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CANCER BUSTING SUPER FATS: A QUEST FOR REAL CANCER KILLER

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Fatty acids with conjugated double bonds such as Conjugated Linoleic Acid (CLA; cis-9, trans-11)) and Conjugated Linolenic Acids (CLNAs) such as □-eleostearic acid (ESA; Cis-9, trans-11, trans-13) and punicic acid (PA; cis-9, trans-11, cis-13) are reported with potent anti-cancer properties and this effect has been proven by *in vitro* and *in vivo* studies. The anti-cancer action of CLNAs is far more potent than that of CLA though only difference accounted to be the presence of an additional double bond at Δ^{13} position.

Interestingly, by numerous studies performed since 1999 revealed that these CLNAs would not survive within a cell (*in vitro* studies) or an animal (*in vivo* studies), hence rapidly converted in to CLA by a quick saturation of Δ^{13} double bond by an unknown mechanism and then incorporated into cellular lipids. Then the question arises on how the CLNAs could exhibit more potent anti-cancer properties than its metabolic end product, CLA. Thus, it reveals the fact that potent anti-cancer property of CLNAs are not mediated by themselves, but possibly though another metabolite.

On the other hand, retinoids, especially, the *all-trans*-retinoic acid reported with potent anti-cancer properties. Malignancies are associated with deficiencies of retinoids. Retinol saturase (RetSat) specifically reduces the C13-C14 double bond in *all trans* Retinol to form *all trans* 13-14 dihydroretinol, which will subsequently be converted to *all-trans*-13-14 dihydroretinal and then to *all-trans* -13-14 dihydroretinoic acid. RetSat reported to have a ubiquitous distribution in animal body. If the *all-trans*-Retinol can evade this saturation reaction by RetSat, it can be converted into its active form, *all-trans* -Retinoic acid (atRA). The *all-trans*-Retinoic acid is far superior ligand than all-trans -13-14 dihydroretinoic acid form for RAR and RXR receptors in nucleus. Cellular differentiation, cell proliferation and apoptosis, which play a key role in tumor growth suppression are efficiency regulated by *all-trans* -Retinoic acid signalling, but not by 13-14 dihydroretinoic acid. Thus RetSat activity may downgrade the potency of cellular level retinoid signalling.

Some researchers speculate that the hydrogenation or saturation of Δ^{13} double bond of CLNAs is mediated by RetSat. Thus, it can be hypothesized that CLNAs may compete for RetSat for quick hydrogenations of Δ^{13} double bond as fake substrate with its usual substrate *all-trans*-Retinol which, may be favoured by the RetSat activity. Then *all-trans*-Retinol may evade its conversion to all-trans-13-14 Dihydroretinol and further metabolized into active *all-trans*-Retinoic acid which could control numerous cellular processes helping the suppression of carcinogenesis.

For these reasons, it is hypothesized that the potent anti-cancer property of CLNAs may be mediated by this quick conversion of it to CLA, creating a competitive inhibition on hydrogenation of *all-trans*-Retinol by RetSat. As a result, abundance of *all-trans*-Retinoic acid would be increased at cellular level and it would induce key cellular mechanisms such as differentiation, apoptosis and controlling the proliferation of cells and thereby suppresses the occurrence of malignant cell growths.

Keywords: Fatty acids, cancer, CLA

Fatty acids play a key role in cellular metabolism by acting as an energy source, a key component in cell membrane, as precursors for eicosanoid synthesis and as a

regulator of gene expression. Thus, imbalances and deficiencies of different classes of fatty acids could impart a significant impact on gene expression, which will subsequently influence the related physiological processes and induce numerous pathological sequelae in vertebrate organisms (Kitajka K *et al*. 2004., Jayasooriya *et al*. 2005).

