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Polyunsaturated Fatty Acids from Astrocytes Activate PPAR Gamma Signaling in Cancer Cells to Promote Brain Metastasis

Yongkang Zou¹, Andrea Watters¹, Nan Cheng¹, Caroline E Perry², Ke Xu³, Gretchen M Alicea¹, Joshua L.D Parris², Ezra Baraban⁴, Pulak Ray⁵, Anupma Nayak⁴, Xiaowei Xu⁴, Meenhard Herlyn⁶, Maureen E Murphy², Ashani T Weeraratna¹, Zachary T. Schug², Qing Chen¹

¹ Immunology, Microenvironment and Metastasis Program, The Wistar Institute, Philadelphia, PA 19104

² Molecular and Cellular Oncogenesis Program, The Wistar Institute, Philadelphia, PA 19104

³ MD/PhD Program, Boston University School of Medicine, Boston, MA 02215

⁴ Departments of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, 19014

⁵ Delaware Neurosurgical group, Christiana Care Health System, Newark, DE 19713

⁶ Melanoma Research Center and Molecular and Cellular Oncogenesis Program, The Wistar Institute, Philadelphia, PA 19104

Abstract

Brain metastasis, the most ominous form of melanoma and carcinoma, is the consequence of favorable interactions between the invaded cancer cells and the brain cells. Peroxisome proliferator-activated receptor γ (PPAR γ) has ambiguous functions in cancer development and its relevance in advanced brain metastasis remains unclear. Here, we demonstrate that astrocytes, the unique brain glia cells, activate PPAR γ in brain metastatic cancer cells. PPAR γ activation enhances cell proliferation and metastatic outgrowth in the brain. Mechanistically, astrocytes have a high content of polyunsaturated fatty acids that acts as ‘donors’ of PPAR γ activators to the invaded cancer cells. In clinical samples, PPAR γ signaling is significantly higher in brain metastatic lesions. Notably, systemic administration of PPAR γ antagonist significantly reduces brain metastatic burden *in vivo*. Our study clarifies a pro-metastatic role for PPAR γ signaling in cancer metastasis in the lipid rich brain microenvironment and argues for the use of PPAR γ blockade to treat brain metastasis.

Correspondence: Qing Chen, MD, PhD, 3601 Spruce Street, Philadelphia, PA 19104, Phone: 215-898-3844; qichen@wistar.org.

AUTHOR CONTRIBUTIONS

Conception and design: Y. Zou, Q. Chen

Development of methodology: G. Alicea, C.E. Perry, A. Weeraratna, Z. Schug, J. L.D. Parris, M. Herlyn, M.E. Murphy

Acquisition of data (provide animals, acquired and managed patients, provided facilities, etc.): Y. Zou, A. Watters, N. Cheng, C.E. Perry, J.L.D. Parris, E. Baraban, P. Ray, A. Nayak, X. Xu, M.E. Murphy, Z. Schug, Q. Chen

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K. Xu

Writing, review and/or revision of the manuscript: Y. Zou, K. Xu, M.E. Murphy, Q. Chen

Study supervision: Q. Chen

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INTRODUCTION

Metastasis, the spread of cancer from primary tumor sites to distal organs, is the cause of 80% of deaths from cancer. The brain is one of the common metastasis locations for carcinoma (e.g., breast and lung carcinoma) and melanoma (1). Compared to carcinoma, melanoma has a much higher propensity to metastasize to the brain: over one-third of metastatic melanoma patients develop a clinically apparent brain metastasis (2). Brain metastasis typically occurs at a late stage of the disease progression after patients have already survived primary tumors and metastatic disease in other organs. Therapeutic strategies, including novel chemotherapies and targeted inhibitors, have historically focused on controlling the disease at primary sites and visceral organs (3). However, these therapies have shown limited efficacy in brain metastatic lesions. As a consequence, brain metastasis is a significant problem in the clinic due to its rising incidence and its resistance to existing therapies (1,3,4). There is an urgent need to expand our knowledge on the mechanistic underpinnings of brain metastasis as a disease, from which new targeted therapies can be developed.

As envisioned in the “seed and soil” hypothesis, cancer metastasis depends on the complex interplay between cancer cells and the microenvironments in distal organs (5–8). The ‘soil’, representing the microenvironment, not only regulates the outgrowth of metastatic cancer cells, but also contributes to therapy resistance. The brain has a unique microenvironment. At the cellular level, it is composed of brain-specific cell types: functional neurons and supporting glial cells. Astrocytes are the most abundant glial cells in the brain and contribute to the pathogenesis of many brain disorders (9,10). A common hallmark of brain pathologies is reactive astrogliosis, where astrocytes increase glial fibrillary acidic protein (GFAP) expression and cellular processes (9,10). Examination of the very early steps of brain colonization in experimental mice revealed that activated astrocytes associate with invading cancer cells and this interaction persists throughout the formation of large metastatic lesions (11,12). At the molecular level, one distinct feature is that the brain is the fattiest organ in the body. Lipid constitutes ~50% of the brain (13). Unlike adipose tissue, the fatty acid component of the brain is enriched in polyunsaturated fatty acids (13). Of note, glial cells, but not neurons, regulate fatty acid synthesis and metabolism in order to maintain the normal function of the brain (14–16). The ‘seed’, the invaded cancer cells, needs to survive, proliferate and eventually form metastatic lesions. It is still unclear how the brain-tropic cancer cells adapt to the unique brain microenvironment.

