Review

Interaction of brain fatty acid-binding protein with the polyunsaturated fatty acid environment as a potential determinant of poor prognosis in malignant glioma

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ABSTRACT

Malignant gliomas are the most common adult brain cancers. In spite of aggressive treatment, recurrence occurs in the great majority of patients and is invariably fatal. Polyunsaturated fatty acids are abundant in brain, particularly ω-6 arachidonic acid (AA) and ω-3 docosahexaenoic acid (DHA). Although the levels of ω-6 and ω-3 polyunsaturated fatty acids are tightly regulated in brain, the ω-6:ω-3 ratio is dramatically increased in malignant glioma, suggesting deregulation of fundamental lipid homeostasis in brain tumor tissue. The migratory properties of malignant glioma cells can be modified by altering the ratio of AA:DHA in growth medium, with increased migration observed in AA-rich medium. This fatty acid-dependent effect on cell migration is dependent on expression of the brain fatty acid binding protein (FABP7) previously shown to bind DHA and AA. Increased levels of enzymes involved in eicosanoid production in FABP7-positive malignant glioma cells suggest that FABP7 is an important modulator of AA metabolism. We provide evidence that increased production of eicosanoids in FABP7-positive malignant glioma growing in an AA-rich environment contributes to tumor infiltration in the brain. We discuss pathways and molecules that may underlie FABP7/AA-mediated promotion of cell migration and FABP7/DHA-mediated inhibition of cell migration in malignant glioma.

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Contents

1. Introduction

Gliomas are tumors that arise from glial or glial progenitor cells in the central nervous system. These tumors most commonly occur in the brain. A subtype of glioma, called astrocytoma, is characterized by expression of glial fibrillary acidic protein (GFAP), normally found in astrocytes. Anaplastic astrocytoma (grade III astrocytoma) and glioblastoma multiforme (grade IV astrocytoma) are collectively called high-grade astrocytomas or malignant gliomas. Malignant gliomas are the most common cancers of the central nervous system, accounting for ~70% of malignant primary brain tumors [1]. The prognosis of patients with malignant glioma is dismal with median survival times of 3 years and 15 months for patients with anaplastic astrocytoma and glioblastoma multiforme, respectively [2]. Although patients with grade II astrocytomas have a better prognosis, >35% of these tumors recur as high grade astrocytomas, further compounding the challenges associated with the treatment of astrocytoma tumors [3]. Surgical resection and adjuvant radiotherapy followed by chemotherapeutic agents such as temozolomide or nitrosourea is the standard treatment for malignant gliomas. In spite of this aggressive treatment, almost all (>90%) malignant gliomas recur, most commonly within 3 cm from the margin of the original tumor, suggesting that recurrence is due to infiltrative rather than invasive properties of the tumor cells [4–5]. Survival time is very short once recurrence has been diagnosed, usually 3–6 months [4].

Treatment options for recurring malignant glioma are limited because of toxicity and detrimental effects on brain function. For example, radical resection often cannot be considered for the treatment of recurrent tumors because of the associated decline in brain function, as measured by Karnofsky Performance Status, and/or surgery-related morbidity and infection [4,6–7]. A combination of chemotherapy or stereotactic radiosurgery with repeated surgery was shown to improve survival of patients with recurrent glioblastoma compared to surgery alone, although none of the patients in this study survived beyond 44 weeks after treatment [8]. Furthermore, the use of radiotherapy is limited in recurrent tumors because of associated irreversible brain tissue damage and radiation-induced necrosis of normal brain [9]. The recommendation to wait at least 6 months before initiating a second round of radiation treatment further limits this treatment option [4]. Despite the above limitations, the standard treatment for recurrent malignant glioma is still a combination of radiotherapy and chemotherapy [10], highlighting the need of finding new therapeutic strategies that will limit or prevent tumor infiltration and recurrence.

There are reports indicating that lipid metabolism is deregulated in malignant glioma and that altered lipid metabolism is associated with a worse prognosis in these tumors [11–12]. Brain fatty acid-binding protein (B-FABP or FABP7), involved in the intracellular transport of polyunsaturated fatty acids (PUFA), is up-regulated in glioblastoma compared to normal brain tissue and low grade astrocytomas [13–14]. Furthermore, elevated levels of FABP7 in the nucleus are associated with a worse prognosis in glioblastoma [15–16]. We and others have shown that expression of FABP7 in malignant glioma cell lines increases cell motility and migration [13,17]. Importantly, altering the DHA:AA ratio in the culture medium affects cell migration in a FABP7-specific manner, with an increased DHA:AA ratio associated with reduced cell migration [18]. In this review, we discuss how alterations in the lipid environment

together with FABP7 expression may affect malignant glioma tumor growth. We propose that a better understanding of the consequences of lipid alterations in malignant glioma may shed light on the mechanisms driving tumor recurrence thereby revealing new approaches for the treatment of malignant glioma.

2. Normal brain lipid composition

Lipids constitute ~2% of the total cell mass in most organs. However, in the brain, lipids are major structural components with fatty acids making up about 50% of the total mass of neural membranes [19–20]. Long chain PUFA such as docosahexaenoic acid (DHA, C22:6, ω-3) and arachidonic acid (AA, C20:4, ω-6) are abundant in brain, constituting close to 20% of the dry weight of the brain, including 6% for AA and 8% for DHA [20]. The fatty acid composition of the three major types of brain cells (neurons, oligodendrocytes and astrocytes) has been reported in rats [21]. In 60-day old rats fed a soya oil diet, ω-3 and ω-6 fatty acids constitute ~30%, ~20% and ~29% of the total neuron, oligodendrocyte, and astrocyte lipid content, respectively, including 8%, 5% and 12% for DHA and 10%, 9% and 10% for AA [21].