Discovery of anti-carcinogenic conjugated linoleic acid (18:2, cis-9, trans-11; CLA) in fried ground beef back in 1987 (Ha *et al*. 1987) initiated the interest of scientific community on these conjugated fatty acids and their metabolic effects. Thus, now It is well known that conjugated fatty acids have anti-cancer actions and the said effect has been proven by substantial evidence generated by numerous *in vitro* and *in vivo* studies (Moon *et al*, 2014., Pariza *et al*, 1999., Hagen *et al*, 2013., Zhuo *et al*, 2014., Tsuzuki *et al*, 2008., Yuan *et al*, 2014) as well by a limited number of human trials (Faramarzi, E *et al*, 2017). Conjugated Linolenic acids (CLNAs) such as a-eleostearic acid (18:3, cis-9, trans-11, trnas-13) present in Bitter melon (*Momordica charantia*) seeds (Fig.1) and Punicic acid (18:3, cis-9, trans-11, trnas-13) present in pomegranate seeds (*Punica granatum*) contain an additional double bond at Δ^{13} as compared with CLA. Interestingly, the anti-cancer action of these CLNAs having an extra double bond at Δ^{13} position is far more potent than that of CLA (Tsuzuki et al, 2004 & 2008). The only structural difference between aforementioned CLA and CLNAs is the presence of an additional double bond at Δ^{13} position in those two CLNAs. Thus, cancer busting super power of these CLNAs could have been attributed to this additional Δ^{13} -double bond.

Evidences are emerging on an another bizarre metabolic scenario related to a rapid conversion of CLNAs with Δ^{13} double bond into CLA (cis-9, trans-11) *in vivo* and *in vitro* (Jayasooriya, 2000., Tsuzuki *et al*, 2003., 2004 and 2006., Ghomdim Nzali, *et al*, 2012., Yuan *et al*, 2009., Schneider et al, 2013).

Fig.1 Bitter melon (*Momordica charantia)* seeds and fleshy part; a rich source of both CLNA (ESA: α-eleostearic acid) and β-carotene (Pro-Vitamin A)

Interestingly, this rapid but relatively a complete saturation of Δ^{13} of □-eleostearic□ acid □by forming CLA once given orally into experimental rats was first observed 17 years ago (Jayasooriya, 2000). According to the observations made in this study, not even a trace of the dietary CLNA; □-eleostearic acid was detected in hepatic lipids such as triglyceides, cholesteryl ester or phospholipids or the total lipids in adipose tissues (Fig 3 D, E). Instead, the metabolic end product, CLA (cis-9c, trans-11) was detected. It clearly indicated the fact that Δ^{13} double bond of □-eleostearic has been rapidly hydrogenated by a specific enzyme possibly within different locations firstly at intestinal cells and then at other locations such as liver due to involvement of hydrogenation reaction. To further ascertain this *in vivo* fate of α-eleostearic acid, an administration of □□eleostearic acid into experimental was done. After 1h of feeding, blood and lymph samples were isolated to analyse the fatty acid profile of lipids in those tissues. Interestingly CLA appeared and □-eleostearic acid itself also was detected in the lymph sample (Fig. 3 B). Thus, those observations suggested the fact that some of α-eleostearic acid were

absorbed after its conversion to CLA within the intestinal cells, and a major proportion was absorbed readily without any conversion and incorporated into lymph in the form of chylomicrons. The proportion of □-eleostearic acid declined drastically in blood in comparison with that in the lymph (Fig. 3C). Thus, these observations further suggested a possibility of conversion of dietary □-eleostearic acid in the two places; initially at the intestinal lumen or intestinal cells, and secondly at another location probably the liver. Similar observations regarding this *in vivo* fate of CLNAs have been made by a series of above mentioned other studies too (Tsuzuki *et al*, 2003., 2004 and 2006., Ghomdim Nzali, *et al*, 2012., Yuan *et al*, 2009., Schneider *et al*, 2013). Furthermore, it has also been reported that this type of quick saturation of a double bond only occurs at double bond position of Δ^{13}. The administration of another CLNA with cis-9, trans-11, cis-15 arrangement of double bonds has not ended up in CLA *in vivo* by the saturation of Δ^{15} double bond as it has happened at Δ^{13} double bond of CLNAs (Schneide *et al*, 2013). This indicates the fact that this quick metabolic reaction occurred possibly by a saturation of double bond at Δ^{13} and that reaction is specific to Δ^{13} position.