In this study, we observed that brain metastatic cancer cells took advantage of the high fat microenvironment in the brain for metastatic outgrowth. As a major cellular source of fatty acid synthesis, astrocytes supplied arachidonic acid (AA, 20:4) and mead acid (20:3) to activate the proliferator-activated receptor γ (PPAR γ) pathway in the surrounding cancer cells. PPAR γ has diverse functions in different cancer types, stages and when combined with different therapeutic strategies (17–19). Here, we identified a pro-proliferative function of PPAR γ pathway in brain metastatic cancer cells. Furthermore, systemic blockage of PPAR γ pathway specifically decreased brain metastases, but not extracranial tumor growth, in the pre-clinical mouse models.

RESULTS

Pro-metastatic effect of astrocytes on brain metastatic cancer cells

To develop clinically relevant brain metastasis models, we used patient-derived xenografts (PDX) established from surgically removed melanoma brain lesions (20) and performed *in vivo* selection to isolate brain tropic melanoma cells, which we termed BrM cells (21,22) (Fig. 1A and Supplemental Fig. 1A). To track the growth of cancer cells in the experimental mice, we stably labeled the melanoma cells with far-red luciferase and fluorescent protein (Supplemental Fig. 1A). Of note, WM5265.2 cells from the brain metastasis PDX model remained brain tropic in the experimental mice with very limited ability to form metastases in other organs (i.e., lung) (Supplemental Fig. 1B). In contrast, WM1366 or WM793 cells, both from the primary melanoma PDX models (Supplemental Fig. 1A), either formed no metastatic outgrowth or massive metastases throughout the whole body (Supplemental Fig. 1B). In parallel, we developed a syngeneic melanoma brain metastasis model using mouse Yumm1.7 melanoma cell line, established from a BRAF^{V600E}/PTEN^{-/-}/CDKN2A^{-/-} transgenic mouse (Fig. 1A and Supplemental Fig. 1A) (23).

In the brain lesions formed by WM4265.2-BrM1 cells and Yumm1.7-BrM cells, we detected GFAP⁺ astrocytes surrounding the cancer cells (Fig. 1B and Supplemental Fig. 1C). This is consistent with observations in the breast cancer brain metastasis model using MDA231-BrM cells, in which activated astrocytes associate with invading cancer cells and this interaction persists throughout the formation of large metastatic lesions (12). We further confirmed the presence of activated astrocytes in the brain metastatic lesions from melanoma patients (Fig. 1C). To detect the contribution of astrocytes on the growth of BrM cancer cells, we established cancer-astrocyte co-culture assays under both 2-dimensional (2D) and 3D conditions (Fig. 1D–K). We tracked and quantified the growth of cancer cells by their fluorescence (Fig. 1E), luciferase labeling (Fig. 1F,J) and cell number counts (Supplemental Fig. 2A) in the co-culture experiments. In nutrition-restricted culture media (1% serum), astrocytes promoted the growth of both melanoma WM4265.2-BrM1, Yumm1.7-BrM cells as well as breast cancer MDA231-BrM cells (Fig. 1D–K, Supplemental Fig. 2A). In complete media (10% serum), this astrocyte-promoted growth was abolished or much less significant, as the cancer cells grow relatively faster than the nutrition-restricted condition (Supplemental Fig. 2B,C). Notably, astrocytes elicited more pro-growth effects on brain metastatic cancer cells in physiologically relevant 3D co-cultures. We confirmed that the 3D co-cultured spheroids mimicked the cancer cell-astrocytes interactions in the brain metastatic lesions *in vivo* (Fig. 1I and Supplemental Fig. 2D). In addition, in consistent with previously published work (24), astrocytes protected MDA231-BrM cells from apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (Supplemental Fig. 2E). Overall, our data indicate that astrocytes promote a pro-growth and pro-survival effect on brain metastatic cancer cells.

Gene expression profiling predicts PPAR signaling as a brain metastasis mediator

We established two BrM derivatives from parental WM4265.2 cells, designated WM4265.2-BrM1 and WM4265.2-BrM2. WM4265.1-BrM2 cells showed significantly lower brain metastasis potential relative to WM4265.2-BrM1 despite the fact that they were selected

