

**Can Dehydroepiandrosterone (DHEA) target PRL-3 to
prevent colon cancer metastasis?**

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Running title:

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Abstract:

Colorectal cancer (CRC) is a frequently diagnosed cancer and causing significant mortality in the patients. Metastasis caused by CRC is mainly responsible for this cancer-related deaths. Despite recent advancements in the treatment methods, prognosis remains poor. Therefore, effective treatment strategies need to be designed for successful management of this disease.

Dehydroepiandrosterone (DHEA), a 17-ketosteroid hormone produced by adrenal glands, gonads and including gastrointestinal tract is required for several physiological processes.

Deregulation of DHEA levels leads to various disease conditions including cancer. In fact, several experimental studies strongly suggest that DHEA could be used as a chemopreventive agent against colon cancer. Prenylation of certain membrane proteins such as phosphatase of regenerating liver-3 (PRL-3) is crucial for metastatic progression of colon cancer cells. The ability of DHEA to target prenylation pathway could be utilized to inhibit PRL-3 prenylation for successful prevention of CRC metastases. As DHEA is a widely consumed drug for various ailments, incorporation of DHEA in the treatment regimen may be beneficial to prevent or delay the occurrence of metastasis resulting from CRC.

Introduction/Background:

Colorectal cancer (CRC) is the third most common cancer in the United States and is second only to lung cancer as a cause of cancer-related mortality. Occult metastasis is a frequent issue encountered in CRC. The liver is the most common and often the only site of metastatic disease. The presence of colorectal liver metastases (CLM) is associated with a poor prognosis, with a median survival ranging between six and twelve months. However, what causes metastases in CRC patients remains poorly understood. CRC treatment depends on the grade and the stage of the tumor diagnosed in patients. Stage 1 tumors are associated with a good prognosis. Surgical intervention followed by chemotherapy is the standard treatment module currently employed with an outcome of five year survival rate ranging between 25% and 58% [1].

Despite recent advances in diagnostic and therapeutic measures, the prognosis of CRC remains poor and the treatments are associated with serious morbidity and mortality. Hence there is a need to develop alternative effective and non-toxic treatment strategies for successful management of CRC. The identification of biomarkers which might capture tumor properties not reflected by clinicopathological variables has become essential to improve prognosis and will aid clinicians to efficiently manage the disease [2]. Colonic carcinogenesis is characterized by the accumulation of proto-oncogene and tumor suppressor gene mutations. Consistent genetic alterations associated with the transition from primary colorectal cancers to liver metastases was studied by Saha et al [3] who performed the serial analysis of gene expression profiles. This led to the identification of PRL-3 (phosphatase of regenerating liver-3/PTP4A3) which was found to be frequently overexpressed in the liver metastases, but expressed at lower levels in primary

tumors and normal colorectal epithelium [3]. Subsequent studies have re-affirmed that PRL-3 is a metastasis related protein and a promising therapeutic target for cancer metastasis [4, 5].

PRL-3 belongs to the PRL class of proteins which constitutes (PRL-1, PRL-2 & PRL-3) and they share at least 75% amino acid sequence similarity [6]. Of all PRLs, PRL-3 plays a major role in cancer metastasis [7]. PRL-3 has two functional domains, the catalytic domain functions as a protein tyrosine phosphatase (PTP) for dephosphorylation (Figure 1). The specific substrate for PRL-3 *in vivo* has not been clearly identified, although it has been addressed in prior studies about the importance of the PTP domain in promoting cancer cell growth, invasion and metastasis [8], The other functional domain of PRL-3 is the CCVM motif for prenylation. Isoprenylation is a common post-translational modification for proteins that are targeted to membranes. The CAAX sequence on the C-terminal of PRL-3 constitutes a conserved feature of this type of protein family.

Metastatic functions of PRL-3 depends on its association with the inner surface of the plasma membrane [5]. It is initially synthesized as a soluble protein lacking the hydrophobic domain and then they are posttranslationally modified with prenylation for its membrane localization. The prenylation motifs such as CAAX, XXCC, XCXC and CCXX are recognized by farnesyltransferase (FT) or geranylgeranyltransferase (GGT) which aid in the correct localization of target proteins. The CAAX prenylation motif present in the c-terminal region of PRL-3 protein (Figure 1) has shown to be important for membrane interaction of PRL-3 thereby enabling their participation in various signal transduction pathways [5]. Mutation of the prenylation site or inhibition of prenylation substrate synthesis blocks prenylation and thus membrane localization, resulting in loss of normal protein function [9]. Interestingly, disruption of PRL-3 prenylation by mutation studies has shown to eliminate the metastatic potential of

melanoma cells [5]. However to our knowledge there are no studies conducted to date to show prenylation inhibitors could be used to target PRL-3 to manage cancer metastases.