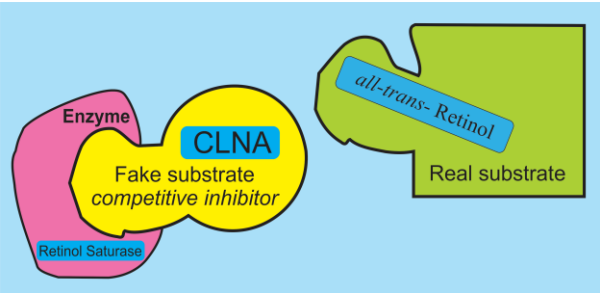

Fig 2. Postulated competitive inhibition by CLNA having a conjugated double bond at Δ^{13} position as the fake substrate for Retinol Saturase (RetSat) enzyme with its real substrate; all *trans*-Retinol.

It has been speculated that most possible candidate enzyme responsible for this quick conversion could be the ubiquitously found Retinol Saturase (RetSat) (Ghomdim Nzali *et al*, 2012). The RetSat is usually involved in hydrogenation of double bond located between 13-14 carbons of *all-trans* Retinol (ATR), to form *all-trans*-13, 14-dihydroretinol (Moise *et al*, 2004), which would result in paving the way to initiate an alternative pathway in retinoid metabolism. *all-trans*- Retinoic Acid (atRA) is an obligatory metabolite of *all-trans*- Retinol pathway, whereas 13-14 dihydroretinoic acid is formed instead of atRA if the RetSat acts on *all-trans*- Retinol and form *all-trans*-13, 14-dihydroretinol. The atRA is the most active ligand among retinoids and it directly influences the cellular functions via nuclear receptors RAR and RXR (Coyle *et al*, 2013). Favourable actions of atRA on control of cell cycle, cellular differentiation and apoptosis have been well documented (YanMei *et a*l, 2010., Mrass *et al*, 2004., Liu *et al*, 2014). Indeed, Inhibition of atRA signaling promoted tumorigenesis, whereas atRA supplementation reduced tumor burden (Coyle *et al*, 2013). Interestingly, the potency in activating RAR-controlled genes by 13-14 dihydro retinoic acid reported to be much lower than that of *all-trans* -Retinoic acid (Moise *et al*, 2009) indicating the fact that dihhydro retinoic acid is a weak ligand for RAR activation. These observation strongly suggest the fact that RetSat may hinder the activity of atRA by diverting its precursor in to an alternative pathway.

Based on the aforementioned observations on this interesting bizarre metabolic fates of CLNAs with Δ^{13} double bond *in vivo* and *in vitro* conditions, and the metabolic fate of *all-trans*-Retinol in the presence of RetSat, a hypothesis is presented on a possible competition between CLNAs with Δ^{13} double bond and the *all-trans*-Retinoic acid for RetSat enzyme (Fig. 2 and Fig. 4). Furthermore, by considering the promptness of hydrogenation of Δ^{13} double bond CLNAs, those fatty acids may be favoured by RetSat enzyme for its biological reduction role (Fig.2 & Fig. 4).