from the high brain metastatic parental WM4256.2 cells (Fig. 2A,B). To form brain metastases from circulating cancer cells, consequential steps are required: 1) cancer cell migration across BBB, 2) cancer cell survival in the brain microenvironment and 3) cancer cell growth. Thus, we compared the parental WM4256.2, the high brain metastatic WM4265.2-BrM1 and the low brain metastatic WM4265.2-BrM2 cell lines for these aspects. We first quantified the number of cancer cells extravasated across BBB in the experimental mice. 7 days are required for the cancer cells to completely pass BBB to get into brain parenchyma (12). There was no difference on the migration of the three WM4256.2 cell lines across BBB (Fig. 2C). We further compared the survival and growth of the cancer cells *in vitro*. None of the WM4256.2 cell lines were sensitive to natural apoptosis inducers (sFasL or TRAIL). Thus, we applied two drugs with different killing mechanisms, cisplatin (DNA damage inducer) and staurosporine (broad protein kinase inhibitor), to induce cell death (Fig. 2D and Supplemental Fig. 3A). We did not detect any difference on either IC50 (Fig. 2E and Supplemental Fig. 3B) or drug induced apoptosis (detected by cleaved caspase 3) (Fig. 2F and Supplemental Fig. 3C) in the three WM4256.2 cell lines. Lastly, we tracked the growth rate of the three WM4256.2 cell lines in culture. Both high brain metastatic parental WM4265.2 and WM4265.2-BrM1 showed a growth advantage over low brain metastatic WM4265.2-BrM2 cells (Fig. 2G). In addition, in low serum condition (1% serum), we observed significant pro-growth effect of astrocytes only in parental WM4265.2 and WM4265.2-BrM1, but not in WM4265.2-BrM2 cells (Fig. 2H). This was not due to the difference in astrocyte interactions during co-culture *in vitro* (Supplemental Fig. 3D) or in brain metastatic lesions *in vivo* (Supplemental Fig. 3E). Thus, the growth of WM4265.2-BrM1 is faster than WM4265.2-BrM2 and can be further enhanced by astrocyte coculture.

To obtain a broad picture of the putative underlying mechanisms, we performed RNAseq to unbiasedly compare gene expression profiles 1) between WM4265.2-BrM1 and WM4265.2-BrM2 cells; and 2) astrocyte-induced changes between WM4265.2-BrM1 and WM4265.2-BrM2 cells. Using Ingenuity Pathway Analysis (IPA), we identified multiple pathways that were differentially activated under each comparison (Fig. 2I and 2J). Between these two comparisons, we identified two shared pathways: PPAR and eukaryotic initiation factor 2 (EIF2) pathways (Fig. 2I,J and Supplemental Fig. 4A–C). EIF2, an enhancer of the translation of specific stress-related mRNA transcripts, has been shown to cancer cell promote proliferation and survival (25). This is consistent with our functional assays showing increased cell growth of WM4265.2-BrM1 both cultured alone (Fig. 2G) and together with astrocytes (Fig. 2H) relative to WM4265.2-BrM2 cells. Another shared activated pathway was PPAR signaling pathway. Therefore, we set out to further validate the activity and the functional relevance of PPAR pathways in brain metastasis.

PPAR γ signaling is a brain metastasis mediator

PPARs are ligand-activated transcription factors of nuclear hormone receptor superfamily comprising of following three subtypes: PPAR α , PPAR β/δ and PPAR γ . All of these PPAR members form heterodimers with nuclear retinoid X receptor (RXR) and bind to the common peroxisome proliferator-activated receptor response element (PPRE) to activate target genes. However, differential activation of PPARs elicit distinct biological activities

(26,27). To validate our IPA analysis and to begin to identify the subtype(s) of PPARs involved, we assessed the binding of individual PPARs to PPRE in three WM4265.2 cell lines. We detected increased PPRE binding by PPAR β/δ and PPAR γ , but not PPAR α , in parental WM4265.2 and WM4265.2-BrM1 relative to WM4265.2-BrM2 cells (Fig. 3A). These results validate our IPA from RNAseq and suggest a correlation between increased PPAR activity and enhanced brain metastasis potential. To further elucidate the nature of the PPAR signaling, we determined the level of protein expression and the location of PPAR γ and PPAR β/δ . We observed elevated PPAR γ expression, but not PPAR β/δ , in parental WM4265.2 and WM4265.2-BrM1 relative to WM4265.2-BrM2 (Fig. 3B). This elevated protein expression of PPAR γ was not due to an increase in its steady-state mRNA level (Supplemental Fig. 5A). Importantly, in all of the melanoma and breast cancer BrM cells tested, the majority of PPAR γ was localized to the nucleus, where PPAR γ binds to RXR to activate gene expression (Fig. 3B and Supplemental Fig. 5B). In contrast, the majority of PPAR β/δ was localized to the cytosol (Fig. 3B and Supplemental Fig. 5B). These data suggest PPAR γ pathway is activated in those cancer cells that possess the high ability to form brain metastases.

To further elucidate the PPAR pathway underlying the growth advantage of BrM cells, we applied specific antagonists of PPAR γ (T0070907) or PPAR β/δ (GSK3787) to assess their effects on the growth of WM4265.2-BrM1 cells. PPAR γ antagonist, but not PPAR β/δ antagonist, inhibited cell growth in complete cell culture media (10% serum) (Fig. 3C). Moreover, the growth advantage provided by the astrocyte co-culture under low serum conditions (1% serum) was inhibited by PPAR γ antagonist, but not PPAR β/δ antagonist (Fig. 3D). In contrast, PPAR γ agonist (Rosiglitazone), but not PPAR β/δ agonist (GW501516), promoted the growth of WM4265.2-BrM1 cells (Supplemental Fig. 5C). We also compared the responses to Rosiglitazone between high PPAR γ -signaling WM4265.2-BrM1 and low PPAR γ -signaling WM4265.2-BrM2 cells. A higher dose of Rosiglitazone was required to elicit pro-growth effect in WM4265.2-BrM2 cells (Fig. 3E). We replicated these experiments to confirm the PPAR γ -activated cell growth in high brain metastatic breast cancer MDA231-BrM and mouse melanoma Yumm1.7-BrM cells (Supplemental Fig. 5C–5E).