Although there is no consensus on how fatty acids are taken up by brain, there is evidence that the unesterified fatty acid (albumin-bound) pool in plasma is a major contributor to the fatty acid pool in brain, at least in the case of AA and DHA [22]. The importance of low-density lipoproteins (LDL) and very low density lipoproteins (VLDL) in brain PUFA uptake was assessed using mice deficient for the LDL receptor (LDLr) or VLDL receptor (VLDLr); however, no differences in PUFA levels were detected between knockout mice and the wild type controls, suggesting that LDL and VLDL do not play a major role in PUFA uptake in the brain [23–24]. Fatty acids have also been postulated to enter the brain by passive diffusion and protein-mediated transport by membrane-associated proteins, such as fatty acid transport proteins and fatty acid translocases (CD36) [22]. Inside the cell, long chain fatty acids are transported by a group of intracellular lipid binding proteins called fatty acid binding proteins (FABPs) which are expressed in most tissues [25–34] (Table 1).

It is well established that the essential fatty acids, cis-linoleic acid (LA, 18:2, ω-6) and α-linolenic acid (ALA, 18:3, ω-3), the precursors of AA and DHA, respectively, have to be obtained from the diet because our bodies cannot synthesize them [20,35]. In the liver, LA is converted to gamma-linolenic acid (GLA, 18:3, ω-6), dihomo-GLA (DGLA, 20:3, ω-6), and AA by different desaturases and elongases (Fig. 1) [35]. Similarly, ALA is converted to eicosa-

Table 1
Names of different fatty acid binding proteins (FABPs) and the tissues from which they were first isolated.

Fatty acid binding protein	Tissue
L-FABP (FABP1)	Liver[26]
I-FABP (FABP2)	Intestine[25]
H-FABP (FABP3)	Heart[27]
A-FABP (FABP4)	Adipocyte[28]
E-FABP (FABP5)	Epidermis[29]
IL-FABP (FABP6)	Ileum[30]
B-FABP (FABP7)	Brain[31]
M-FABP (FABP8)	Myelin[32]
T-FABP (FABP9)	Testis[34]
FABP12	Testis and retina[33]
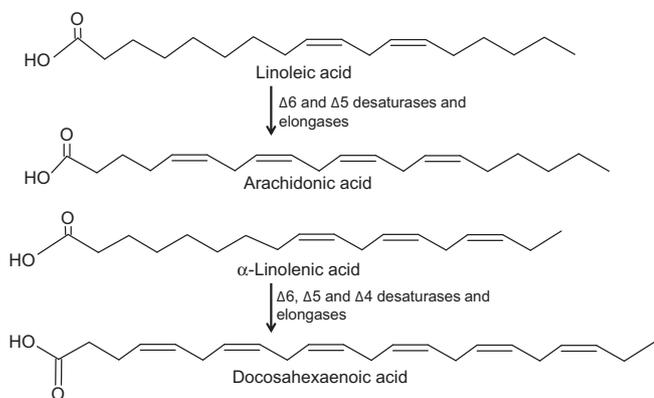
Fig. 1. Outline of the conversion of linoleic acid and α-linolenic acid into arachidonic acid and docosahexaenoic acid, respectively, by a combination of desaturases and elongases. Note that conversions of linoleic acid to gamma-linolenic acid, dihomo-gammalinolenic acid and α-linolenic acid to eicosapentae-noic acid are not shown.

pentaenoic acid (EPA, 20:5, ω-3) and DHA via the same combination of enzymes (Fig. 1). Both liver and diet are important determinants of brain DHA and AA composition. Studies done by Rapoport et al., who used positron emitting tomography to trace intravenously injected radiolabelled AA and DHA, showed that the rates of uptake of AA and DHA by human healthy brain were 17.8 mg/day/1.5 kg brain and 4.6 mg/day/1.5 kg, respectively [36]. Furthermore, incorporation rates of AA and DHA in the brain were approximated by their rates of loss from the brain [36]. The fact that the brain has little if any PUFA synthesis capacity, combined with the specific up-regulation of liver elongases and desaturases in animals fed a ω-3 deficient diet, led to the conclusion that the liver must play an important role in supplying the brain with AA and DHA [36–37].

3. Malignant glioma: lipid metabolism imbalance

Similar to other types of cancers, fatty acid uptake and lipid metabolism is deregulated in malignant glioma [38–39]. Indeed, a recent report indicates marked metabolic differences between low and high grade gliomas with metabolomic differences serving as a reasonably accurate (71%) predictive tool for high versus low grade glioma [11]. Of note, accumulation of long chain fatty acid metabolites was consistently associated with the worst survival in malignant glioma patients [11]. These findings are in agreement with earlier studies showing that the lipid environment is altered

in malignant glioma. Specifically, DHA levels were reduced by ∼50% in malignant glioma samples compared to normal brain tissue [12,40–41]. This reduction was evident whether total lipids or total phospholipids were analyzed. Upon testing the different phospholipid classes, DHA reduction was observed in phosphatidylethanolamine and phosphatidylserine fractions. The decrease in DHA observed in malignant glioma was accompanied by unchanged AA levels and an ∼4-fold increase in LA, resulting in an overall increase in ω-6/ω-3 fatty acid ratio compared to normal brain (Fig. 2) [12]. Although fatty acid composition of phospholipids from different grades of astrocytomas has not been explored in depth, a study published by Albert et al. demonstrates that decreased DHA content in the phosphatidylethanolamine fraction is greater in grade IV astrocytomas (*n* = 6) than in a grade II astrocytoma (*n* = 1) [40].