Hypothesis

It is hypothesized that the saturation of Δ^{13} bond of all the CLNAs such as □-eleostearic acid and punicic acid would impose a competitive inhibition to the usual reaction

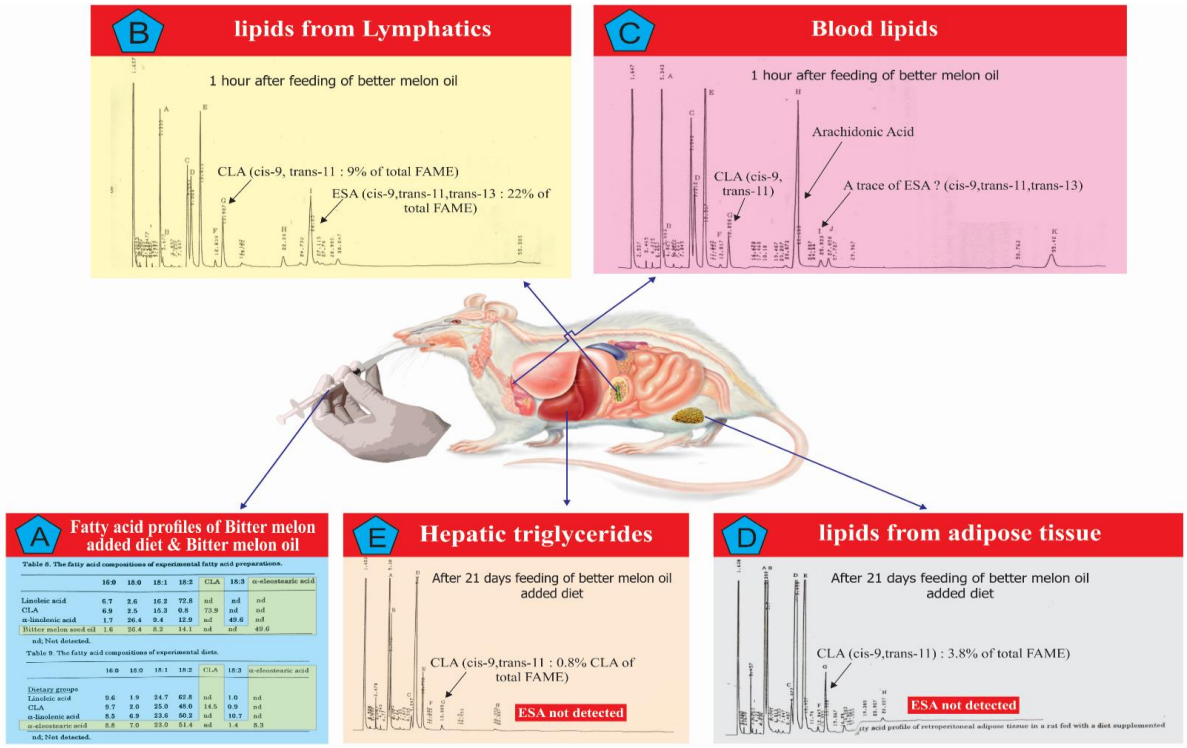

Figure 3. Graphical depiction of rapid in vivo conversion and/or deposition of ESA; α-eleostearic acid (cis-9, trans-11t, trans-13 CLNA) in to CLA (Conjugated Linoleic Acid; cs-9, trans-11) at different time points after oral dose of a Bitter melon oil or added with Bitter melon oil). Highlighted areas (in yellow) in the tables presented in box:A of the figure.3 indicate the absence of CLA in both diet added with Bitter melon fat as well as in the Bitter melon seed oil that were fed to rats to examine the metabolic fate of α-eleostearic acid in vivo . All these figures (B, C, D & E) and tables (A) were extracted from M.Agric thesis of L.J.P. Anura Prasantha Jayasooriya, the author of this article (Jayasooriya, 2000).

that convert *all-trans*-Retinol into *all-trans*-13,14-dihydroretinol. Some postulations have emerged on the saturation of Δ^{13} bond of these CLNAs mediated by Retinol Saturase (RetSat) which is ubiquitous in many tissues with the highest levels in liver, kidney, and intestine. By considering the promptness in the conversion of CLNAs with a double bond at Δ^{13} position to CLA by hydrogenation of Δ^{13} double bond, these CLNAs may have a high affinity to this enzyme than its usual substrate, *all-trans* Retinol. Thus, ultimate result could be a quick saturation of double bond of Δ^{13} of CLNAS by the action of RetSat. This possible competitive inhibition of RetSat may hinder the formation of *all-trans*-13, 14-dihydroretinol and then enhances the chances in survival of *all-trans*-Retinol evading the alternative pathway which is responsible to produce the weaker ligand related to retinoid signalling on RAR and RXR; *all-trans*-13, 14-dihydroretinoic acid.

