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Basic aspects of tumor cell fatty acid-regulated signaling and transcription factors

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Abstract This article reviews the current knowledge and experimental research about the mechanisms by which fatty acids and their derivatives control specific gene expression involved during carcinogenesis. Changes in dietary fatty acids, specifically the polyunsaturated fatty acids of the ω-3 and ω-6 families and some derived eicosanoids from lipoxygenases, cyclooxygenases, and cytochrome P-450, seem to control the activity of transcription factor families involved in cancer cell proliferation or cell death. Their regulation may be carried out either through direct binding to DNA as peroxisome proliferator-activated receptors or via modulation in an indirect manner of signaling pathway molecules (e.g., protein kinase C) and other transcription factors (nuclear factor kappa B and sterol regulatory element binding protein). Knowledge of the mechanisms by which fatty acids control specific gene expression may identify important risk factors for cancer and provide insight into the development of new therapeutic strategies for a better management of whole body lipid metabolism.

Keywords Fatty acids · Signaling · Gene expression · Cancer

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1 Introduction

1.1 Basic concepts of fatty acid signaling and its bioactive derivatives

The molecular basis of the activities regulated by fatty acids (FAs) and their derivatives such as eicosanoids include changes in receptor signaling, the composition of rafts, cell metabolism, and membrane structures that lead to changes in the function of transcription factors and their target genes, a key step essential for the function of FA-activated signaling. Here, we review the basis of FA signaling and its associated gene expression changes that play a role during carcinogenesis.

1.1.1 Lipid metabolism: biosynthesis of fatty acids

Lipid metabolism involves exogenous lipids obtained through the diet and endogenous lipids synthesized *de novo* in the body. Fatty acids are composed of a hydrocarbon chain with a carbonyl group at one end and a methyl group at the other and are fundamental components of the different lipids. They form ester links with an

alcohol group, finally forming the triacylglycerols, phospholipids, and cholesterol esters, among other lipids.

In the cell, endogenous or exogenous FAs are activated first to the acyl-coenzyme A (CoA) thiol-ester and then incorporated into membrane phospholipids. Exogenous FAs require that their transport among and into tissues occurs through specific mechanisms that allow their rapid and controlled distribution so that their uptake can be regulated according to metabolic needs and to avoid the possible harmful effects of excess FA accumulation. The uptake of FAs can be by simple diffusion or principally by a selective expression and/or regulation of specific sets of membrane proteins. The membrane proteins implicated in FA uptake in mammalian cells include fatty acid translocase (FAT/CD36), fatty acid transport protein, and fatty acid-binding protein–plasma membrane [1, 2]. Further evidence suggests that not only proteins but also lipid microdomains play an important part in the uptake process. The increased raft association of FAT/CD36 leads to an increased FA uptake and dynamic association of FAT/CD36 with lipid rafts may regulate the process [3].

De novo synthesis of FAs begins with acetyl-CoA originated either from FA oxidation or carbohydrate and protein metabolism. The synthesis of new FAs is carried out by the successive addition of 2-carbon fragments (acetyl CoA) at the carboxyl end of the growing acyl chain. Polyunsaturated fatty acids (PUFAs) have one or more double bonds in the carbon chain, whereas saturated fats have no double bonds [4]. For instance, the synthesis of 16:0 (palmitic acid) from saturated FAs begins in the cytosol and is catalyzed by FA synthase (FAS). Next, chain elongation and desaturation occur in the membrane of the smooth endoplasmic reticulum. In this way, 16:0 is elongated to form 18:0 SA, (stearic acid). Also, the insertion of double bonds between carbons 9 and 10 of the acyl-chain by Δ9-desaturase transforms 16:0 into 16:1, ω-7, PA, (palmitoleic acid) and 18:0 into 18:1, ω-9 OA, (oleic acid) (Fig. 1).

There are at least four families of PUFAs, depending on the parent FA from which they are synthesized: the ω-3 series derived from α-linolenic acid (ALA, 18:3, ω-3), the ω-6 series derived from linoleic acid (LA, 18:2, ω-6), the ω-9 series derived from OA, and the ω-7 series derived from PA [5]. Plants can insert double bonds between carbons 12 and 13 via conversion of OA to LA by Δ12-desaturase; further desaturation can occur via the insertion by Δ15-desaturase of a double bond between carbons 15 and 16 to form ALA. However, mammals lack Δ12 and Δ15-desaturase; therefore, they cannot synthesize *de novo* LA and ALA. These two FAs are required for mammalian survival and are thus called essential fatty acids (EFAs). Both must be incorporated through the diet before the synthesis of their series PUFAs and derivatives can begin. Mammalian cells metabolize EFAs by successive elongation and desaturation reactions. Implicated in these pathways are the same set of enzymes, elongases

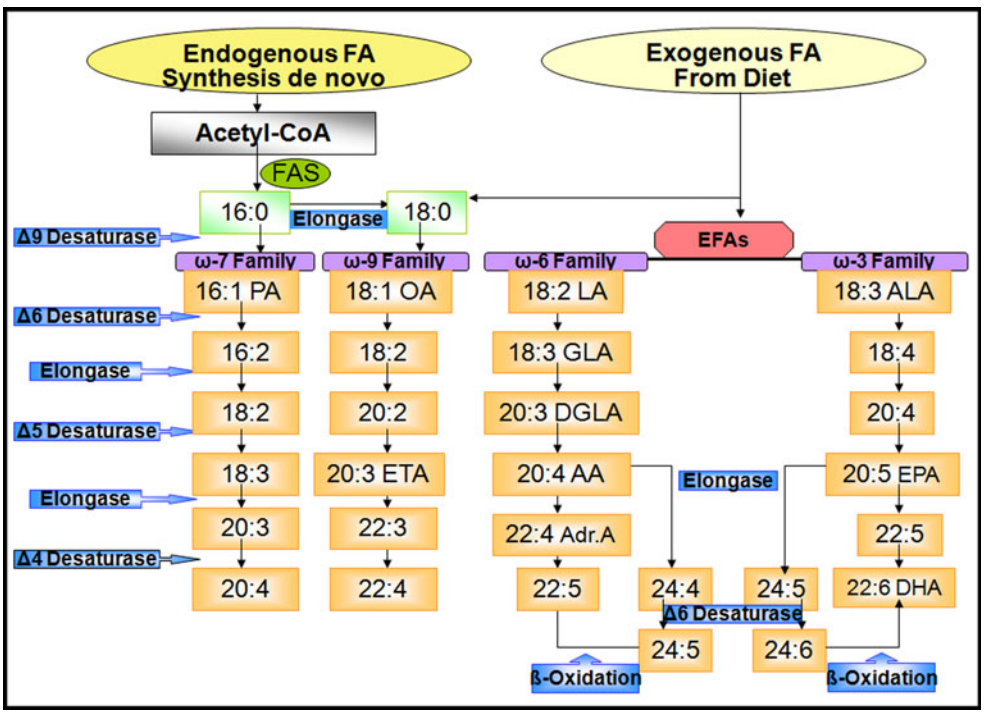

Fig. 1 Fatty Acids Biosynthesis. The figure shows the exogenous (incorporated by diet) as well as the endogenous (*de novo*) saturated and unsaturated fatty acids (FAs) biosynthesis. The endogenous FAs require acetyl-CoA and fatty acyl-synthetase being the 16:0, the first produced FA and elongated to form 18:0. Exogenous FA incorporation through diet includes: 16:0, 18:0 and the essential fatty acids (EFAs) which can not be synthesized de novo as ω-6 and ω-3 families. The implicated enzymes in these pathways are: Δ9, Δ6, Δ5 and Δ4 desaturases and elongases

and the Δ5- and Δ6-desaturases that metabolized the different families of fatty acids (ω-3, ω-6, ω-9, and ω-7) (Fig. 1).

The PUFA precursors compete with each other for the same enzymes, with hierarchical preference given for ω-3 over ω-6, and ω-6 over ω-9 and ω-7. This preference for the higher degree of desaturation in 20-carbon chain PUFA precursors has major implications for dietary habits. Hence, under normal physiological conditions, ω-9 derivatives are formed in small amounts, and a significant increase in 20:3, ω-9 (mead acid), a metabolite of OA, suggests a deficiency of ω-3 and ω-6 essential fatty acids [6].