Prior studies have shown that Dehydroepiandrosterone (DHEA) is a prenylation inhibitor [10] and can confer protection against colorectal cancer [11]. DHEA is a 17-ketosteroid (Figure 2) produced at high levels in the adrenal gland, gastrointestinal tract, gonads, and brain [12]. It is the chief precursor of androstenedione, which gets readily converted into testosterone and 17 β -estradiol in the tissues. Being the most abundant steroid in human circulation, it serves multiple functions in maintaining the physiological homeostasis. Previous studies on animal model systems revealed that DHEA is a powerful inhibitor of mammary, prostate, skin, lung, liver, and thyroid carcinogenesis [13-18]. DHEA has been associated with various beneficial effects such as anti obesity [19], lowering of blood glucose [20], and anti-atherosclerosis [21]. DHEA is also known to be effective in improving the negative, depressive and anxiety symptoms in Schizophrenia patients with no obvious adverse effects reported in the study participants [22]. In another study, DHEA is shown to be useful in enhancing the psychological well-being in male and female hypopituitary patients on maintenance growth hormone replacement [23]. However, few side effects such as greasy skin, diarrhea and muscle aches were reported in some patients of this study depending on the gender [23]. Strikingly, low DHEA levels are associated with increased risks of tumorigenesis as studies show that administration of DHEA prevents spontaneous tumorigenesis in p53-nullizygous mice [24]. However the mechanism of how DHEA decreases carcinogenesis is unclear and may be multifactorial. Previously, Schulz and Nyce [10] found that DHEA can inhibit cholesterol biosynthesis and inhibit posttranslational processing events such as isoprenylation of membrane associated oncogenic p21ras protein in colon adenocarcinoma cells. Therefore, it is possible that

DHEA might target posttranslational processing of PRL-3 protein thereby causing an inhibition of its metastatic function in CRC.

THE HYPOTHESIS:

Dehydroepiandrosterone (DHEA), an adrenal steroid, has been shown to inhibit early steps of prenylation pathway [10]. Isoprenylation is a necessary prerequisite for membrane association of key oncogenic proteins such as PRL-3, RhoA and Ras [25-27]. Therefore, we hypothesize that DHEA can prevent the isoprenylation of PRL-3 protein leading to the disruption of its function in colon carcinoma metastasis.

EVALUATION OF HYPOTHESIS:

The structure of PRL-3 protein:

As PRL-3 belongs to the PTP superfamily, they have an N-terminal catalytic domain containing the signature motif CX₅R (Figure 1). The C-terminal domain of the PRLs contains a polybasic region which immediately precedes a CAAX box, where the C is cysteine, A is an aliphatic residue and X is any amino acid. This box represents the prenylation motif, which is unique only for the PRL-PTP family [28]. The association of the PRLs with the plasma membrane and early endosome in the cells is attributed to this prenylation motif which is subjected to farnesylation, a post-translational lipid modification known as resulting in the membrane localization of protein. The polybasic region may also serve as a nuclear localization signal to direct PRLs to the nucleus in the absence of prenylation [9].

Prenylation of PRL-3 was found to be important for metastatic properties of cancer cells [29]. The localization of PRL-3 to the plasma membrane is a critical requirement for its function. Isoprenylation is the first step in a series of posttranslational processing events promoting the stable association of PRL-3 protein with the cell membrane and will further enable the cell-transforming activity of oncogenic PRL-3 protein.

DHEA and PRL-3:

De novo cholesterol synthesis pathway is of vital importance for providing substrates for protein prenylation. It has been reported that DHEA treatment can inhibit cholesterol biosynthesis in lactating mammary gland [30]. The first and rate-limiting step in this pathway is catalyzed by HMG-CoA reductase. Previous studies show that DHEA inhibits p21ras prenylation in colon adenocarcinoma cells [10]. It functions as a HMG-CoA reductase inhibitor (Figure 3), in a manner similar to Lovostatin [10]. Inhibition of this enzyme leads to the depletion of mevalonate and further inhibits the prenylation of p21ras. Therefore, this drug could be used as a promising chemopreventive agent. In fact, several experimental studies suggest DHEA to be a promising anti-cancer agent [13, 31-33]. In addition, DHEA is also shown to inhibit migration and invasion properties of metastatic cancer cells [34]. However, it is not clear how DHEA targets metastatic properties of cancer cells. Since DHEA is shown to be a prenylation inhibitor, we hypothesize that consumption of DHEA will prevent colon cancer metastases by targeting the prenylation of PRL-3. This hypothesis can be tested using *in vitro* and *in vivo* model of colon cancer metastases. If DHEA shows promise in pre-clinical studies, then these results can be easily translatable in colon cancer patients as DHEA is an approved drug used for various health benefits.