To specifically ascertain the functional relevance of PPAR γ signaling in brain metastasis, we employed genetic approaches to deplete PPAR γ expression in both WM4265.2-BrM cells using short hairpin RNA (shRNA). Because constitutive depletion of PPAR γ may affect proliferation and subsequently the heterogeneity of the WM4265.2-BrM cells, we used doxycycline-inducible shRNA to knock down the expression of PPAR γ (Fig. 3F and Supplemental Fig. 5F). PPAR γ depletion in WM4265.2-BrM1 cells decreased cell growth (Fig. 3G) and astrocyte-induced enhancement in cell growth (Fig. 3H) *in vitro*. In contrast, decreasing the level of PPAR γ did not affect the growth of WM4265.2-BrM2 cells (Fig. 3G). To test the effect of PPAR γ depletion on brain metastasis *in vivo*, we injected these doxycycline-inducible PPAR γ knock-down WM4265.2-BrM1 cells into experimental mice treated with doxycycline-infused food and water. As shown previously (24), doxycycline can pass BBB into the brain metastatic lesions. Depleting PPAR γ in WM4265.2-BrM1 cells significantly decreased brain metastatic burden (Fig. 3I). Thus, PPAR γ signaling facilitates the metastatic outgrowth in the brain.

PPAR γ signaling promotes the proliferation of brain metastatic cancer cells

To determine how PPAR γ pathway contributes to the growth of BrM cells, we tested the effect of a PPAR γ antagonist on cell proliferation and apoptosis. We prelabeled BrM cells with CellTrace dye to track and quantify their proliferation. In complete cell culture media (10% serum), T0070907 inhibited the proliferation of the high brain metastatic melanoma WM4265.2-BrM1 and breast cancer MDA231-BrM cells (Fig. 4A). Compared to WM4265.2-BrM1 cells, this inhibitory effect was less in WM4265.2-BrM2 cells (Fig. 4A). We used Annexin V and DAPI staining to track and quantify the death of BrM cells. Under the more stringent low serum condition (1% serum), T0070907 did not affect the cell death (Fig. 4B). Similarly, neither Rosiglitazone nor T0070907 altered cancer cell apoptosis (detected by cleaved caspase 3) induced by TRAIL or drugs inducing cell death (Fig. 4C and Supplemental Fig. 5G). Lastly, under low serum condition (1% serum), astrocyte co-culture increased the proliferation of high brain metastatic WM4265.2-BrM1 and MDA231-BrM cells, and this effect was diminished by T0070907 (Fig. 4D). In contrast, T0070907 did not change the protective effect of astrocytes on MDA231-BrM cells treated with TRAIL (Fig. 4E). Therefore, we conclude that PPAR γ activation contributes to BrM cell proliferation, but not survival, and is further enhanced by the presence of astrocytes.

Polyunsaturated fatty acids released from astrocytes activates the PPAR γ pathway in brain metastatic cancer cells

PPAR γ can be activated by both naturally occurring ligands (e.g., polyunsaturated fatty acids) and pharmacologically synthesized agents (e.g. Rosiglitazone). The brain is enriched with polyunsaturated fatty acids (13), which are critical precursors to generate phospholipids for cell membrane. In the brain, fatty acid synthesis in astrocytes is critical for the normal function of the brain (14–16). For example, fatty acids produced by astrocytes are taken up by neurons to support synapse formation and function (28,29). We hypothesize that astrocytes serve as a ‘donor’ of polyunsaturated fatty acids to activate PPAR γ signaling in the surrounding BrM cells. We collected whole cell lysates from WM4265.2-BrM1, MDA231-BrM cancer cells and human astrocytes to quantify 70 different types of fatty acids by mass spectrometry. We detected reliable peaks for 45 fatty acids (Fig. 5A). Overall, astrocytes have a much higher content of detected fatty acids than our brain metastatic cells (Fig. 5A). Compared to both melanoma and breast cancer BrM cell lines, the top 3 enriched fatty acids in astrocytes were arachidonic acid (AA) (20:4), mead acid (20:3) and docosahexaenoic acid DHA (22:6) (Fig. 5A,B). When directly added into the culture media, AA and mead acid, two structurally similar fatty acids, promoted the growth of WM4265.2-BrM1 and MDA231-BrM cells (Fig. 5C). Compared to WM4265.2-BrM1, the WM4265.2-BrM2 cell line, which shows modest PPAR γ -signaling, had a markedly decreased response to AA (Supplemental Fig. 6A). In contrast, DHA did not change the growth of any of the BrM cells (Fig. 5C and Supplemental Fig. 6B). Furthermore, the pro-growth effect of AA and mead acid was abolished by the specific PPAR γ antagonist T0070907 (Fig. 5D). The combined data support the premise that AA and mead acid enhance BrM cell growth by activating PPAR γ signaling.