Controversy exists about the mechanisms involved in the last part of FA synthesis, in particular about the roles played by desaturation and elongation of arachidonic acid (AA, 20:4, ω-6) and eicosapentaenoic acid (EPA, 20:5, ω-3) to form other compounds. It was long accepted that AA and EPA were first elongated and then, via Δ4-desaturation, transformed into 22:5, ω-6, and docosahexaenoic acid (DHA, 22:6 ω-3), respectively. However, an alternative pathway involving the peroxisome has been proposed in mammals—the so-called Sprecher pathway [7]. This pathway involves sequential elongations of EPA or AA to C24 substrates followed by a second Δ6-desaturation step to 24:6, ω3, and 24:5 ω6, which would finally undergo ß-oxidation in the peroxisome to produce DHA 22:6 ω-3 and 22:5 ω-6. The current literature suggests that both the direct mechanism involving elongation followed by Δ4 desaturation and the Sprecher pathway that assumes a role for peroxisomes are implicated in the biosynthesis of the long-chain PUFAs [8, 9].

The activities of desaturases and elongases involved in the metabolism of PUFAs can be influenced by different factors, including saturated fats, cholesterol, transfatty acids formed by vegetable oil processing, alcohol, adrenaline, and glucocorticoids, which inhibit Δ6- and Δ5-desaturases. Pyridoxine, zinc, and magnesium are also necessary for normal desaturase activity [10].

In summary, FA families differ in their structure, including the quantities of carbons in the acyl-chain and the presence of double bonds. The two families of PUFAs that cannot be synthesized *de novo*—ω-6 and ω-3—are EFAs and must be obtained via dietary sources to ensure survival known as EFAs The following section discusses the key roles of FAs in cells, not only as part of the phospholipid membrane but also as eicosanoids and other biolipids and as regulators of signaling in gene expression.

1.1.2 Major functions of FAs in cells

In addition to serving as energy sources in cells, FAs have many other functions. They serve as membrane constituents, playing a vital role in the maintenance of membrane fluidity. They determine and influence the behavior of membrane proteins and membrane-bound enzymes and receptors. They also may act as precursors for the synthesis of eicosanoids and other biolipid derivatives of PUFAs, which in turn influence intracellular signaling processes affecting transcription factor activities, such as the regulation of gene expression.

As membrane components, endogenous or exogenous FAs incorporated into membrane phospholipids play important roles in membrane structure, potentially influencing membrane functions and transmembrane and intracellular protein activity via a number of mechanisms. Changes in lipid composition alter the physical properties of the membrane bilayer, a process which in turn regulates a wide range of membrane protein function in a seemingly non-specific manner [11, 12].

Many functional proteins require a specific lipid to provide a proper microenvironment around hydrophobic regions [4, 13–15]. A number of membrane-bound enzymes, transporters and receptors have been shown to be particularly sensitive to their FA environments. These include adenylate cyclase, 5′-nucleotidase, the Na^+/K^+ ATPase and the insulin receptor [16]. Saturated FAs such as myristic 14:0 and PA are the predominant FAs found attached to proteins in eukaryotic cells [17]. Both FAs are attached to distinct classes of proteins, some of which are involved in signal transduction, such as Src family kinases, G-proteins, growth factor receptors, mitogenic-activated protein kinase (MAPK), protein kinase C (PKC), and a number of oncogene products [18]. These proteins are targeted to specific regions of the plasma membrane known as "rafts," which act as signal platforms for various signaling components [19]. Lipid rafts are discrete patches of plasma membrane with distinct lipid composition that differ from other regions of the membrane. These microdomains are rich in sphingolipids and cholesterol and the FA chains of lipids are more saturated and tend to be tightly packed. Lipid rafts seem to be involved directly or indirectly in lipid-mediated cell regulation for generation of intracellular signals in response to extracellular stimuli.

It is known that the reactivity of PUFAs with molecular oxygen depends on the number and position of their double bonds. This can happen non enzymatically, contributing to oxidative stress, or through the actions of different oxygenases. The enzymatic oxygenation of 20-carbon PUFAs, particularly the EFAs, generates a series of bioactive metabolites broadly termed eicosanoids [20].

Arachidonic acid, a relatively minor PUFA found at the n-2 position of cell membrane glycerophospholipids, is released either by the action of phospholipase A2 (PLA_2) or from membrane phosphatidylinositol-4,5-biphosphate by the actions of phospholipase C (PLC) and diacylglycerol lipase (DAGL) [21]. It also may be directly supplied to tissues from dietary sources or formed from the EFA

