss of the expression of CRBP-I.




Northern blot analysis of RAR (β2, α2, γ2) and CRBP-I expression in human breast cancer cells.

## CRBP-I and breast cancer

### 1. CRBP-I cellular localization:

CRBP-I localizes primarily to lipid droplets, so a major function of CRBP-I is the promotion of retinol storage in these organelles.

### 2. CRBP-I function in retinol storage and RAR activation:

There is a link between a target cell's ability to store retinol and its ability to use retinol to locally activate RARs under physiologic conditions.

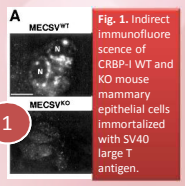



**Fig. 1.** Indirect immunofluorescence of CRBP-I WT and KO mouse mammary epithelial cells immortalized with SV40 large T antigen.

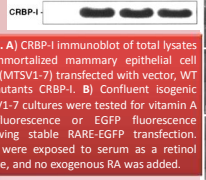
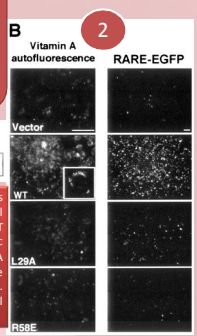



**Fig. 2. A)** CRBP-I immunoblot of total lysates of immortalized mammary epithelial cell lines (MTSV1-7) transfected with vector, WT or mutants CRBP-I. **B)** Confluent isogenic MTSV1-7 cultures were tested for vitamin A autofluorescence or EGFP fluorescence following stable RARE-EGFP transfection. Cells were exposed to serum as a retinol source, and no exogenous RA was added.




### 3. CRBP-I function in epithelial differentiation:

CRBP-I, acting via its effect on retinol storage and through the downstream activation of RAR, promotes breast epithelial cell differentiation and growth inhibition, both *in vitro* and *in vivo*.

### 4. CRBP-I and tumorigenicity *in vivo*:

The somatic loss of CRBP-I function in human breast cancer is an event that contributes to tumor progression by chronically depressing RAR activity and allowing tumor cells to escape differentiation and gain greater growth autonomy.

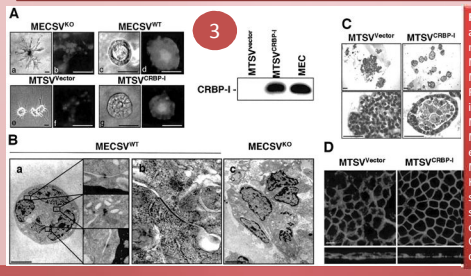



**Fig. 3. A)** Phase contrast and Nile Red fluorescence micrographs of MECSV$^{WT}$, MECSV$^{KO}$, MTSV$^{vector}$ and MTSV$^{CRBP-I}$ cells. Rightmost panel: immunoblot of CRBP-I in MTSV$^{CRBP-I}$ and in primary MECs. **B)** Transmission electronmicrograph of an MECSV$^{WT}$ cell. **C)** Hematoxylin and eosin-stained paraffin thin sections of MTSV1-7 colonies in 3D Matrigel. **D)** Confocal microscopy of E-cadherin immunostaining.

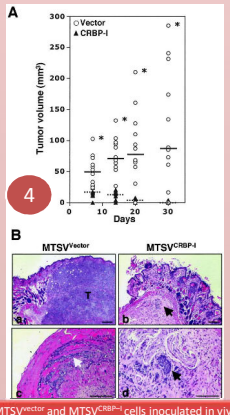



**Fig. 4. A)** MTSV$^{vector}$ and MTSV$^{CRBP-I}$ cells inoculated in vivo. **B)** Hematoxylin and eosin-stained paraffin of tumors formed by MTSV$^{vector}$ (a,c) and MTSV$^{CRBP-I}$ cells (b,d). T: relatively large tumor mass; b and d: small residual tumor cell clusters; c: tumor cell interdigitation into adjacent muscle tissue.

## Conclusions

- Retinoic acid (RA), the active metabolite of retinol, by binding to its nuclear receptors RAR and RXR regulates the transcription of genes involved in anti-proliferative, pro-apoptotic and anti-oxidants processes.
- CRBP-I, acting via its effect on retinol storage and through the downstream activation of RAR, promotes breast epithelial cell differentiation and growth inhibition. Therefore, the somatic loss of CRBP-I function in human breast cancer is an event that contributes to tumor progression by chronically depressing RAR activity and allowing tumor cells to escape differentiation and gain greater growth autonomy.
- This decrease of CRBP-I levels could be due to hypermethylation of DNA, causing their epigenetic silencing. These mechanisms are not known well enough yet, so they should be further investigated.
- Many studies have shown that retinoids can suppress carcinogenic process as well as its prevention, but epigenetic changes can make cells resistant to retinoids. Successful cancer treatment with retinoids may require a combination with drugs that regulate the epigenome, including DNA methyltransferase inhibitors and classic chemotherapeutic agents.
- Ideally, cancer treatment with retinoids would be a personalized therapy for each patient, in order to precisely know if some mechanism of the retinoids pathway is altered and, in that case, what is the most appropriate drug to provoke the desired anti-proliferative and anti-apoptotic effects.

## References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