Implications of the hypothesis:

DHEA is the most abundant adrenal steroid with apparent anticarcinogenic properties. Our hypothetical study suggests that DHEA could potentially inhibit isoprenylation of PRL-3 protein, involved in colon cancer metastasis, at a point in the protein prenylation pathway distal to HMG-CoA reductase activity. Our predictions might provide a plausible explanation for the antitumor action of DHEA on PRL-3 mediated metastasis in colon carcinoma. Hence, the use of DHEA as

an inhibitor of isoprenylation will represent a rational, targeted approach for the development of DHEA as a mechanism-based chemopreventive drug for CRC.

Conflict of Interest Statement:

The authors have no conflict of interest to declare.

Acknowledgment

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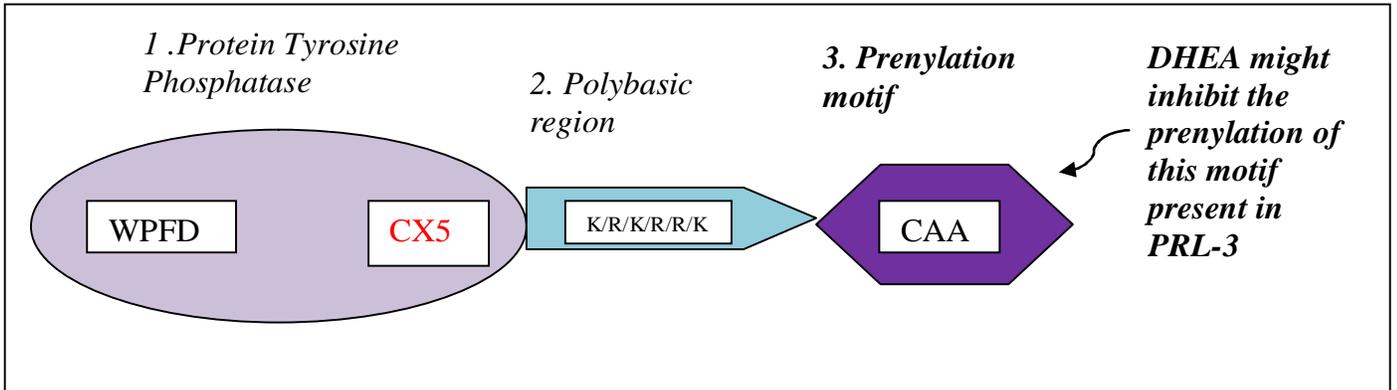


Figure 1. *Domain structures in PRL-3 Protein*

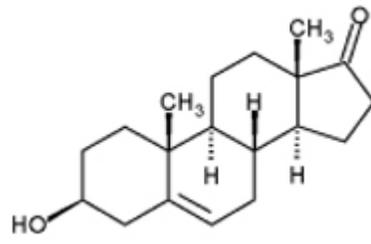


Figure 2. *Structure of DHEA*

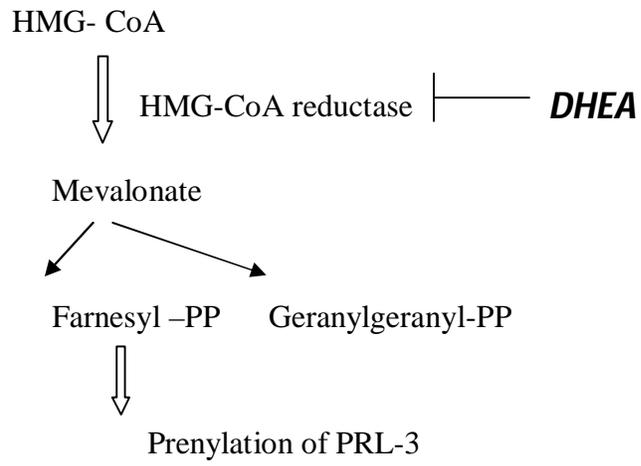


Figure 3. *Prenylation pathway*