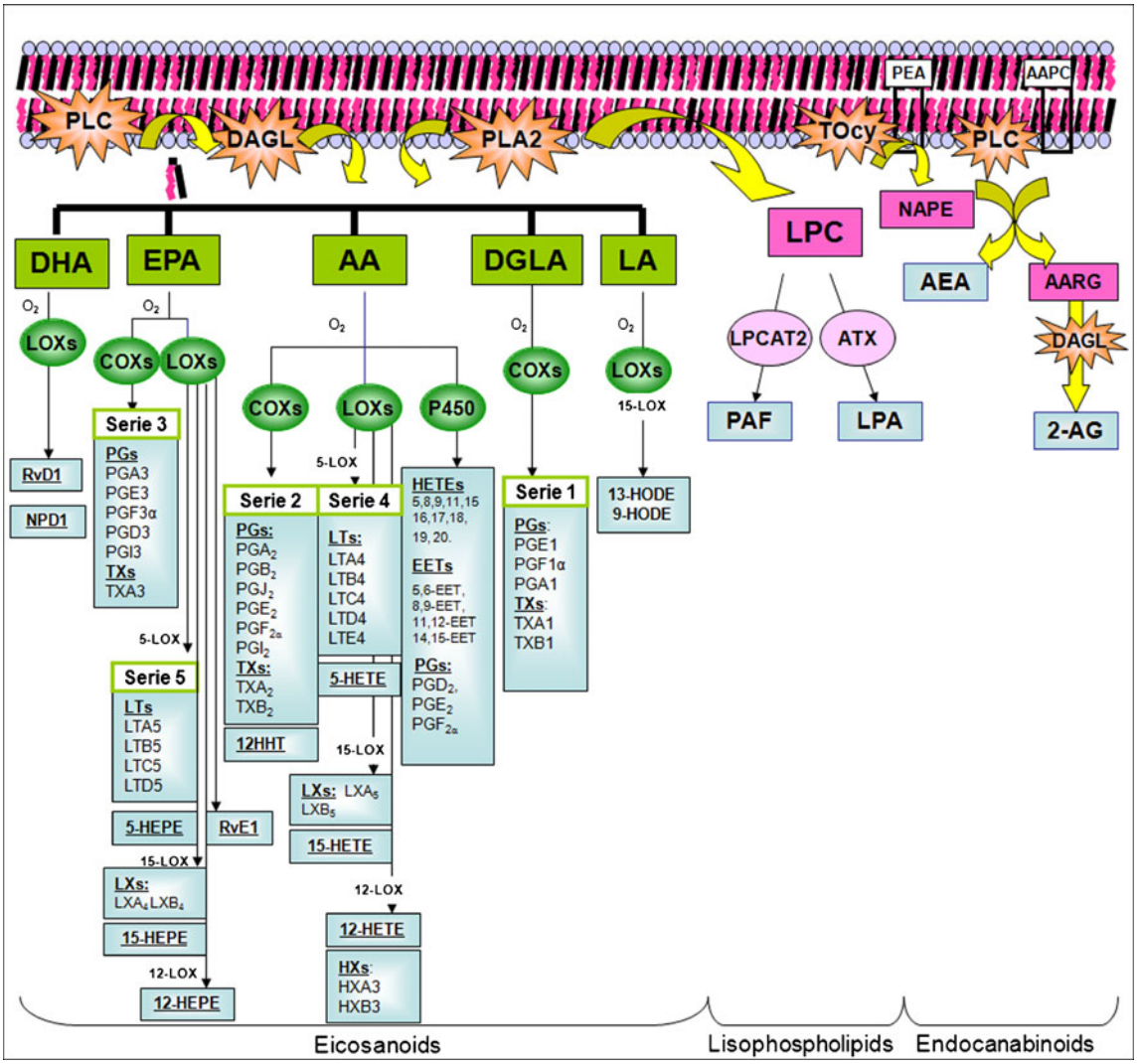

Fig. 2 Biosynthesis of Lipids Derivatives. The figure shows the biosynthesis of some lipid derivatives as eicosanoids, lisophospholipids and endocanabinoids. After phospholipase C (PLC) activation diacylglycerol (DGA) is liberated from membrane phospholipids. DGA lipase and phospholipase A2 (PLA_2) activities produced fatty acids (FA) liberation from membrane phospholipids as well DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid, AA: arachidonic acid, DGLA: dihomo-gamma-linoleic acid and LA: linoleic acid. These unsaturated fatty acids can be function as substrate of lipoxigenases (LOXs), cyclooxygenases (COXs) and cytochrome P-450 (CYP-450) giving rise to resolvins (Rvs), neuroprotectins (NPs), prostaglandins (PGs), leukotrienes (LTs), thromboxanes (TXs), hydroxyeicosatetraenoic acids (HETEs), hydroxyeicosapentaenoic acids (HEPEs), lipoxins (LXs) and hepoxilins (HXs). By another way, PLA_2 is implicated in lisophospholipid production. The activity of this enzyme yields to the 1-O-alkyl-lysophosphatidyl coline (LPC). This molecule is metabolized by LPC acetyl-transferase2 giving the platelet-activating factor (PAF) and by autoxine enzyme (ATX) giving lisophosphatidic acid (LPA). Indeed, the endocannabinoids synthesis takes place when a transacylase (TRCY) catalyses the phosphatidyl-ethanolamine (PEA) in N-acyl-phosphatidylethanolamine (NAPE). These molecules are substrate for PLC and for PLD, resulting in the liberation of arachidonylethanolamide (anandamide: AEA) and 2-arachidonylglicerol (2-AG), considered the two main endogenous endocannabinoid compounds

linoleic acid (LA). In Western-fed populations, AA is the main precursor of eicosanoids, and these bioactive lipids have more potent biological functions than those released from dihomo-γ-linolenic acid (DGLA) or eicosapentaenoic acid (EPA). In contrast to other more abundant unsaturated FAs such as oleic, linoleic or linolenic acid, levels of unesterified AA are strictly controlled within mammalian cells, and pathways of AA uptake, incorporation and remodeling in glycerolipids are well documented [22]. As shown in Fig. 2, eicosanoids belong to a family of biolipid mediators that are oxygenated derivatives of the 20-carbon PUFAs such as prostaglandins (PGs), thromboxanes (TXs) formed by the enzymatic activity of cyclooxygenases (COXs) or leukotrienes (LTs), lipoxins (LXs), hepoxilins (HXs), dihydroxyeicosatrienoic acid (DiHETEs), and hydroxyeicosatetraenoic acids (HETEs derived from enzy-

matic activity of lipoxygenases (LOXs). Eicosanoids are also formed by the cytochrome P450 enzymes that metabolize AA in PGs, epoxyeicosatrienoic acid (EET), and HETEs, among others [23–25]. LA is the precursor of hydroxyoctadecadienoic acids (HODEs). The DGLA 20:3, n-6 is the precursor of the one series of PGs and TXs (Fig. 2).

By another pathway, AA is the precursor of the two series of PGs, TXs and 12- HHT and the four series of LTs, LXs, HXs, DiHETEs, HETEs, and EETs [26] (Fig. 2). EPA forms the three series of PGs and TXs and the five series of LTs, LXs, and hydroxyeicosapentaenoic acids (HEPEs), as well as the E series of new compounds known as resolvins [12, 13, 27]. Docosahexaenoic acid (DHA) is also a precursor of resolvins of the D series. Other bioactive members from DHA with conjugated triene structures are docosatrienes, termed neuroprotectins [28] (Fig. 2). Indeed, when PLA_2 catalyzes the hydrolysis of FA ester linkage at the 2 position of glycerol, a variety of free FAs, usually unsaturated, and a variety of lysophospholipids are formed. The first recognized lysophospholipid-type lipid derivative was platelet-activating factor. Its biosynthesis involves production of 1-O-alkyl-lysophosphatidil choline (LPC) by PLA_2, followed by its acetylation by LPC acyltranferase-2 (LPCAT-2). The other derivate is lysophosphatidic acid, obtained from LPC by autotoxin enzyme activity [24] (Fig. 2).

Interestingly, other eicosanoids resulting from the oxidative metabolism of related lipids are those of the endocannabinoid system (ECBS). These are endogenous AA-derived lipids that are produced in the brain and other tissues. They bind and activate the cannabinoid receptors CB1 and CB2 and have been implicated in a wide array of physiological and pathological processes, including cancer [29]. Although various new molecules with ECBS activity have been identified, arachidonoyl-ethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) are considered to be the two main endogenous cannabinoid compounds. ECBS are released from cells when levels of calcium rise inside the neuron or when certain G-protein-coupled receptors are activated. Increasing calcium activates trans-acylase, which catalyzes the first step of ECBS biosynthesis converting phosphatidylethanolamine, a membrane-resident phospholipid, into N-acyl-phosphatidylethanolamine (NAPE). Both phospholipase C (PLC) and phospholipase D (PLD) cleave NAPE to yield AEA [30]. However, when 2-AG biosynthesis was studied in PLD knockout mice, the PLD-mediated cleavage of NAPE was only reduced, not abolished, with low resultant calcium concentrations, suggesting the involvement of multiple and distinct pathways. The other endocannabinoid, 2-AG, is produced by action of PLC on the sn-1, 2-diarachidonoyl-phosphatidylcholine; the product formed by sn-1, acyl-2-arachidonyl glycerol is degraded by the diacylglycerol lipase to 2-AG. Dietary uptake of various PUFAs may modify ECBS production, potentially deeply affecting ECBS activities [31, 32]. It is interesting to consider that cells can use either free FAs taken up from the extracellular pool or etherified FAs released from cell membrane phospholipids by PLA_2 for eicosanoid production [20]. For example, studies of the relative utilization of exogenous versus endogenous estherified AA studied in collagen-stimulated platelets under physiological conditions (in plasma) have shown that endogenous EFA is used for thromboxane production, but that exogenous plasma lipid is preferentially used for 12-HETE production by 12-LOX [33, 34].

This difference may be due to the efficiency of coupling of exogenous versus endogenous AA to downstream enzymes depending on their subcellular localization [35] (Fig. 2).

Substantial epidemiological and experimental evidence shows that FAs such as PUFAs and their derivatives modulate many metabolic processes. Indeed, PUFAs and their LOXs, COXs and CYP-450 products activate cellular signaling pathways to produce specific changes in gene expression affecting proliferation, cell death, migration, and extracellular matrix production. When the lipids are modified by dietary manipulation, this can give rise to pathological processes such as carcinogenesis in part by altering the activity of transcription factors regulating gene expression implicated in carcinogenesis.

1.2 Role of transcription factors in FA signaling—regulated gene expression

1.2.1 Direct effects of FAs on transcription factors

To modify the function of a transcription factor, one of the common approaches is to affect the direct binding between the ligand and the protein. Indeed, several FAs have been identified to interact with transcription factors and mediate the process of transcription. In this section, we will summarize a classic lipid-activated transcription factor, the peroxisome proliferator-activated receptor (PPAR), as an example of the direct effects of FAs.