It is well known that among all retinoids, *all-trans*-Retinoic acid plays a critical role regulation of cell differentiation, proliferation, and apoptosis via retinoic acid receptors (RARs), which works as heterodimers with retinoid X receptors (RXRs) (Coyle et al 2013). The conversion *all-trans*-Retinol to 13-14 dihydroretinol could act to avoid the formation of active *all-trans*-Retinoic acid and reduce the activation of RAR. Thus, if the RetSat diverts *all-trans*-Retinol towards an alternative pathway, formation of *all-trans*-Retinoic acid would be hindered and result in loss of control of a number of key cellular regulatory functions such as cell differentiation, proliferation and apoptosis.

Deficiency of *all-trans*-retinoic acid in cells have a direct link to the carcinogenesis and subsequent restoration of cellular retinoic

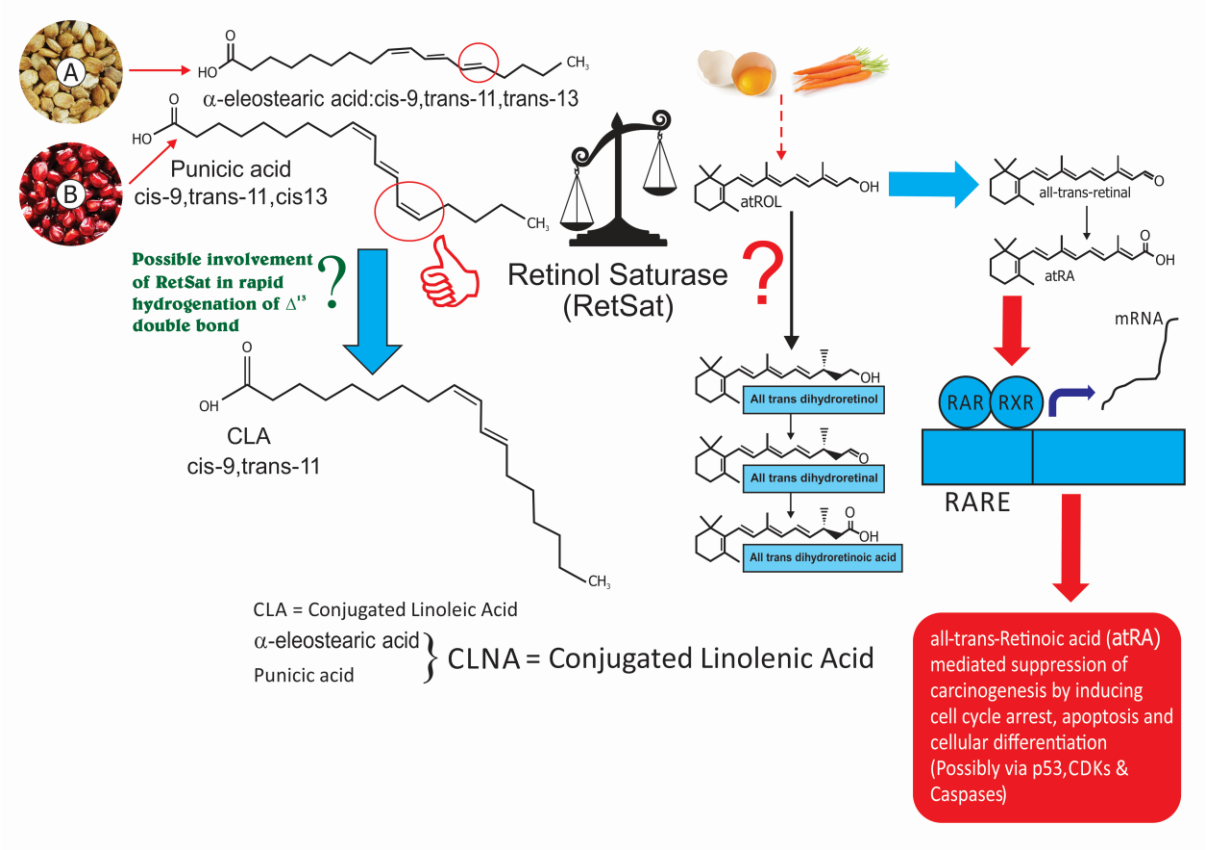

Fig 4: Hypothetical competitive inhibition of RetSat inhibition by CLNAs by diverting its reducing action of RetSat towards the hydrogenation of Δ^{13} double bond of CLNA. It will evade the conversion of *all-trans*-Retinol in to 13-14 dihydroretinol and make more active *all-trans*- retinoic acid abundant for controlling cellular mechanisms such as cell cycle arrest, differentiation and apoptosis. The ultimate outcome would be suppression of tumorgenesis and then ultimately the malignant cell growth throughout the body. RAR: Retinoic Acid Receptor, RXR: Retinoid X Receptor, RARE: Retinoic Acid Response Element. A: Bitter melon (*Momordica charantia*) seeds, B: Pomegranate (*Punica granatum*) seeds.

acid has resulted in disappearance of tumors indicating a key role of *all-trans*-Retinoic acid in supressing carcinogenesis (Bhattacharya et al 2016). Thus, by its possible competitive inhibitory reaction on usual reaction of RetSat by CLNAs with Δ^{13} double bond would enhance the survival of *all-trans*-Retinoic acid, the active ligand of retinoid metabolism and thereby favourably mimic the usual cellular control mechanisms which are otherwise induce carcinogenesis by losing proper control on cellular mechanisms by *all-trans*-Retinol. This hypothesis is well depicted in Fig.4.