Using rat as a model, Igarashi et al. showed that in cases of DHA dietary deficiency, normal brain DHA content was maintained via liver conversion of ALA to DHA, provided that sufficient ALA was present in the diet [42]. In cases of ω-3 PUFA deficiency (greatly reduced ALA, no DHA) for 15 weeks, brain DHA content was reduced by 37% [37,43]. To maintain brain DHA content, brain DHA metabolism, as measured by iPLA$_2$ and COX-1, was reduced in ω-3 PUFA-deficient rats. In contrast, AA metabolism was up-regulated as the result of increased activity of AA-selective cytosolic phospholipase A$_2$ (cPLA$_2$), secretory PLA$_2$ (sPLA$_2$) and COX-2 [36]. These data suggest that there is a tight balance between brain AA and DHA turnover that serves to maintain the relative content of these two fatty acids at a favorable ratio (Fig. 2). In support of this idea, deprivation of ω-6 PUFAs was shown to increase brain DHA metabolism while maintaining AA levels by down-regulating enzymes involved in its metabolism [44]. Specifically, ω-6 PUFA deprivation resulted in up-regulation of the DHA selective intracellular phospholipase A$_2$ (iPLA$_2$ VIA) and 15-lipoxygenase (15-LOX). In a separate study, SREBP-1, a transcription factor involved in iPLA$_2$ regulation, was also shown to be up-regulated upon deprivation of ω-6 PUFAs [45]. Predictably, expression of proteins responsible for AA-metabolism such as cPLA$_2$ IVA and COX-2 were significantly reduced in ω-6-deprived rats, along with the transcription factors AP-2α and NF-κB p65 which are involved in the regulation of COX-2 and cPLA$_2$ IVA [45]. Collectively, these data indicate that brain turnover of AA and DHA is closely linked and maintenance of a favorable or homeostatic DHA:AA ratio is critical for normal brain function.

An increase in the AA:DHA ratio has been consistently reported in malignant glioma (Fig. 2) [12,40–41] suggesting that tumorigenicity is associated with enhanced DHA loss/metabolism, to the extent that brain DHA loss cannot be compensated for by liver or diet. The mechanisms driving DHA loss while maintaining AA levels and

	ω-3 Deficiency	AA metabolism ↑	DHA metabolism ↓
ω-3 Deficiency	AA metabolism ↑	DHA metabolism ↓
ω-6 Deficiency	AA metabolism ↓	DHA metabolism ↑
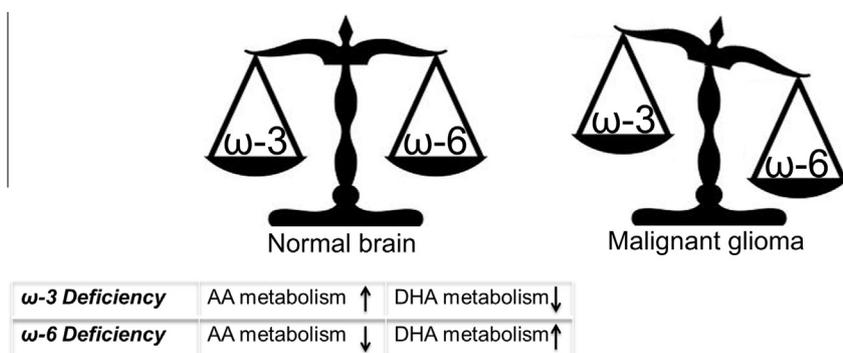
Fig. 2. Diagrammatic representation of the balance between ω-3 and ω-6 fatty acids in normal brain and the deregulation of this balance in malignant glioma. A tightly controlled AA:DHA ratio is maintained in normal brain, with ω-3 deficiency and ω-6 deficiency resulting in enhanced AA/decreased DHA metabolism and enhanced DHA/decreased AA metabolism, respectively. This ratio is deregulated in malignant glioma tumors, with decreased DHA content and increased linoleic acid resulting in a significantly decreased ω-3:ω-6 ratio.

increasing LA content are not clear. However, recent studies have shed some light on the inhibitory effects of DHA on pro-inflammatory pathways such as COX-2, which is believed to be important for cancer proliferation and invasion [46–47]. Therefore, decreased DHA levels may be important for tumor survival and infiltration. As brain fatty acid-binding protein (FABP7) is negatively correlated with survival in glioblastoma patients and binds AA and DHA depending on their relative availability (although it has a higher affinity for DHA), it is reasonable to postulate that this lipid metabolic imbalance is linked to FABP7 overexpression.