Peroxisome proliferator-activated receptors The PPARs are a group of nuclear receptor proteins belonging to the sterol/thyroid superfamily, and function as transcription factors regulating the expression of genes. Three subtypes of PPAR (α, β/δ, and γ) have been described, each with organ-specific expression. PPAR contains a DNA-binding domain and a ligand-binding domain (LBD) that interacts with various ligands. The first identification of the PPAR activator was done by Green's group in the early 1990s [36]. Green's group transfected cultured cells with reporter construct containing the PPAR ligand-binding domain. By treating the cells with fibrate hypolipidemic agents, they

were able to stimulate reporter gene expression, suggesting that fibrate serves as a PPAR activator [37]. Following this finding, more natural compounds, including several FAs, have been found to potentiate PPAR activation [36, 38–40]. The LBD of PPAR contains a large Y-shaped cavity with a total volume of 1,300–1,400 Å. The cavity branches off two extended arms from the surface of the protein and forms a hydrophobic entrance and a polar pocket. In this pocket, Thr289, His323, His449, and Tyr473 of PPARβ/δ (respectively Ser280, Tyr314, His440 and Tyr464 of PPARα, and Ser289, His323, His449 and Tyr473 of PPARγ) construct a hydrogen network that interacts with the carboxyl group of FAs or eicosanoic acids [41]. Moras' group proposed a "mouse trap" model to explain the regulation of ligand-stimulated PPAR activation [42]. Based on this model, structural analysis has been conducted on the retinoid X receptor (RXR) and its activating function-2 domain (AF-2). The PPAR–RXR heterodimers bind specifically to PPAR response elements, which are direct repeats of 5′-AGGTCA separated by one or, in a few cases, two nucleotide(s) [42]. In the heterodimer, PPAR occupies the 5′ repeat, whereas RXR occupies the 3′ repeat [43]. The activation of transcription by the PPARs relies on two activation domains, i.e., activation function one (AF-1) located in the N-terminus and activation function two (AF-2) located in the C-terminal LBD [44]. The activity of AF-2 is regulated by the binding of ligands, such as fatty acids and fatty acid derivatives. This type of binding leads to a conformational change of the C-terminal domain, the AF-2 helix in particular favoring the interactions with transcriptional coactivators [45]. Because PPARs function as sensors for FAs and their derivatives, this family mostly controls the gene expression involved in lipid metabolism and energy homeostasis [46].

Substantial evidence suggests that PPAR plays a role in cancer development. Activation of PPAR-β/δ is involved in the development of breast, prostate, and liver cancers. However, considerable controversy remains as to whether PPAR-β/δ stimulates or inhibits cancer cell proliferation [47–49].

PUFAs such as LA, ALA, γ-linoleic acid (GLA), AA, EPA, and DHA are natural ligands and activators of PPAR-α and PPAR-δ. Several CLA isomers have also been shown to bind and activate PPAR-α [50]. As described before, the binding capacity of different PUFAs to PPARs may prevent cancer development. In contrast, saturated FAs do not bind or activate these transcription factors.

PPARα is required for the induction of genes involved in mitochondrial and peroxisomal β-oxidation [51]. In rats and mice, these PPAR ligands have been associated with rapid peroxisome proliferation and liver enlargement, and their long-term administration led to liver carcinogenesis, perhaps as a result of the enhancement of lipid peroxidation products [52]. However, the anti-neoplastic effects of PPARs have been shown in several types of cancer cells. These effects are thought to be related to programmed cell death because, apart from its well-established metabolic actions, PPARγ induces apoptosis through an upregulation of certain pro apoptotic proteins by the release of cytochrome C and subsequent activation of several effector caspases [53].

Imbalance of peroxisome proliferator levels results in the sustained activity of PPARα, which causes DNA damage and hepatocellular proliferation by deregulating responsive genes [54, 55], thereby, leading to the development of liver cancer [56].

Although a variety of compounds have been shown to activate PPARs, identification of physiologically relevant ligands has been difficult [57]. The PPARs regulation is directly done by FAs interacting with PPAR ligand-binding domain and potentiates the transcription activity. The behavior of PPAR in cancer may be modulated by PUFAs and their derivated biomolecules [58] (Fig. 3).

1.2.2 Indirect effects of FAs on transcription factors

In addition to directly affecting transcription factors, as with PPAR (described in the previous section), FAs can also regulate transcription factors by interacting with their regulatory pathways. Other signaling molecules, such as PKC, also have indirect effects on FAs and transcription factors. We will discuss the indirect effects of FAs, using nuclear factor kappa B (NFκB) and sterol regulatory element—binding proteins (SREBPs) as examples.

Protein kinase C signaling PKC is a family of phospholipids-dependent serine/threonine kinases that play important roles in signal transduction associated with a variety of cellular responses, including cell growth and differentiation, gene expression, hormone secretion, apoptosis and membrane function. PKC consists of at least 11 isoforms that show diversity in structure, cellular distribution, and biological functions and that have been divided into three groups based on their structures and cofactor requirements: conventional, novel, and atypical. The conventional PKC isoforms α, βI, βII, and γ require phosphatidylserine, and Ca2+ for activity. The novel PKC isoforms δ, ε, η, and θ do not require Ca2+ as a cofactor, but bind to phosphatidylserine when activated. Both conventional and novel PKCs are activated by phorbol 12-myristate 13-acetate or 1,2-diacyl-sn-glycerol (DAG) [59]. The interaction of DAG with PKC has implications for many PKC-dependent cellular functions because PKC is a substrate of neuromodulin, myristoylated alanine-rich C kinase, G-protein-coupled receptors, growth factor receptors, metabolic

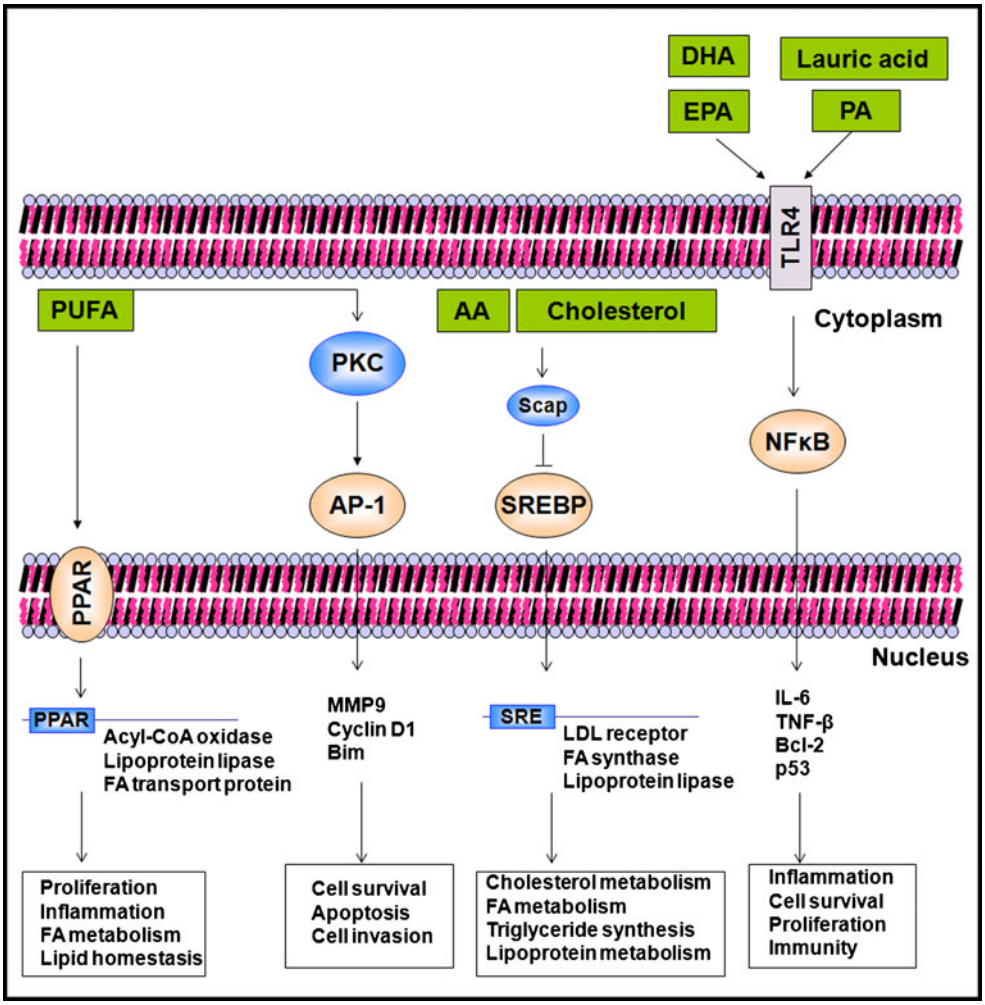

Fig. 3 Interaction of Fatty Acids and Transcription Factors. The figure summarizes examples of the effect FAs have on transcription factors. In peroxisome proliferator-activated receptor (PPAR) regulation, FAs directly interact with PPAR ligand-binding domain and potentiate the transcription activity. Active PPAR associates with PPAR response element (PPRE) in the target gene promoter and recruits transcription-coactivator complex to initiate gene expression. AP-1, sterol regulatory element-binding protein (SREBP), and NFκB represent the indirect effect of FAs to transcription factors. FAs and cholesterol modulate the upstream molecules (e.g. PKC, Scap, TLR4) and cause activation and nuclear translocation of the downstream transcription factors, and as a result change the target gene expression