Theoretical evidences for the hypothesis

It has now become a well-established fact that CLNAs with double bonds at Δ9, Δ11 and Δ13 positions are rapidly converted into CLA with Δ9 and Δ11 by a quick reduction (hydrogenation) of double bond at Δ13 position *in vivo* as well as *in vitro* (Jayasooriya, 2000., Tsuzuki *et al*, 2003., 2004 and 2006., Ghomdim Nzali, *et al*, 2012., Yuan *et al*, 2009., Schneider et al, 2013) It has also been reported an existence of the retinol saturase enzyme (RetSat), which is ubiquitous in different tissues including high levels in intestine and liver that saturates the C13-C14 double bond of *all-trans* Retinol to make *all-trans*-13,14-dihydro retinol, paving the way to create an alternative pathway of retinoid metabolism. This would create retinoids with low affinity and activity towards the RAR and RAX nuclear receptors that regulate cell

proliferation and differentiation as well as apoptosis (Moise *et al*, 2009).

Ghomdim-Nzali, *et al* (2012) postulated citing the previous work (Moise *et al*. 2004, 2009) which described that the RetSat enzyme which carries out the saturation of the C13-C14 double bond of the *all-trans*-retinol to produce *all-trans*-13, 14-dihydroretinol could be the candidate enzyme responsible for the conversion of the CLNAs with Δ^{13} double bond to form CLA with cis-9, trans-11 structure. There are speculations that different lipid molecules compete for same pathways. For example, CLA and retinoids may compete for same catabolic enzymes, hence CLA can increase the accumulation of retinoids in tissues such as liver by an interference by CLA on the catabolism of retinoids (Carta *et al*, 2014). Thus, there is a greater possibility to compete CLNAs with retinoids for RetSat enzyme.