4. FABPs, their fatty acid ligands and interacting partners

FABPs are intracellular fatty acid binding proteins involved in the binding and the intracellular trafficking of fatty acids and retinoids to different subcellular compartments: cytoplasmic, for metabolism and energy production, or nuclear, for regulation of gene transcription *via* activation of peroxisome proliferator-activated receptors (PPARs) [48]. Studies indicate that FABPs may serve as major determinants of the pharmacokinetics and metabolism of fatty acids and their metabolites. For example, FABP1 (L-FABP) (Table 1) expression strongly correlates with hepatic uptake of palmitate and the production of palmitic acid metabolites [49].

Different FABP genes are expressed at different stages of brain development, each with demonstrated binding preference for certain fatty acids [50]. For example, FABP3 (or H-FABP) is expressed after birth with its levels gradually increasing until adulthood [51]. FABP3 preferentially binds ω-6 PUFA [52]. FABP5 (E-FABP) is expressed in mid-term embryonic rat brain and reaches its peak at birth, then gradually decreases in postnatal life [51]. FABP5 preferentially binds saturated fatty acids [53]. FABP7 is expressed mainly in radial glial cells at early stages of brain development [51,54–55]. Based on Isothermal Titration Calorimetry, FABP7 binds DHA and AA with dissociation constants (K_d) of 53 nM and 207 nM, respectively [56], indicating that a preferred ligand for FABP7 is DHA. The expression of FABP7 decreases dramatically in neonatal and adult brain [51]. When mixed cultures of rodent glial cells and neurons were incubated with an anti-FABP7 antibody, inhibition of glial fiber formation and neuronal migration was observed, indicating a role for B-FABP in the establishment of the radial glial fiber system [55]. FABP7 has garnered attention because of its association with decreased survival in glioblastoma patients [13,15–16,57]. Another FABP, FABP4, has recently been shown to be preferentially expressed in grade IV astrocytomas compared to low grade astrocytomas and normal brain tissue [58]. FABP4 does not co-localize with FABP7 in astrocytoma tumors but rather is expressed in endothelial cells and other yet-to-be-identified cell types [58]. Like FABP7, FABP4 binds AA with high affinity (K_d of ~250 nM) [59–60]. The role of FABP4 in astrocytoma tumors remains to be elucidated.

PPARs, especially PPARα, have been shown to interact with and regulate FABPs. Specifically, FABP1, FABP2 and FABP3 were induced in liver, intestine and heart, respectively, in response to treatment with the PPARα agonist, Wy14643 [61]. The authors showed that interaction with PPAR response elements (PPRE) was required for transcriptional up-regulation of FABP1 by Wy14643 in the liver [61]. Using recombinant proteins and Fluorescence Resonance Energy Transfer (FRET), PPARα was shown to bind FABP1 with high affinity (K_d 56.5 nM) and at close proximity, with an average intermolecular distance of 52 Å [62]. Furthermore, double immunogold labeling electron microscopy revealed significant co-localization of FABP1 and PPARα in both the nuclei and cytoplasm of hepatocytes from wild-type FABP1$^{+/+}$ mice [62]. These combined data suggest that PPARs and FABPs interact and that FABP expression is regulated through this interaction.

5. Brain fatty acid binding protein

5.1. FABP7 and its transcriptional regulators

PPAR ligands have been implicated in FABP7 induction during zebrafish development, with FABP7 levels significantly induced in the liver and intestine, but not the brain, of zebrafish treated with clofibrate, a specific ligand for PPARα and to a lesser extent for PPARγ [48,63]. There is evidence suggesting that natural PPAR ligands such as fatty acids can also affect FABP7 expression. Nasrollahzadeh et al. used a rat C6 glioma model to demonstrate specific up-regulation of FABP7 gene expression in tumor cells when rats were fed a DHA oil-rich diet compared to linoleic acid-rich safflower oil diet [64]. In contrast to DHA, supplementation with the ω-6 fatty acid, γ-linolenic acid, had no effect on the expression of FABP7.

Several transcription factors other than PPARs have been implicated in FABP7 regulation. Among these are the Nuclear factor I (NFI) transcription factors, with NFIA/B/X up-regulating FABP7, and NFIC down-regulating FABP7 in malignant glioma cell lines [65]. PAX6, a transcription factor of the paired box and homeobox family, also up-regulates FABP7 in malignant glioma [66]. The importance of PAX6 in FABP7 regulation is still under investigation as most FABP7 positive malignant glioma cell lines and tumors express little or no PAX6 [66]. In fact, PAX6 is expressed at significantly lower levels in glioblastoma compared to anaplastic astrocytoma tumors, with the latter having PAX6 levels that are equivalent to those found in normal tissue [67].

Examination of 274 astrocytoma specimens has revealed a correlation between Notch1 expression, tumor grade and survival that is similar to that observed for FABP7 [13,15,68]. As Notch1 signaling has been implicated in the regulation of FABP7 in radial glial cells [69–70], it is conceivable that Notch1 may be involved in the up-regulation of FABP7 in astroctyoma tumors. Notch1 ligands, Delta-like-1 and Jagged-1, are also expressed in malignant glioma cell lines and tumor tissues, with levels of Jagged-1 being up-regulated in higher grade gliomas including glioblastoma [69]. Depletion of Notch1, Delta-like-1 and Jagged-1 in U251 malignant glioma cells by specific siRNA transfection resulted in ~90%, 50%, and 25% reduction in Notch1 transcriptional activity, respectively, as measured using the luciferase assay. These combined data demonstrate a potentially important link between Notch1, Notch ligands and FABP7 in malignant glioma. This is important as Jagged-1 has previously been shown to play a role in the activation of the transcription factor Activator Protein 1 (AP-1), which in turn regulates the expression of proteins involved in AA and DHA metabolism such as cyclooxygenase-2 (COX-2) and cytochrome P450 2J2 (CYP2J2) [71–74]. Thus, Notch and/or its ligands may contribute to the deregulation of lipid metabolism in malignant glioma.