enzymes, signaling enzymes such as mitogen-activated protein kinase, cytoskeletal proteins, nuclear proteins, and protooncogenes such as pp60c-src [60]. PUFAs of the ω-3 and ω-6 families, such as LA, AA, GLA, EPA, and DHA, compete to activate PKC-α. Once activated, PKC-α induces mitogenic signals by activating Fos, Jun, and AP-1 transcription factors. In this regard, Field and Murray [59] showed that a diet high in ω-6 fatty acids increased cancer colon cell proliferation. This was associated with an increment in cell membrane PKCβII. In contrast, ω-3 FAs inhibited colon carcinogenesis *in vivo*, by direct inhibition of PKCβII activity which was mediated by COX-2 protein expression and transforming growth factor receptor-β II (TGF-β RII) expression [59] (Fig. 3).

Nuclear factor kappa B NFκB is a transcription factor that controls genes involved in apoptosis, inflammation, cell adhesion, proliferation, the adaptive immune response, the stress response, and tissue remodeling. Disruption of NFκB signaling results in inflammatory diseases and cancer. In the inactive state, NFκB forms a heterodimer complex with the inhibitory factor, IκBα, in the cytoplasm. But, when the IkB is phosphorylated by kinase IκK unlocks the inhibitory effect of NFkB and the active form migrates into the nucleus where it regulates gene expression. Studies of the modulation of NFκB activity by ω-3 PUFAs have shown that impeding phosphorylation of IκB selectively decreased NFκB activation, preventing the nuclear translocation of NFκB [61, 62]. In addition to the effect on the IκB molecule, FAs have a more complex effect on the toll-like receptor (TLR) family and affect downstream NFκB activity. TLRs are membrane-bound receptors that recognize the pathogen-associated molecular patterns of invading microorganisms. Upon recognition of a specific ligand, the responding TLR can trigger various downstream signaling by conjugating to different mediators, including MyD88, MyD88-adapter-like (Mal), TIR domain-containing adaptor protein (TIRAP), TIR-domain-containing adaptor including interferon-beta (TRIF), and TRIF-related adaptor molecule [63]. Among the representative agonists of TLRs, the TLR4 agonist lipo polysaccharide (LPS) and

TLR2 agonist lipopeptides contain a saturated FA moiety (lauric acid, myristic acid, and palmitic acid) [64]. Lee and colleagues identified these saturated FAs (lauric acid and palmitic acid) as inductors of NFκB expression through TLR4 signaling [65]. Interestingly, PUFAs such as DHA and EPA saturated FA activation [66]. These studies suggest that saturated FAs and unsaturated FAs regulate TLR in opposite ways. Even though the detailed mechanism is currently unclear, several lines of evidence indicate that FAs play a role in TLR trafficking to lipid rafts [67, 68]. Consistent with these observations, a recent finding shows that lauric acid, a saturated FA, induced dimerization and recruitment of TLR4 in lipid rafts [69]. In this report, the authors found that lauric acid and LPS enhanced the association between TLR4 and its downstream adaptors, TRIF and MyD88, by gathering them in lipid rafts. Treatment with the ω-3 DHA inhibited this dimerization and recruitment. More research is needed to further elucidate the role of PUFAs in lipid raft regulation of TLR4.

In summary, NFkB is constitutively active in most tumor cells (hematopoietic, prostate, breast cancers). Suppression of NFkB activity in tumors inhibits proliferation, causes cell cycle arrest, and leads to apoptosis, indicating the crucial role of NFkB in cell proliferation and survival. Findings show that PUFAs can selectively affect the TLR family and influence downstream NFκB activity. These interactions form a crucial interface with other signaling pathways in the cell.

In fact, the regulatory ability of PUFAs to behave as either agonists or antagonists provides a plausible explanation for the cell-type- and stimulus-specific effects of NF-κB, such as the ability to either inhibit or facilitate apoptosis. These pathways also provide important routes through which FAs or their derivatives can modulate NF-κB activity (Fig. 3).

Sterol regulatory response element-binding proteins SREBPs are another group of transcription factors that mediate the effects of dietary FAs on gene expression. The three members of the SREBP family (SREBP-1a, SREBP-1c, and SREBP-c) are membrane-bound transcription factors that function as transcription activators for genes involved in the biosynthesis and intake of cholesterol, FAs, triglycerides, and phospholipids [70]. Members of the SREBP family are encoded by two unique genes, *SREBP1* [71–74], and *SREBP2*. All of them share three similar features on the N-terminal basic-helix-loop-helix-leucine zipper (bHLH-LZ) domain—two hydrophobic transmembrane regions and a C-terminal regulatory domain. Tontonoz et al. [75] first implicated a regulatory role for SREBPs in FA synthesis. By screening the rat cDNA of adipose tissue for proteins that contain the bHLH domain to recognize E box, they isolated ADD-1, a rat homologue of human and mouse SREBP-1c, and showed that overexpression of ADD-1 stimulated transcription through a polymerized set of E-boxes located on the 5′-flanking FAS promoter [76]. Subsequently, it was shown that SREBPs directly stimulated the FAS promoter by coordinating with the consensus sequence adjacent to E boxes, a sterol regulatory element-like sequence.

Interestingly, while SREBP plays an important role in the synthesis of FAs and cholesterol, the activation of SREBPs is also a cholesterol-regulated process [77, 78]. When the cells are replete with cholesterol, SREBPs anchor on the ER membrane by two transmembrane segments, forming a hairpin structure with the N-terminal bHLH-LZ domain and the C-terminal regulatory domain extending into the cytoplasm [79]. The C-terminus binds to SREBP cleavage-activating protein (Scap), a variety of protein that associates with cholesterol, and promotes the conjugation between the SREBP-Scap complex and the ER-resident insulin-induced gene (Insig) [80]. This process retains SREBP on the ER membrane, preventing interaction with Golgi-localized proteases [81]. When the cells are lacking in cholesterol, decreased levels of sterols are released, allowing binding between SREBP-Scap and Insig and the exit of SREBP-Scap to the Golgi by COPII-coated vesicles through interaction with Sec23/24 [82, 83]. In the Golgi, SREBPs are proteolytically cleaved by site 1 protease and site 2 protease, releasing the N-terminal transcription factor domain into the nucleus [84]. Nuclear SREBPs include ~480 amino acids on the protein N-terminus that form an acidic domain followed by an activation domain, bHLH-LZ. This segment endows SREBPs with the ability to bind to SRE and activate transcription. As would be expected given the function of the bHLH-LZ domain, SREBPs in which this domain is deleted retain their DNA-binding ability but have an attenuated transcriptional activity, turning them from transactivators into inhibitors [85]. Furthermore, the shorter acidic domain in SREBP-1c makes it a weaker transactivator compared with the other two family members [86], suggesting that the length of the acidic head also affects the activity of SREBPs. To function as a transcription factor, nuclear SREBPs first recognize SRE in the promoter and bind to the DNA elements as dimers. After binding occurs, the activation domains of the nuclear SREBPs recruit coactivator CBP/p300 and SWI-SNF, a nucleosome remodeling complex, to modulate target gene expression [87].