The aforementioned hypothesis on possible advantages of competition between CLNAs and *all-trans*-Retinol for RetSat that leads to a hinder the formation of *all-trans*-13-14 dihydro retinoic acid, but leads to an increased abundance of *all-trans*-Retinoic acid in cells would be further reinforced by the evidence published on a possible strong relationship between carcinogenesis in different tissues with the deficiency of retinoids or abnormalities of retinoid metabolism in those locations. For an example, it has been clearly indicated that in colorectal cancer condition, there had a marked deficiency in colonic *all-trans*-Retinoic acid (Bhattacharya *et al*, 2016) Retinoids influence the cell differentiation, proliferation, and apoptosis and play an important physiologic role in a wide range of biological processes (Doldo *et al*, 2015., di Masi, *et al*, 2015).

Furthermore, studies on retinoid signalling during cancer progression has attracted a great interest (Doldo *et al*, 2015). A recent study (Bazhin *et al*. 2016) reported a relationship between *all-trans*-13,14-dihydroretinoic acid and the occurrence of Pancreatic Adenocarcinoma (PDAC) and they suggested that carcinogenesis, including PDAC development may be related with tissue levels of *all trans* 13-14 dihydroretinoic acid and retinoid signalling. Interestingly, they have found that there is a strong correlation between tissue level of *all-trans*-Retinoic acid and *all-trans*-13,14-dihydroretinoic acid in both murine and human PDAC conditions indicating a ubiquity of an active dihydroretinoid pathway in carcinomatous tissues. This correlation was not found in healthy murine or human pancreatic tissues. Hence, a possible contribution of the process that convert *all-trans*-Retinol into 13-14 dihydroretinol by RetSat enzyme and then into dihydroretinal and then into 13-14 dihydroretinoic acid forms in the pathophysiology of carcinogenesis may be unravelled by the aforementioned finding. Furthermore, there are reports that the normalizing deficiency of *all-trans* Retinoic acid would imparts a protection against the occurrence of colorectal cancer (Bhattacharya *et al*, 2016).

Overall, the existence of a ubiquitous RetSat would hinder the availability of cellular level active all-trans Retinoic Acid, which would impair the control of cellular differentiation and apoptosis. Thus concomitant presence of all-trans-Retinoic and CLNAs acids with Δ^{13} double bond would make more and more *all-trans* Retinoic acid available at cellular level for a proper control of cellular differentiation and induce the apoptosis as well in controlling cell proliferation. This would ultimately supresses the unwanted cell growth that leads tumorgenesis.

Testing the hypothesis

1) By *in vivo* studies

Moise *et al* (2010) reported that that RetSat-null mice have normal levels of retinol and retinyl palmitate in liver, serum, and adipose tissue, but, in contrast the wild-type mice, are deficient in the production of all-*trans*-13,14-dihydroretinol from dietary vitamin A. It indicates the fact that RetSat activity is supressed to a certain levels and it would help the organism to produce at least a minimal level of all-trans-Retinoic (atRA) needed for vital functions at cellular level. However, in cancerous tissues, RetSat

activity seems to be functioning at a higher rate to generate more and more *all-trans*-diydroretinoic acid resulting a better correlation between atRA and *all-trans*-diydroretinoic acid in Pancreatic Adenocarcinoma (Bazhin *et al*. 2016).

Thus, we must ascertain the fact that whether or not the wild type mice have a normal level of retinol, retinyl palmitate in above mentioned tissues. These findings could be extrapolated to human subjects who are under a normal dietary intake of retinoids or beta-carotene. It would be interesting to find out that whether or not such kind of situation is prevalent in various human populations across the globe with different socio-economic status that determine the Vitamin A intake. Indeed, it is needed to see whether or not the RetSat activity, which is ubiquitous throughout the body tissues would create a deficiency of active retinoids by the conversion of all-*trans*-Retinol into the less active all-*trans*-13,14-dihydroretinol throughout the body tissues, where the RetSat activity is present and thereby activates the alternative retinoid pathway to produce all-trans-13,14-dihydroretinoic acid which is regarded as a weak ligand for RAR and RXR. This scenario would pre-dispose such tissues for abnormalities such as defects in control of cell cycle, cellular differentiation and apoptosis which would lead to various type of malignancies.