5.2. FABP7: link to prognosis, migration and lipid environment

In grade IV astrocytoma, FABP7 is highly expressed at sites of infiltration and surrounding blood vessels [15,17]. Of note, a negative correlation was found between nuclear FABP7 and survival in glioblastoma patients [15–16]. Borderline ($p = 0.084$) negative association of survival with cytoplasmic FABP7 has also been reported [16]. Furthermore, FABP7 is highly expressed in glioblastoma neurospheres [57,66], with evidence of radiation-induced up-regulation of FABP7 [57]. Manipulating FABP7 levels by either expressing it in the FABP7-negative malignant glioma cell line U87 or depleting it from the FABP7-positive cell line U251 had a striking effect on malignant glioma growth properties, with FABP7 expression correlating with reduced proliferation and increased

cell migration [13,17–18]. Similar results were observed upon depleting FABP7 in glioblastoma neurospheres [57]. When combined with immunostaining data showing the presence of FABP7 at sites of tumor infiltration in grade IV astrocytomas, these data suggest a role for FABP7 in tumor infiltration and recurrence [17]. Intriguingly, the effect of FABP7 on malignant glioma cell migration was found to be dependent on the fatty acid composition of the culture medium, with a high DHA:AA ratio inhibiting cell migration, and a high AA:DHA ratio promoting cell migration [18]. Transfection of malignant glioma cells with FABP7 mutant expression constructs that either do not bind PUFAs or do not localize to the nucleus revealed that FABP7 bound to AA promotes cell migration even when FABP7 does not localize to the nucleus. In contrast, the inhibition in cell migration observed upon increasing the DHA:AA ratio was only observed when FABP7 localized to the nucleus [18]. FABP7/AA-mediated cell migration was accompanied by up-regulation of COX2 and increased levels of prostaglandin E_2 (PGE$_2$), whereas FABP7/DHA-mediated inhibition of cell migration appeared to be at least partially driven by PPARγ [18].

As mentioned earlier, DHA levels are decreased in malignant glioma tumor samples resulting in a higher AA:DHA in tumor compared to normal brain tissue [12,41]. The up-regulation of FABP7 combined with the higher relative availability of AA may thus favor tumor infiltration. Therefore, it is conceivable that strategies aimed at correcting the DHA:AA ratio may result in the inhibition of tumor cell migration/infiltration and reduce tumor recurrence. The inhibitory effect of DHA on the migration of FABP7-positive malignant glioma cells *in vitro* lends further support to the idea that it may be possible to control malignant glioma infiltration through manipulation of the lipid environment. As glioblastoma tumor tissues also have elevated levels of the ω-6 LA, it would be worthwhile to investigate the effect of LA on the migration of FABP7-positive and FABP7-negative malignant glioma cells.

5.3. FABP7/fatty acid mechanisms controlling malignant glioma migration

To understand how AA and DHA affect malignant glioma growth, it is important to first understand the roles and utilization of these fatty acids in normal brain. Most AA and DHA are incorporated in the *sn*-2 position of phospholipids, with AA and DHA released from phospholipids by selective phospholipases (PLA$_2$) upon activation [75–76]. Most of the released AA and DHA undergo reincorporation into available *sn*-2 positions of lysophospholipids via acyl-CoA synthetase and acyltransferase [36]. Normally this deacylation–reacylation occurs rapidly in association with neurotransmission involving receptors coupled to PLA$_2$. A small fraction of the released AA and DHA is lost via either beta-oxidation or conversion into eicosanoids (for AA), or docosanoids (for DHA) [36]. It is worth mentioning that AA may also be cleaved from 1,2-diacylglycerol (which is cleaved from membrane phospholipids by the action of phospholipase C) through the action of diacylglycerol lipase [77].

Based on gene sequence comparisons, there are nine groups of PLA$_2$ isoforms which are expressed in different species. PLA$_2$ proteins are divided into three main groups based on their basic biochemical properties: (i) cytosolic Ca^{+2} dependent PLA$_2$ or group IV PLA$_2$ (cPLA$_2$, 85 kDa), (ii) Ca^{+2}-dependent extracellular secretory PLA$_2$ or (groups IB, IIA, IID, IIE, V, and X) (sPLA$_2$, 14 kDa), and (iii) Ca^{+2}-independent intracellular PLA$_2$ or group VI (iPLA$_2$, 85–88 kDa) [78]. Unsaturated fatty acids are highly vulnerable to lipid peroxidation and it is thought that PLA$_2$ preferentially releases peroxidized fatty acids from membranes. If these peroxidized fatty acids were to be retained in the membrane structure, this would lead to disruption of the membrane. PLA$_2$s are therefore critical for the integrity of the membrane structure under normal physio-

logical conditions [75]. Peroxidized fatty acids are then reduced by reaction with glutathione peroxidase and membrane repair is completed by reacylation with long-chain fatty acyl-CoA. Different PLA$_2$ isoforms show different substrate selectivity with cPLA$_2$ and iPLA$_2$ being selective for AA and DHA-containing phospholipids, respectively. sPLA$_2$ has no apparent selectivity requirement *in vitro* [75,78–79]. Furthermore, iPLA$_2$ appears to be involved in membrane phospholipid homeostasis and is up-regulated in response to increased phosphatidylcholine synthesis [80–81].