In this context, AA is almost the unique PUFA that significantly affected the SREBP-1 transcription factor, which controls genes for the low-density lipoprotein receptor and 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) synthase. Indeed, it has been shown that AA had inhibitory effects on SREBP-1a and SREBP-1c in cultured human cells [5]. Moreover, alterations of SREBP and their downstream-regulated genes such as FAS were involved in the

development and progression of cancer. Indeed, SREBP-1 overexpression is highly linked to some cancer tissues.

In summary, the cholesterol-dependent ER localization and transcriptional activity of SREBPs, in which AA and other FAs may competitively inhibit activation of the endogenous SREBP-1 gene, suggest that intracellular levels of FAs and cholesterol should regulate preneoplastic changes. The dependence of SREBP-ER localization on cholesterol could explain the intracellular accumulation of cholesterol observed in some types of cancers. Consequently, disrupting the mechanisms that increase sterol levels has become an important checkpoint implicated in cancer development (Fig. 3).

1.2.3 Lipid derivatives and cell signaling

LOX derivatives Some AA-LOX metabolites, such as leukotriene B4, 8-HETE, 15-HETE, 9 HODE, and 13-HODE, activate signal transduction pathways that lead to cell proliferation or elicit a positive modulation of cell behavior and phenotype that include: (1) cell surface G-protein activation, (2) nuclear PPAR activation, (3) Ras signaling pathway through activation of regulation of Ras-GAP, (4) activation of immediate early genes such as c-fos and Egr-1, (5) PKC activation, (6) activation of the Raf-1/Mek/Erk pathway, (7) ROS-induced NF-κB activation, (8) activation of P21-activated kinase, (9) phosphoinositol 3 kinase (PI3K) activation, and (10) interaction with steroid receptor coactivator-1 [88, 89]. It is generally acknowledged that abnormal expression of specific PKC isoforms is closely associated with cellular transformation and cancer progression. Also, 12-HETE may act as a second messenger activating PKC [90].

A different mechanism was proposed by Zeng et al. [91], who reported that 5-HETE and 12-HETE stimulate endothelial cell growth via Jak-2/STAT and phosphatidylinositol-3-kinase/AKT signaling, inducing the expression of fibroblast growth factor-2. Furthermore, 12 (HETE) activated ERK ½ and p38 MAPk pathways in 3T6 fibroblast cultures [92], whereas 20-HETE also induced the Ras/MAPk pathway in vascular smooth muscle cells [93].

COX derivatives COX-derived products, such as PGE_2, $PGF2_\alpha$ and the arachidonate product of thromboxane synthase (TXA_2), may stimulate cell growth and proliferation by enhancing the self-production of PGs. TXA_2 has a number of potent and diverse bioactivities, including platelet aggregative activity and smooth muscle constrictive activity, that are important both to the maintenance of health and the development of disease and are mediated by TXA_2 receptors (TPs) [58]. Two isoforms of TP, TPα, and TPβ have been identified. Activation of TPs also initiates signaling cascades, leading to activation of various kinases known to be involved in mitogenic responses, such as epidermal growth factor receptor (EGFR), extracellular signal-regulated kinase (ERK), Akt/protein kinase B, and glycogen synthase kinase. TXA_2 receptor signaling is very much related to the mitogenesis of target cells [94]. It has been shown that TXA_2–TP signaling axis may regulate cell migration through Rho factor activation and cytoskeleton reorganization in prostate carcinoma cells [95].

Indeed, it was demonstrated that PGE_2 increased the activity of the a2b1 integrin via EP1, PLC, PKC, c-Src, and NFkB-dependent pathways and enhanced migration of human chondrosarcoma cells [96]. Also, PGE_2 is known to play an important role as a physiological stimulator of cAMP synthesis in the activation of mechano growth factor, a second messenger implicated in the synthesis of myoblasts and differentiated myotubes in murine and human cells [97]. In HCA-7 human colon cancer cells, PGE_2 enhanced the upregulation of vascular endothelial growth factor (VEGF) receptor-1 (VEGFR-1) expression. The PGE_2-stimulated increase in VEGFR-1 expression was accompanied by an increase in the cellular migration of HCA-7 cells. This PGE_2 activity included a signaling pathway involving a receptor-mediated activation of phosphatidylinositol 3-kinase (PI_3K) and other extracellular signal-regulated kinases [98]. Yang et al. [99] showed that PGE_2 treatment of oral cancer cells produced cell migration and ICAM-1 expression-linked to an upregulation of PKCδ and c-Src and activation of the AP-1 signaling pathway.

The human gastric carcinoma cell line MGC803 exhibits high expression levels of PPAR-γ, an agonist of 15-deoxy-delta12,14-prostaglandin J_2 (15d-PGJ_2) that has been shown to inhibit growth and induce apoptosis and G(1)/G(0) cell cycle arrest in these cells in a concentration-dependent and time-dependent manner. The inhibition of PPAR-γ function by COX derivatives may be a potentially important and novel modality for the treatment and prevention of gastric carcinoma [100].

P450 derivatives P450-derived eicosanoids have activities closely linked to inflammation, angiogenesis, and cardiovascular function rather than to cancer pathways [101]. Nevertheless, among AA p450 derivatives, 20-HETE is the major metabolite of the ω-hydroxylation by cytochrome-P4A (CYP4A) and the CYP4F pathway. This metabolite is a proinflammatory mediator that markedly stimulates the production of inflammatory cytokines/chemokines in endothelial cells, including IL-8, IL-13, IL-4, and PGE_2. 20-HETE activates vascular endothelial cells by its participation in the cellular mechanisms that trigger activation of the signaling pathways NFkB and MAPK/ERK [102] Also, 20-HETE induces angiogenic responses *in vivo* and *in vitro*, and CYP ω-hydroxylase promotes tumor angiogenesis and metastasis

by upregulation of VEGF and MMP-9 via PI3 K and ERK1/2 signaling in human non-small cell lung cancer cells [103].

The above-discussed roles played by PUFAs and their derivatives in molecular mechanisms could explain the *in vitro*, *in vivo* and clinical effects observed after diverse PUFA treatments. In animals, FAs are key molecules that participate in various biological processes. The structural properties of FAs, such as their chain length, the degree of desaturation, and the position of the double bonds, largely determine the nature of these processes [4].

Summing up, eicosanoids derived from LOXs, COXs, and CYP450 enzyme activity can interfere with different transcription factors activating or inhibiting the expression of gene products involved in carcinogenesis

1.3 PUFAs: a context-dependent oncogene–tumor suppressor pathway

Perturbation of lipid metabolism is an early event in carcinogenesis and a central hallmark of many cancers. However, the precise molecular lipid profile in tumor cells remains poorly characterized because variability is a common feature of cancer cells. Nonetheless, in the following section, we attempt to characterize the major lipid profile characteristics of a tumor cell.

1.3.1 Lipid profile of tumor cells

Cancer cells synthesize *de novo* large amounts of FAs and cholesterol, irrespective of the circulating lipid levels, and benefit from these increased levels in terms of growth advantage, self-survival, and drug resistance. Key lipogenic alterations that are common in cancer cells include over-expression of the FAS enzyme and deregulation of the 5-AMP-activated protein kinase [104]. It is well-known that many tumors and cancer cell lines are deficient in Δ5, Δ6 desaturase and/or contain low levels of Δ5, Δ6 desaturated EFAs because loss of Δ5 and Δ6 desaturating ability is relevant to the process of malignant transformation [105, 106]. Remodeling the FA composition of membrane lipids can alter cell metabolism as well as affect the complex interacting array of signaling cascades that govern a range of physiological responses. Fatty acids can function as signaling molecules that not only trigger FA immune response but also elicit the general "cancer" signaling network [107]. Recent studies on the lipid profile of cancer membrane cells carried out in gastric cancer cell lines have shown that a lipid kinase sphingosine kinase-1 [Sphk1] is overexpressed in this line and is linked to tumor growth, cell transformation, and poor prognosis [108]. The determination of the global lipid profile in 267 human breast cancer cells was correlated to protein expression. It was observed that the increased products of *de novo* FA synthesis incorporated into membrane phospholipids were mainly palmitate-containing phosphatidylcholine, which renders membranes less fluid and more rigid. The increased concentration of these lipids was associated with cancer progression and a higher expression of lipid metabolism-related genes. It was also shown that the expression of many genes in mammals is positively or negatively modulated by FAs through changes in the rate of transcriptional or post-transcriptional modifications that could be dependent on the viscosity of the plasma membrane [109–111].