Initially, possible involvement RetSat in conversion of CLNAs with Δ^{13} double bond to conjugated linoleic acid (CLA) only with Δ^{9}, Δ^{11} double bonds can be confirmed. According to the hypothesis presented in this paper, CLNAs with Δ^{13} competes with all-*trans*-Retinol for RetSat (Fig. 2 and Fig.4) and it may hinder the usual conversion of *all-trans*-Retinol to all-trans-13,14-dihydroretinol. Thus, it would be very interesting to evaluate the impact of these conjugated trienoic fatty acids with Δ13 double bonds on preserving the active retinoids by preventing it being converted into dihydroretinoids which are reported to be weak ligands in controlling mechanism of cell division, differentiation and apoptosis as well as immune-modulatory actions.

Furthermore, it will be possible to induce the cancer in RetSat null mice and its wild type to compare the impact of formation of all-*trans*-Retinol and then all-*trans*-Retinoic acid at sufficient levels possibly in RetSat null mice to prevent the carcinogenesis induced by a cancer inducing agents as compared with its wild-type which, may experiences a deficiency of these active forms of retinoids due to RetSat activity.

It is also possible to design and synthesize (artificially) a potent inhibitor of RetSat and evaluate its inhibitory actions on carcinogenesis.

RetSat reported to be an enzyme specific to the substrate, all-trans-Retinol. According to some postulations by other workers (Ghomdim Nzali *et al*, 2012) and the hypothesis presented in this article, RetSat could intervene the hydrogenation of conjugated double bonds in other structures as well. Thus, would be interesting to see whether or not the all-trans-Retinoic acid also act as a substrate for the RetSat enzyme and thus, it is also converted to its dihydro form with a low affinity towards the RAR and RXR receptors. Understanding of this scenario would pave the way to understand the limited success of retinoic acid in treating some cancer types (Coyle *et al*, 2013) and possibly be able to rectify the problem associated with those treatments.

CONCLUSIONS

Under normal conditions, dietary retinoids are metabolized through an alternative pathway due to the activity of a ubiquitous enzyme named RetSat. This may hinder the formation of all-*trans*-Retinoic, a strong ligand for RAR and RXR. It plays a key role in control of number of essential cellular level activities such as control of cell cycle, differentiation and apoptosis. Defects in the above mechanisms would result in uncontrolled proliferation of cells that lead to tumorgenesis. However, presence of CLNAs with a Δ^{13} double bond may hinder this possibly unfavourable conversion of all-trans- Retinol into all-*trans*-13,14-dihydroretinol by competing for RetSat enzyme. It is evident by the rapid and complete *in vivo* conversion of CLNAs

to CLA by the saturation of Δ^{13} double bond possibly by the mediation by RetSat enzyme. Thus, by preserving all-*trans*-Retinol, cells could get an increased the levels of all-*trans*-Retinoic acid, a strong ligand for RAR and RXR, in the expense of weak ligand, *all-trans*-13,14-dihydroretinoic acid. This scenario would pave the way to maintain the normalcy in cell proliferation and turnover to avoid any possible uncontrolled cell divisions and thereby supress the process of tumorgenesis. Aforementioned action would eliminate possibilities of malignant cell growths throughout the body. The mechanism of action that may responsible for the reported potent anti-cancer properties of CLNAs may be interrelated to the aforementioned circumstances related to metabolism of retinoids and CLNAs by RetSat enzyme activity. Proper understanding on these metabolic scenario would create a great opportunity to tackle the menace of uncontrolled cell growth that leads to deadly malignancies.

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