A fraction of the AA and DHA released from phospholipids is metabolized *via* three pathways, namely cyclooxygenases-2 (COX-2), lipoxygenases (LOX) and cytochrome P450 (CYP) epoxygenases and hydroxylases, thereby generating eicosanoids or docosanoids from AA or DHA, respectively. Eicosanoids include prostaglandins (PGs), leukotrienes, epoxyeicosatrienoic acids (EETs) and hydroxyeicosatrienoic acids (HETEs) [76]. Docosanoids include docosatrienes, protectins and resolvins [82–83].

6. Arachidonic acid-related mechanisms

6.1. Cytosolic phospholipase A2

cPLA$_2$ is of special interest since cPLA$_2$α knockout mice show defects in inflammation and generation of eicosanoids [84–85]. *cPLA$_2$α$^{-/-}$* mice have fertility defects and are partially protected in models of brain injury. When brain ischemia-reperfusion injury was induced in *cPLA$_2$α$^{-/-}$* mice, the infarct volume in the knockout mice was smaller (by 34%) than that of wild-type mice, with fewer functional neurological deficits observed in *cPLA$_2$α$^{-/-}$* compared to *cPLA$_2$α$^{+/+}$* mice [86]. The activity of cPLA$_2$ is regulated by Ca^{+2} levels and by phosphorylation. cPLA$_2$ is mainly phosphorylated and activated by mitogen-activated protein kinase (MAPK or ERK1/2) which phosphorylates cPLA$_2$ at Ser-505 [87]. Of note, MAPK has been reported to activate AP-1 in rat brain astrocytes through either direct phosphorylation of AP-1 proteins (Jun and Fos families) or phosphorylation of different factors involved in their transcription [88–89]. Importantly, MAPK is believed to be activated by Notch1 in some cancer types such as breast cancer and hematologic malignancies [90–91]. Activation of MAPK and cPLA$_2$ through Notch in FABP7-expressing malignant glioma cells may therefore result in increased AA metabolism, resulting in increased cell migration and infiltration.

AA and its eicosanoid metabolites play important roles in actin remodeling, a process that underlies the shape and structural changes associated with cellular migration and response to inflammation and angiogenesis [92]. In NIH3T3 mouse fibroblasts, the initial transient burst in AA resulting from cPLA$_2$ activation is responsible for cell adhesion, with the AA metabolites leukotrienes and PGs regulating cell spreading and cell migration, respectively [93–95]. Other reports have shown that cPLA$_2$α and its Ser-505-phosphorylated form are rapidly and specifically recruited at sites of actin polymerization such as membrane ruffles and leading edges [92]. A role for cPLA$_2$α in actin remodeling may be highly relevant in the context of FABP7/AA inducing malignant glioma cell migration [17–18], especially in light of the fact that: (i) the increase in FABP7-mediated malignant glioma cell migration is accompanied by increased expression of AA-metabolizing enzymes such as COX-2 and its metabolite PGE$_2$ [18] and (ii) malignant glioma tumor tissues have a readily available supply of AA probably through an increased load of LA [12,41]. However, studies of the detailed mechanisms underlying these observations and potential involvement of cPLA$_2$ have yet to be carried out. We have used cDNA microarray analysis to compare gene expression in highly migratory U87 FABP7-positive versus non-migratory U87 FABP7-negative cells cultured in an AA-rich environment. Interestingly,

cPLA$_2$ IVA (group IV A) was up-regulated by 27-fold in the FABP7-positive cells. These results are in keeping with FABP7 promoting an AA cascade starting at the earliest step of AA release and ending with eicosanoid production.

6.2. Cyclooxygenase

AA is a major substrate for COX (prostaglandin-endoperoxide synthase) enzymes which catalyze its conversion to the key upstream prostanoid precursor prostaglandin H$_2$ (PGH$_2$). There are two main COX isoforms, namely COX-1, which is constitutively expressed and is responsible for production of physiological levels of PGs, and COX-2, which is inducible and up-regulated by different growth factors and cytokines [96]. Transcription factors NF-κB and AP-1 regulate COX-2 transcription in the brain [73,97] and mitogen-activated protein kinase (MAPK) cascades (ERK1/2, JNK/SAPK, and p38) contribute to its induction [96].

COX-2's importance in carcinogenesis, progression and survival of patients with different types of cancers is well established [98–100]. Clinical studies designed to assess the efficacy of combined COX-2 inhibitors and chemotherapy versus chemotherapy alone showed potential benefits only in those patients with elevated levels of COX-2 in their tumors [101–102]. A correlation between COX-2 expression and glioma grades has been described, with one report indicating that 71% of glioblastoma tumors had >50% COX-2-positive cells compared to 44% of anaplastic astrocytomas and 30% of low-grade astrocytomas [103–104]. Additionally, a significant correlation was observed between COX-2 expression and survival in glioblastoma patients [103]. We showed that COX-2 and PGE$_2$ levels were increased in FABP7-positive U87 malignant glioma cells [18]. FABP7-positive U87 cells also had increased levels of PPARβ/δ compared to their FABP7-negative counterparts. Interestingly, PPARβ/δ activation was shown to induce COX-2 and PGE$_2$ levels which in turn induced the phosphorylation of cPLA$_2$α, in human cholangiocarcinoma cells [105]. Given the interaction of COX-2 with AA and PPARβ/δ, and the involvement of FABP7 with AA and PPARβ/δ in malignant glioma [18], it is possible that FABP7 is playing an important role in regulating COX-2 activity through PPARβ/δ activation.