Exogenous and endogenous PUFAs and their metabolites can significantly alter protein expression and metabolism to influence the cellular tissue microenvironment and cell–cell interactions. However, it was also proposed that ω-6 FAs enhance tumor cell growth, whereas ω-3 Fas are beneficial because they arrest cell growth. The cytotoxic effects of ω-3 PUFAs on various cancer cells depends on the type and concentration of FAs used and of the cancer cell being tested.

We will next discuss experimental approaches *in vivo* as well as clinical research on the effects of treatment with different PUFA families on cancer development.

1.3.2 Targeting cancer cells with omega-6 PUFAs: LA, AA, and γ-linoleic acid

In vitro studies It is interesting to note that different cell lines showed diverse sensitivity to LA or AA depending on the cell line as well as the dose of FAs. Lu X et al. [112] showed that low concentrations of LA (≤200 μM) promote colorectal cancer cell growth, whereas high levels (≥200 μM) induce apoptosis *in vitro* in these cancer cells by inducing differential formation of lipid peroxides. After exposure to increasing concentrations of AA, hepatomoma cancer cell lines showed decreased cell growth and increased apoptosis. These effects were linked to downregulation of the gene expression of lipogenic enzymes such as FAS and hydroxy-methyl-glutaryl CoA-reductase [113].

Jiang et al. [114] showed that GLA reduced adhesion of colon and breast cancer cells to the endothelium by inhibiting the tyrosine phosphorylation of connnexin-43, a protein that formed the gap junction communication in these cells with the endothelium. GLA was shown to increase e-cadherin expression in a human squamous cell carcinoma line. This cadherin is a cell adhesion molecule highly concentrated at the *zonulae occludens*. It´s expression is decreased in highly malignant carcinoma. Of these reasons, it is considered an accurate parameter of tumor differentiation. Interestingly, 20:3,ω-9 is increased in the EFA deficiency, selectively and blocks the beneficial pro differentiation effects of GLA [115].

Using cultures of human tumor cells, our group examined the effects of exposure to GLA and EPA, ω-6 and ω-3 EFAs, respectively, on e-cadherin expression and 15-HETE and 13-HODE liberation. In bladder (T-24), colon (HRT-18), and mammary gland (MCF-7) cancer cell lines, we found significant levels of 15-HETE and 13-HODE synthesis following GLA or EPA treatment. In addition, the predominant metabolites produced by the three types of cancer cells via the LOX pathway were 15-HETE and 13-HODE [116]. The high levels of 15-HETE and 13-HODE in cancer cells may be explained by the preferential activity of Δ 5 desaturase on exogenous and endogenous EPAs and the partial suppression of Δ 6 desaturase in these cancer lines. We observed that levels of both LOX products were well correlated to e-cadherin expression.

Another group showed that AA is a potent stimulator of prostate cancer cell (PC-3) invasion by inducing bone marrow adipocyte formation. PC-3 cells treated by AA induced destruction of the adipocyte and subsequent formation of a bone metastasis [117]. Other studies with fresh surgical explants of tumors from 22 patients with five varieties of malignancy exposed to different concentrations of GLA and ALA using an *in vitro* analysis of chemo-sensitivity testing system and fluorescent cytoprint assay demonstrated anti-tumor activity by both PUFAs. The anti tumor activity mechanism observed for PUFAs was the selective perturbation of the biophysical properties of the malignant plasma membrane [118].

Other *in vitro* experiments showed that GLA induced apoptosis of tumor cells without harming normal cells. GLA increased free radicals and lipid peroxides, suppressed the expression of the oncogenes ras and Bcl-2, and enhanced the activity of p53. GLA also seemed to produce mitochondrial depolarization, lipid accumulation, and over-expression of c-Myc and p53 to bring about their tumoricidal action [119].

Exogenous AA and inhibitors of AA metabolism that lead to the accumulation of unesterified AA are cytotoxic to the colon cancer cell line, HCT-116. Additionally, exogenous AA and triasin C, an inhibitor of AA acylation, induced apoptosis by caspase-3 activity in a transcriptionally dependent manner [120].

Animal studies Our group studies with experimental animals showed the effects of the manipulation of dietary lipids on the generation of eicosanoids linked to metastatic growth in a murine lung alveolar carcinoma model. Feeding the mice with a diet containing corn oil rich in n-6 PUFAs modulated the synthesis of eicosanoids (increasing 12-HETE and decreasing 12 HHT). These findings were positively correlated with an increment in apoptosis, diminution of mitosis, and concomitantly with a decrease in tumoral growth [121]. Experimental studies on implanted C6 glioma cells into rat brains locally infused with 200 μM-2 mM of GLA have been shown the most active local concentration of GLA for anti-tumor activity was 2 mM, infused at 1 μl/h over 7 days. This concentration of GLA induced tumor regression, increased apoptosis, and decreased proliferation in tumors of rats being small the effect detected on normal neuronal tissue [122]. Also, these findings were recently confirmed in a similar model of glioma in rats (orthotopic C6 glioma model) [123].

Clinical studies Clinical were studies carried out in more than 30 patients with intractable glioma tumor who had different grades of advanced disease (grades III or IV glioma). All were intra tumorally injected with GLA, given at increased rates (0.5–1 mg/day for 7–10 days) inducing GLA produced a significant reduction in tumor size with significant increases in survival without any acute adverse clinical effects [119, 124]. In an extension of this study, the authors using intra-cerebral injection of GLA on the brain of patients with malignant gliomas showed that this PUFA can induce regression of cancer cells without having cytotoxic effects on normal cells suggesting that GLA is a safe anti tumor agent and recommend its use in the management of human gliomas [125].

1.3.3 Tumor cell treatment with omega-3: ALA and EPA

In vitro studies The molecular basis for the health benefits of omega-3 fatty acids is poorly understood. The most immediate effect is an increased incorporation of EPA and DHA into the *sn*-2 position of plasma membrane phospholipids, where AA is usually esterified. Thus, EPA and DHA replace LA and AA, the main substrates for the production of eicosanoids by the COX, LOX, and cytochrome P-450 pathways [126]. Numerous subsequent studies confirm that AA and EPA compete at all steps of prostanoid biosynthesis, resulting in specific shifting in metabolite production and action because respective ω-3 or ω-6 metabolites are synthesized [127].

Similarly, the competition between AA and EPA for the production of leukotrienes provides an interesting explanation for the anti-inflammatory effect of dietary ω-3 PUFAs. Arachidonic acid is metabolized by the 5-lipoxygenase to leukotriene B4 (LTB4) that induces inflammation and acts as a powerful chemo-attractant of neutrophils. In contrast, the same pathway yields LTB5 from EPA, a metabolite at least 30 times less potent than LTB4 [128]. However, recent studies reveal that the anti-inflammatory effects of EPA and DHA are not only due to an exchange of the classical AA-derived pro-inflammatory eicosanoids but also for their less potent ω-3 counterparts. EPA and DHA are the precursors

of novel lipid mediators, termed resolvins and protectins which have potent anti-inflammatory and pro-resolution properties and may play a major role in the protection against various inflammatory diseases [27].

In other *in vitro* studies using BT-474 and SKBr-3 breast cancer cells, which naturally exhibit amplification of the *HER2* oncogene, Menéndez et al. [129] showed that ALA suppresses overexpression of the *HER2* oncogene at the transcriptional level and thereby interacts synergistically with anti-*HER2* immunotherapy. It has been suggested that ALA may be a potential dietary alternative or adjunct to currently used drugs in the management of *HER2*-positive breast carcinomas. Moreover, a low ω-6/ω-3 PUFA ratio and elevated levels of mono unsaturated fats (e.g., ω-9), the two prominent fat features of the Mediterranean diet, should be extremely efficient at blocking *HER2* expression in breast cancer cells [129]. Other investigators sought to determine whether EPA or DHA was more responsible for the increased apoptosis in cancer cells that was observed with the higher consumption of fish oils.