We next investigated whether the fatty acids can be released from astrocytes to activate PPAR γ signaling in the surrounding BrM cells. We first quantified AA, mead acid and DHA

in the conditioned media (CM) of cultured astrocytes and BrM cells. Serum-free culture media were used to collect CM to avoid any exogenous contamination of fatty acids. In astrocyte CM, AA was secreted at the highest concentration, followed by DHA and mead acid (Fig. 6A), which was consistent with our findings of high AA content in astrocyte lysates (Fig. 5A). About 2-fold more DHA than mead acid was detected in astrocyte CM (Fig. 6A), even though astrocyte lysates had higher mead acid content (Fig. 5A, B). These data suggested that the secretion of DHA maybe more efficient than mead acid. Notably, compared to BrM cells, astrocytes secreted higher amounts of these detected fatty acids (Fig. 6A). Secondly, we tested whether astrocyte CM could activate PPAR γ signaling in BrM cells. Astrocyte CM increased PPAR γ -dependent PPRE binding in the BrM cells (Fig. 6B). Similarly, exogenous AA increased PPAR γ -PPRE binding when directly added to the BrM cells (Fig. 6C). Lastly, astrocyte CM promoted their growth and this pro-growth effect was diminished by the specific PPAR γ antagonist T0070907 (Fig. 6D). PPAR γ activation has been shown to be regulated by CDK5-dependent phosphorylation of PPAR γ at serine273 in adipocytes (30). However, in our BrM cells, neither astrocyte CM nor exogenous AA treatment altered this specific phosphorylation (Supplemental Fig. 6C). Overall, our data indicate that polyunsaturated fatty acids, including AA and mead acid, are released from astrocytes and activate PPAR γ signaling in BrM cells to enhance their growth.

PPAR γ is a therapeutic target for brain metastasis

To validate the activation of PPAR γ pathway in clinical samples of brain metastasis, we performed immunohistochemical staining of PPAR γ to detect the expression as well as its nuclear location in the cancer cells. For melanoma, we compared normal skin, benign nevus, primary tumors, extracranial metastases (including lymph node and gastrointestinal tract) and brain metastases. Our results showed a significantly higher proportion of PPAR γ -positive samples in brain metastasis lesions (Fig. 7A). Notably, in all PPAR γ -positive brain metastasis lesions, we detected distinct nuclear distribution of PPAR γ in melanoma cells (Fig. 7A). For breast cancer, we obtained 13 paired samples of primary and brain metastatic tumors from the same patients. These paired samples were processed in same pathology department and stained with PPAR γ at the same time. Thus, we scored of the expression of PPAR γ by the staining intensity (Supplemental Fig. 7A) and confirmed significantly increased PPAR γ staining in the brain metastases (Fig. 7B). However, the nuclear localization of PPAR γ in the breast cancer brain metastatic samples was less distinct relative to the melanoma brain metastasis samples (Fig. 7B). Our data confirm the increased expression and nuclear localization of PPAR γ in brain metastases compared to primary tumors, lymph node and GI tract metastases in clinical samples.

Lastly, we systemically administered the PPAR γ antagonist T0070907 in our melanoma and breast cancer brain metastasis animal models to assess its therapeutic potential on brain metastatic outgrowth. Daily administration of T0070907 significantly decreased brain metastatic burden in both melanoma and breast cancer metastases, using both female and male experimental mice (Fig. 7C and Supplemental Fig. 7B). Our *in vitro* data suggested that T0070907 inhibits BrM cell proliferation, the step after extravasation to establish brain metastatic lesions. Thus, we initiated treatment of the experimental mice 7 days after initial cancer cell inoculation, which is required for the extravasation of cancer cells cross BBB

(12). We observed a similar inhibitory effect of T0070907 on brain metastases (Fig. 7D). In contrast, systemic application of T0070907 did not change the growth of subcutaneously implanted tumors (Fig. 7E) or lung metastases (Fig. 7F). Thus, the therapeutic effect of PPAR γ antagonist was specific to the polyunsaturated fat-rich brain microenvironment. PPAR γ signaling is one of the key pathways that regulate metabolism, particularly glucose homeostasis and fat metabolism. Thus, we assessed for any changes in body weight of the experimental mice as a potential side-effect. Our data showed that daily administration of T0070907 for 28 days did not significantly decrease the body weight of either male or female experimental mice (Fig. 7G and Supplemental Fig. 7C), indicating that this drug is well tolerated in mice. Our combined data strongly support the premise that inhibiting PPAR γ pathway may be a viable therapeutic strategy to control brain metastases.