6.3. Cytochrome P450

Recent studies indicate that some CYP-mediated metabolites of AA possess pro-tumorigenic, pro-angiogenic, pro-migratory and pro-invasive properties in different cancer types [106–109]. For example, the CYP epoxygenase 2J2 transforms AA into four regioisomeric epoxyeicosatrienoic acids (EETs). Overexpression of CYP2J2 or direct addition of EETs to four cancer cell lines derived from liver, lung, and tongue resulted in a 4.5–5.5× increase in migration based on the Transwell assay and a 3–3.5X increase in invasion based on the Matrigel assay [108]. Furthermore, MDA-MB-231 human breast carcinoma cells infected with adeno-associated viral vector containing CYP2J2 showed 60% more lung metastases compared with control mice [108]. It is noteworthy that the expression of some members of the CYP family is altered in U87 FABP7-positive versus U87 FABP7-negative malignant glioma cells. CYP2J2 in particular is up-regulated >100× at the RNA level based on our cDNA microarray data.

AA metabolism occurs mainly in the cytoplasm, at the luminal surface of the endoplasmic reticulum and on the inner and outer membranes of the nuclear envelope, where COX-2 and different CYP isoforms are expressed [110–111]. As we have shown that nuclear localization of FABP7 is not required, whereas fatty acid binding is essential, for induction of cell migration in U87 cells [18], our results suggest that cytoplasmic FABP7 bound to AA drives the increase in cell migration observed in FABP7-positive
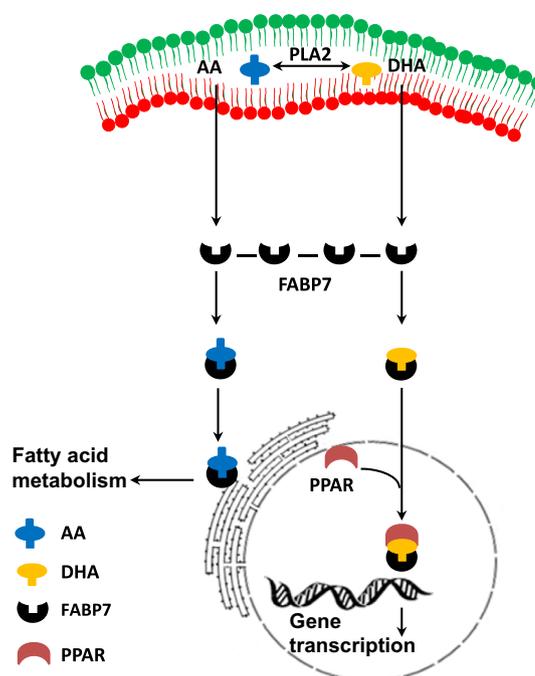
Fig. 3. Diagram depicting the role of FABP7 in the intracellular trafficking of fatty acids. Fatty acids AA or DHA are cleaved from membrane phospholipids depending on which PLA$_2$ isoform is activated. Upon release, AA or DHA binds to FABP7 which then translocates the fatty acid either to the nucleus, where it activates PPARs (for DHA) or to the endoplasmic reticulum for fatty acid metabolism (for AA).

U87 cells. We propose that the effect of FABP7 on cell migration is driven by enhanced translocation of AA, mediated by FABP7, to the endoplasmic reticulum as well as the overexpression of AA metabolism enzymes discussed above (Fig. 3). It would be interesting to examine the effect of blocking FABP7 localization in the endoplasmic reticulum on AA-induced migration and eicosanoid production.

7. Docosahexaenoic acid-related mechanisms

Inhibition of cell migration in U87 B-FABP-positive cells is observed upon increasing the DHA:AA ratio in the culture medium. Our laboratory has shown that the inhibitory effect of DHA on cell migration is partially mediated by PPARγ [18]. A documented natural ligand for PPARγ is DHA [112]. Furthermore, FABP7/DHA-dependent inhibition of migration is dependent on the nuclear localization of FABP7 [18]. These results suggest that DHA-bound-FABP7 is translocated to the nucleus where the complex interacts with and activates the PPARγ transcription factor (Fig. 3). In support of this hypothesis, Adida and Spener showed interaction between FABP7 and PPARγ in the monkey kidney (COS) cell line [113]. Target genes previously shown to be down-regulated by PPARγ include NF-κB and COX-2, involved in inflammation, and VCAM and ICAM, involved in cell adhesion [47]. Our results indicating that COX-2 is up-regulated and PPARγ is down-regulated, in FABP7-positive U87 cells cultured in an AA-rich environment, are in keeping with COX-2 being down-regulated by PPARγ in DHA-treated malignant glioma [18]. It is worth mentioning that some DHA metabolites such as 17-hydroxy DHA are more potent activators of PPARγ than DHA itself [112,114] and therefore could contribute to the DHA effect.