They measured a number of endpoints, including phosphatidylserine staining with Annexin-V, Bcl-2 expression and Bid, caspase 3, 8 and 9 expression as well as PARP cleavage, in LT97 human colon adenoma and HT29 human colon cancer cells treated with EPA, DHA, or LA. They observed that DHA was more effective at inducing apoptosis than EPA and that LT97 cells were more prone to DHA- and EPA-induced apoptosis than HT29 cells. These authors concluded that cancer cells are highly susceptible to ω-3 PUF-induced apoptosis and that longer chains and higher numbers of double bonds produced greater anticancer effects [130].

In an experimental study Siddiqui et al. [131] induced Jurkat leukemic programmed cell death by DHA treatment, suggesting that one mechanism through which DHA may control cancer cell growth is through apoptosis involving PP1/PP2B protein phosphatase (PP) activities .Activation of these PPs increases the susceptibility of apoptotic cell death [131]. Indeed, introduction of DHA and EPA into the plasma membrane led to disruption of the lipid raft domains, perhaps causing an increasing fluidity of the bilayer, thus displacing signaling metastatic proteins such as CXCR4 in MDA-MB-231 breast cancer cell lines [132]. Human mammary cancer cells were treated with ω-3 FAs to assess the potential of these FAs as an adjuvant therapy against the transcription factor Sp1 [133]. The transcription factor Sp1 governs genes involved in cell cycle regulation, apoptosis, and lipogenesis. Sp1 is a target of acetylation and is associated with loss of DNA binding at promoters related to cell cycle arrest and cell death in a colon cell line. The findings of this study suggest that the use of ω-3 FAs as adjuvant therapy prevents recurrence and metastasis of breast cancer.

Animal studies Changes in dietary lipids may modify the eicosanoid production. In this way, experimental data from our laboratory with murine models of mammary cancer fed with Chia oil (*Salvia hispanica*) rich in ω-3 showed that this diet modifies tumor cells, enriching membranes with ω-3 PUFAs with a concomitant decrease in the AA-derivative eicosanoids 12-HETE and 12-HHT, both associated with cancer cell proliferation and lymphocytes migration. Increased apoptosis and infiltration of T-lymphocyte and inhibition of tumor growth and metastasis formation were also observed in association to minor production of these eicosanoids [134].

Studies by other investigators in Lewis lung carcinoma-bearing C57BL/6 mice have shown that a diet containing 5% fish oil (rich in ω-3 PUFAS) resulted in the substantial slower growth of primary tumors, a lower mortality rate, and decreased metastatic spread, compared with tumor-bearing mice on a diet containing 5% soybean oil rich in ω-6 PUFAS [135].

Clinical studies In patients with prostate cancer, consumption of fish rich in ω-3 PUFAs was inversely related to cancer development [136]. In contrast, low levels of ALA 18:3, ω-3 a precursor of EPA and DHA in mammary adipose tissue, were associated with an increased risk of breast cancer in women [137]. Liang et al. [138] examined the effects of omega-3 FA parenteral supplementation postoperatively on clinical outcomes and immunomodulation in colorectal cancer patients. They evaluated routine blood tests, biochemistry, systemic levels of IL-6 and TNF-α and the percentage of CD3+, CD4+, and CD8+ lymphocytes preoperatively and on postoperative days 1 and 8. Patient outcome was evaluated considering mortality during the hospital stay, the length of the postoperative hospital stay, and the occurrence of infectious complications. They concluded that postoperative supplementation with omega-3 FAs may have a favorable effect on outcomes in colorectal cancer patients undergoing radical resection by lowering the magnitude of inflammatory responses and modulating the immune response [138].

1.3.4 Tumor cell treatment with omega-9

In vitro studies Our laboratory examined the effects of the administration of abnormal PUFA: ETA (20:3, n-9) affected parameters linked to tumor progression and metastases in several human cancer cells lines. In the T-24 bladder cancer cell line, cell peroxidation and expression of the cell–cell adhesion molecule e-cadherin were decreased. In the MCF-7 breast cancer cell line, e-cadherin expression was increased, lipid peroxidation was enhanced, and cell proliferation was reduced. In the HRT-18 colon cancer cell

line, ETA produced an increment in e-cadherin expression and lipid peroxidation but also increased proliferation. We concluded that this 18:1 derivative produced some pro carcinogenic effects on three human cancer cell lines [139]. Other experiments in which ETA was added at a low concentration resulted in a reduction in the expression of e-cadherin, and to a lesser degree, of desmoglein, along with increased invasion of Matrigel using human squamous cell carcinoma (SCC) cells *in vitro*. At higher concentrations, ETA stimulated the growth of SCC cells [140]. The promoting effects of OA and its metabolites on cancer cells were recently confirmed. Soto-Guzman et al. [141] demonstrated that stimulation of MDA-MB-231 breast cancer cells with 200 μM of OA (18:1, n-9) induced an increase in MMP-9 secretion through a PKC-, Src-, and EGFR-dependent pathway (as revealed by gelatin zymography assays), increasing the invasiveness of breast cancer cells. In contrast, experimental studies in human cancer cells showed that stimulation of MDA-MB-231 breast cancer cells with OA promoted an increase in focal adhesion kinase (FAK) phosphorylation, as revealed by site-specific antibodies that recognize the phosphorylation state of FAK at tyrosine-397 (Tyr-397), Tyr-577, and *in vitro* kinase assays. Oleic acid also promotes the migration of MDA-MB-231 cells. These findings led the authors to describe a new signal transduction pathway, in which OA mediates the production of AA, and then AA metabolites mediate FAK phosphorylation and cell migration in MDA-MB-231 breast cancer cells [142].

The pro-carcinogenic action of OA was demonstrated by experimental research studying the activity of stearoyl CoA desaturase (SCD), the enzyme that produces monounsaturated fatty acids, in tumoral cells. This enzyme impairs lung cancer cell proliferation, survival, and invasiveness and dramatically reduces tumor formation in mice. In this way, Hess et al. [143] have demonstrated that exogenous OA treatment in human lung cancer cells restored the decreased membrane lipids levels resulting from inhibition of SCD activity, a key enzyme involved in the biosynthesis of unsaturated fatty acids. These data suggest that active lipid synthesis is required for the FA-mediated restoration of proliferation in SCD1-inhibited cancer cells. Although some evidence exists for the anti-proliferative potential of OA *in vitro*, most studies have shown OA and its derivatives to have pro-carcinogenic effects.

Animal studies Recent studies in our laboratories using an experimental murine model of mammary gland adenocarcinoma showed that diets rich in ω-9 FAs reduce tumor growth, metastasis, and tumor leukocyte infiltration by inhibiting LOX activity, thereby reducing the formation of pro tumorigenic eicosanoids such as 12 (S)-HETE and 15 (S)-HETE, increasing the synthesis of apoptosis-inducing 12 (S)-HHT, and decreasing the production of pro-inflammatory PGE_2 [144]. Our group also conducted other studies examining the modulating effects of diets rich in ω-9, ω-6, and ω-3 on the development of murine mammary gland adenocarcinoma. Different tumor parameters were analyzed. Our findings showed that a diet rich in OA induced an essential FA deficiency and increased the incidence and the number of metastases when compared with the controls [145].

2 Conclusion

Understanding the mechanisms of FA-regulated signaling and its molecular bioactive derivatives is a critical issue to determine their involvement in cell proliferation and apoptosis impacting of the promotion, progression, or regression of the carcinogenic process. The intervention on dietary FAs induces changes in membrane lipids modifying their bioactive lipids derived from specific biosynthetic pathways in response to extracellular stimuli. These molecules in turn modulate transcription factors involved in many physiological processes, and their deregulations have been linked to diseases such as inflammation, metabolic syndrome, and cancer. Knowledge of the mechanisms by which FAs control specific gene expressions may provide insight into the development of new therapeutic strategies for better management of whole body lipid metabolism, which, is discussed in this review, are important risk factors for cancer. Finally, to better understand the complex roles played by PUFAs in tumorigenesis, further research will be needed using a wide variety of experimental models and cell tumor lines as well as different FA concentrations.

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