DISCUSSION

Astrocytes have diverse functions in brain metastasis (12,24,31–36). On one hand, activated astrocytes release the killing factor, soluble FasL, in the microenvironment to induce cancer cell death (12). On the other, most studies suggest pro-metastatic function of astrocytes. Astrocytes have been known to facilitate brain metastasis by increasing the survival, trans-BBB migration and stemness of the invaded cancer cells (12,24,33–37), as well as by modulating the immune cells in the brain metastatic lesions (31,32). Here, we identified a pro-proliferative function of astrocytes by supplying unsaturated fatty acids to activate PPAR γ signaling in the invaded brain metastatic cancer cells (Fig. 7H). This PPAR γ activation in cancer cells is not through CDK5-dependent phosphorylation of PPAR γ at serine273. As the most abundant glial cells, astrocytes are a major source for fatty acid synthesis in the brain (14–16). Fatty acids produced by astrocytes are taken up by neurons to support synapse formation and function (28,29). Our data suggest that once migrated across the BBB to the brain parenchyma, cancer cells take advantage of this high fatty acid microenvironment to proliferate, which is the ultimate step required for metastatic outgrowth to form macrometastasis. Moreover, inflammation-activated astrocytes have been shown to increase the production and secretion of polyunsaturated fatty acids (38). This raises an intriguing possibility, in which the metastatic cancer cells themselves instigate the manipulation of the surrounding astrocytes to obtain PPAR γ activators in the microenvironment.

The role of PPAR γ in cancer remains controversial, and may depend on cancer types and stages (17,18). Most studies show that activating PPAR γ pathway suppresses cancer development. Perhaps, this is why all relevant cancer clinical trials (from <https://clinicaltrials.gov>) are using PPAR γ agonists to prevent cancer development or treat primary tumor. One of the identified suppressive mechanisms shows that PPAR γ pathway modulates the transition between epithelial and mesenchymal phenotype of cancer cells. PPAR γ activation induces reverse epithelial-mesenchymal transition (EMT), also termed MET, by directly increasing the expression of E-cadherin (39,40) and indirectly inhibiting the canonical Wnt/ β -catenin pathway (41–43). Consequently, cancer cells exhibit decreased invasion and migration, blocking the spreading of metastatic cells from the primary tumor to secondary metastatic organs. Little is known about the effect of PPAR γ -activated MET on the proliferation of cancer cells after they achieved migration into distal metastatic organs.

Moreover, increasing evidence indicates that PPAR γ can act as a cancer promoter, particularly in a lipid rich environment (44,45). A recent study on primary brain tumors shows that PPAR γ induces the production of reactive oxygen species in glioblastoma to promote tumor growth (45). Our current work focuses on the most aggressive form of cancer, the brain metastasis, and identify PPAR γ as a promoter for cancer cell proliferation in the brain. The results not only expand our knowledge on the mechanistic underpinnings of cancer brain metastasis, but also highlight that therapeutic strategies using targeted agonists or inhibitors will have to be more context dependent and personalized.

METHODS

Cell culture

Human WM4265.2, WM793, WM1366, MDA-MB-231 (MDA231), murine Yumm1.7, and their brain metastatic derivatives (BrM) were cultured in DMEM with 10% fetal bovine serum (FBS) and 2 mM L-Glutamine. For lentivirus production, 293T cells were cultured in DME media supplemented with 10% fetal bovine serum and 2 mM L-glutamine. Human and mouse primary astrocytes were cultured in media specified by the supplier (ScienCell), and used between passages 2–6. Patient-derived WM4265.2, WM793 and WM1366 cells were obtained from Dr. Meenhard Herlyn's lab. Yumm1.7 cells were obtained from Dr. Ashani Weeraratna's lab. MDA231-BrM cells were generated in Joan Massagué's lab. All these cells have been well characterized by fingerprint or exon sequencing (25,46,47). Cell authentication on newly generated BrM cells was not performed. All cells tested negative for *mycoplasma*. *Mycoplasma* testing with MycoAlert™ Mycoplasma Detection Kit (Lonza) was performed at Cell Center Services, University of Pennsylvania. Each cell line was tested after isolation from the experimental mice and routinely retested every 3–6 months.

Animal studies

All experiments using animals were done in accordance to protocols approved by the Wistar Institutional Animal Care and Use Committee. Athymic NCR nu/nu mice (Charles River Laboratories), C57BL/6J mice (Jackson Laboratory) NSG mice (The Wistar Institute) were used at 5–6 weeks of age. To establish the BrM cells by *in vivo* selection, we followed previously described procedures (21,22). In brief, 5×10^4 cancer cells suspended in 100 μ L of PBS were injected into the left cardiac ventricle. Metastasis growth was monitored by bioluminescent imaging (BLI) after retro-orbital injection of the experimental mice with D-luciferin (150mg/kg). At the experimental endpoint, we anesthetized mice (ketamine 100mg/kg, xylazine 10 mg/kg), retro-orbitally injected D-luciferin, identified the brain colonization by *ex vivo* BLI, cultured the single cell suspension from the brain metastatic lesions, and sorted out fluorescent labeled cancer cells two weeks after *in vitro* culture. For both WM4265.2 and Yumm1.7 cells, we performed two rounds of *in vivo* selection to obtain WM4265.2-BrM1, WM4265.2-BrM2 and Yumm1.7-BrM cells. For brain metastasis assays, we followed previously described procedures (24). In brief, 10^4 MDA231-BrM2 cells, 5×10^4 WM4265.2-BrM cells, or 5×10^4 Yumm1.7-BrM cells suspended in 100 μ L of PBS were injected into the left cardiac ventricle. At the experimental endpoint, brain colonization was quantified by *ex vivo* Bio-luminescent imaging (BLI). For inducible knockdown experiments, mice were given doxycycline hyclate (Sigma-Aldrich) in the drinking water (2