Pathways other than PPARγ likely contribute to the inhibition of cell migration observed in FABP7-expressing U87 cells cultured in DHA-rich medium as efficient knock-down of PPARγ does not

fully restore cell migration [50]. For example, production of anti-migratory DHA metabolites and inhibition of eicosanoid production, and hence migration, by direct competitive inhibition are possible mechanisms. A report from 30 years ago showed that DHA acts as a potent competitive inhibitor of AA metabolism by COX-2 [115]. More recent reports indicate that DHA also inhibits AA metabolism by CYP epoxygenases and hydroxylases [82]. In brain-metastatic melanoma and in a neuroblastoma cell line [116], DHA competition with AA resulted in decreased PGE_2 levels, with COX-2-mediated DHA metabolite PGE_3 significantly decreasing invasion in a dose-dependent manner [46]. DHA was also metabolized by recombinant COX-2 to 13-hydroxy DHA which has anti-inflammatory properties and down-regulates cytokine production and neutrophil recruitment to sites of inflammation [117]. Others have shown that metabolites derived from AA-COX and AA-LOX were significantly decreased in brain microvessels upon feeding marine fish oil to weanling rats [118]. In light of a recent report indicating that CYP2J2 is 4-fold more active in DHA metabolism than AA metabolism [82], it will be interesting to assess how DHA treatment of FABP7-positive malignant glioma cells affects CYP2J2 activity. These examples demonstrate the complexity of the interrelationship between the molecules and enzymes that utilize and process AA, DHA and their metabolites. We suggest that upstream regulators of genes encoding enzymes involved in AA and DHA metabolism such as Notch1 and PPARs may coordinate cellular responses depending on levels and availability of AA and DHA (Fig. 4).

8. Neuroimaging of AA and DHA: potential for astrocytoma detection and grading

The rates of uptake of some fatty acids in brain tumors are different from that of normal brain tissue. For example, Nariai et al. [39] used autoradiogrady to examine the uptake of intravenously-injected radiolabeled [9,10-^{3}H] palmitate, [1-^{14}C] AA and [1-^{14}C] DHA into the lipids of Walker 256 carcinosarcoma cells intracerebrally implanted in rats. The authors showed that brain incorporation rates of these fatty acids was equal to their rates of metabolism by brain and that brain tumors had higher rates of uptake compared to normal brain tissue [39].

More recently, 1-^{11}C-AA and 1-^{11}C-DHA radiotracers have been used to image AA and DHA metabolism in the brain by positron emission tomography (PET) [36,119–122]. Studies are underway to develop ^{18}F-fluoro tracers, as ^{18}F has a half life of 110 min compared to 20 min for ^{11}C, thus allowing the capture of higher resolution images. While AA and DHA-based radiotracers have not yet been used to image brain tumors, PET imaging using 1-^{11}C-acetate and 2-^{18}F-fluoro-2-deoxy-D-glucose tracers have shown promise

for the detection of primary astrocytomas in humans [123]. Furthermore, standardized uptake values that correlated with astrocytoma histological grade were observed with 2-^{18}F-fluoro-2-deoxy-D-glucose [123]. We propose that the altered uptake/turnover of AA and DHA in malignant glioma compared to normal brain tissue could be exploited for the imaging and grading of astrocytomas. High resolution imaging of radiolabeled AA and DHA may also allow detection of small tumor lesions in brain as well as identify multiple sites of tumor growth in recurrent astrocytoma.

9. Conclusion

The PUFA-rich lipid environment of the brain is critical to our understanding of malignant glioma behavior. Key proteins in AA and DHA metabolism have already been identified in brain, resulting in the production of AA and DHA metabolites with opposing roles in migration and invasion. We propose that FABP7 coordinates the utilization of AA and DHA in the brain and in malignant glioma depending on the relative availability of these two PUFAs. In turn, the amount of AA and DHA available for binding to FABP7 may be determined by specific PLA_2's which release AA and DHA from phospholipids. Furthermore, PLA_2's and other enzymes involved in AA and DHA metabolism may themselves be regulated through AA-and DHA-dependent pathways (e.g. PPARs). A better understanding of the opposing effects of AA and DHA in malignant glioma may lead to the identification of new therapeutic approaches based on modifying the lipid environment of the tumor.

Acknowledgements

Fig. 4. Diagrammatic representation of pathways that may be involved in FABP7-induced malignant glioma cell migration. Pro-migratory and anti-migratory pathways controlled through AA-FABP7 and DHA-FABP7, respectively, are indicated. $^+$ PPARβ/δ enhances COX-2 activity whereas PPARγ down-regulates COX-2.

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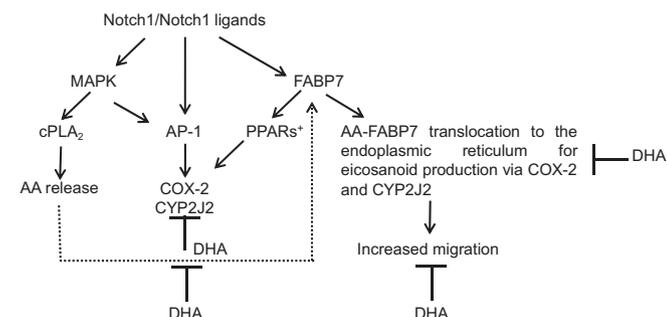
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