mg/mL) and the diet (Harlan). For lung metastasis assays, 4×10^5 cancer cells in 100 μ L PBS were injected into the lateral tail vein. For subcutaneously implanted tumor growth, 5×10^5 cancer cells in 50 μ L PBS were injected. Female athymic NCR nu/nu mice were used for MDA231-BrM cells. Male NSG mice were used for WM4265.2-BrM1 cells since these cells did not grow subcutaneously in athymic NCR nu/nu mice. For drug treatment experiments, mice were intraperitoneally injected with T0070907 (Selleck Chemicals or synthesized by Wistar Molecular Screening and Protein Expression Facility) (5 mg/kg/day). Vehicle (5% DMSO, 45% Polyethylene glycol 300 in water) was used in control mice. Body weight of every experimental mouse was measured on day 1 and every 7 days. Normalized body weight was calculated as the measured weight divided the initial body weight from day 1. BLI was performed using an IVIS SpectrumCT, In vivo Imaging System form (Perkin Elmer) and analyzed using Living Image software. In the selected experiments to count the number of lesions in the brain, a DLIT (Diffuse Light Imaging Tomography) sequence was setup within the Living Image software in conjunction with the IVIS SpectrumCT. A combination of five 2D luminescent images was obtained at varying filters between 560–640nm on the specimen. Then the specimen was lowered to the CT portion of the IVIS SpectrumCT, where a microCT image was obtained of the specimen to showcase the regions and surface of the brain. The count values were used to determine correctly adjusted camera settings and exposure times, with a minimum of 600 counts to reach a quantifiable luminescent signal. For brain metastasis assays, 8–10 mice were used in each group. For drug treatment experiments, mice were inoculated with cancer cells and randomly assigned to treatment groups. Following the established approach to quantify the number of BrM cells migrate across BBB to enter brain parenchyma (12), short-term brain metastasis assay was performed by injecting 5×10^5 cells into the left cardiac ventricle. 7 days after cancer cell inoculation, whole mount staining of GFP was applied to 1/10 of the whole brain tissues and number of GFP positive cancer cells were quantified under fluorescent microscope.

Knockdown and cancer cell labeling constructs

For inducible knockdown, control and *PPARG* shRNAs in TRIPZ lentiviral vector (Dharmacon) were used. 1 μ g/mL doxycycline hyclate (Sigma-Aldrich) was added to induce the expression of shRNA. Targeted sequences of *PPARG* shRNAs and sequence of non-silencing control shRNA are listed in Supplementary Fig. 8. For stably labeling the cancer cells, we used pLenti-UBC vector to express far red luciferase-GFP or far-red luciferase-RFP fusion protein. After stably labeling the cancer cells, GFP positive cells were sorted using Astrios EQ (MoFlo).

mRNA and protein detection

Total RNA was extracted using the Direct-Zol^{RM} RNA MiniPrep Plus (Zymo Research). To prepare cDNA, 1 μ g of total RNA was treated using the RevertAid RT kit (Thermo Fisher). Sequences of primers are listed in the Supplementary Fig. 8. Relative gene expression was normalized relative to *ACTB* (human β -actin). Reactions were performed using Powerup SYBR Green Master Mix (Applied Biosystems). Quantitative expression data were analyzed using QuantStudio 6 Flux and QuantStudio Real-Time PCR Software v.1.2 (Applied Biosystems). For total protein lysates, cell pellets were lysed with RIPA buffer and protein concentrations determined by BCA Protein Assay Kit (Pierce). Cytosol and nucleus

fractions were isolated from cell pellets using NE-PER™ Nuclear and Cytoplasmic Extraction kit (Pierce). For western immunoblotting, Proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes (BioRad). Antibodies used for immunostaining are listed in Supplementary Fig. 8.

Cancer cell-astrocyte coculture experiments

Astrocytes and cancer cells were mixed at ratio of 1:1 or 2:1. For experiments to detect the growth effect of astrocytes on cancer cells, cancer cells ($2.5\text{--}5\times 10^3$ cells/well) were seeded with or without astrocytes in tissue culture–treated 96-well plates. In 3D culture condition, 96-well plates were coated with 50 μL 1.5% Difco Agar Noble (Becton Dickinson). For astrocyte conditioned media (CM) experiments, astrocytes were culture until 90% confluent and continued cultured in 1% FBS containing media. CM was collected after 48 hours and went through 0.22 μm filter before added to cancer cells. For fatty acid experiments, arachidonic acid (AA), mead acid or docosahexaenoic acid (DHA) (Cayman Chemical Company) was added in the cancer cells. Same amount of ethanol was used as vehicle control. In all these co-culture, CM and fatty acid treatment experiments, the growth of cancer cells was quantified after 48 hours by BLI imaging (by IVIS SpectrumCT) or Alamar Blue staining (Thermo Fisher; by Synergy HT from BioTek). Data were analyzed by Living Image software or Gen5 3.05. Final results were normalized by cancer cell culture alone samples. To validate this approach to measure the growth of cancer cells, the number of GFP or RFP labeled cancer cells was quantified under fluorescent microscope or by flow cytometry in the indicated experiments. For cell proliferation assays, cancer cells were prelabeled with Cell Trace™ Violet dye before culture. The initial label and the final label intensity in GFP⁺ cancer cells were measured by flow cytometry (BD FACS Aria II from BD Biosciences). Proliferation index was calculated by dividing the measured intensity after 48 hours by the initial intensity. For cell death measurement, cancer cells were treated with TRAIL (Peprotech), cisplatin (Acros Organics), staurosporine (Selleckchem) for 24 hours. Cell death were detected by caspase 3 cleavage (using western blotting) or Annexin V/DAPI staining (Thermo Fisher) (the apoptotic and necrotic cells were detected by flow cytometry). Specific antagonists and agonists, T0070907 (final concentration: 10 μM), GSK3787 (5 μM), Rosiglitazone (10 μM) and GW501516 (1 μM), were all purchased from Tocris Bioscience added to the culture cells. Same amount of DMSO was used as vehicle control. The doses of the agonists and antagonists were selected based on the related references recommended by Tocris Bioscience. We tested different doses of fatty acids, agonists and antagonists, based on previously published work or recommended dose from Tocris Bioscience, in our initial experiments in the lab to optimize the doses for our BrM cells. For RNAseq experiments, BrM cells were co-cultured with astrocytes for 24 h and the GFP⁺ cancer cells were sorted using Astrios EQ (MoFlo).

RNAseq and bioinformatics analysis

mRNA purified from cancer cells ($n = 4$ biologically independent experiment) were used. Sequencing libraries were prepared from RNA samples using QuantSeq (Lexogen). Samples were aligned using hg38 and 2-pass STAR alignment. Gene and transcript level counts were calculated using RSEM using Ensembl v75 annotation. All reads within any transcript's coding part of a gene were counted to get expression for each gene. Raw counts were tested

for differential expression using DESeq2 method after filtering out lowly expressed genes (genes with at least 10 raw counts in at least one sample were considered). DESeq2 normalized count values were used for expression differences. Differentially expressed genes (FDR = 0.05, as the cut-off) under each indicated comparison were used for Ingenuity Pathway Analysis (QIAGEN Bioinformatics). Gene set enrichment analysis (GSVA) was performed on normalized raw counts using gene sets (PPAR and EIF2) curated from the IPA results. All these were performed by Wistar Genomics and Bioinformatics Facilities.

PPAR binding assay

Nucleus fractions, isolated from cell pellets using NE-PER™ Nuclear and Cytoplasmic Extraction kit (Pierce), were used in PPAR α , δ , γ Complete Transcription Factor Assay (Cayman Chemical). In the astrocyte conditioned media (CM) experiments. CM are collected 48 hours after treating human astrocytes with 1% serum culture media. BrM cells were harvested after treated with CM for 8 hours.

Immunohistochemical staining

Mouse brains were fixed with 4% paraformaldehyde, 70 μ m sections were cut by cryostat (Thermal Fisher) and whole mount staining was applied following established protocols (12). For immunostaining of 3D cultured cell spheroids, cells were fixed with 4% paraformaldehyde and stained. For clinical samples, paraffin sections were stained by Wistar Histotechnology Facility. Antibodies used for immunochemical staining are listed in Supplementary Fig. 8. Images were acquired with Nikon 80i microscope (Nikon Instrument) or TCS SP5II upright confocal microscope (Leica), and analyzed with LAS AF and NIS-Elements software.

Fatty Acid Analysis

Human astrocytes and BrM cancer cells were cultured in 1% serum culture media for 48 hours. The cells were washed in PBS and then scraped into ice-cold methanol and transferred to glass vial for lipid extraction. The same volume of SPLASH! LIPIDOMIX (Avanti Polar Lipids) dissolved in methanol was introduced to each sample as an internal standard. To measure secreted fatty acid, cells were cultured in serum free media for 48 hours. Chloroform and deoxygenated ice-cold PBS were added and centrifuged. The lower phase was collected and an equivalent volume from each sample was dried under a nitrogen stream. Samples were then redissolved in 0.3M KOH in 90% methanol and incubated at 80°C for one hour. Formic acid and hexanes were added. The upper phase was dried under a nitrogen stream before resuspension in methanol. LC-MS analysis was performed using HILIC chromatography and Thermo Q-Exactive HF-X mass spectrometer (Thermo Fisher Scientific). Raw data analysis was performed using TraceFinder software (Thermo Fisher Scientific). Peak areas were normalized to internal standard. We used deuterated oleic acid as the internal standard. Fatty acid content in cell lysates were normalized by the total protein amounts. Synthetic AA, mead acid and DHA (Cayman Chemical Company) was used to generate standard curve to quantify and calculate the amount of the fatty acid secreted in the conditioned media.

Clinical sample analysis

Normal skin, benign nevus, primary tumors, lymph node and GI tract metastases melanoma samples were purchased as tissue arrays from US Biomax. Paraffin embedded tissues of melanoma brain metastases (14 cases) and paired primary/brain metastasis (13 pairs) were obtained from UPENN Departments of Pathology and Laboratory Medicine and Christiana Care Health System, in compliance with the UPENN or Wistar Institutional Review Board. The the studies were conducted in accordance with recognized ethical guidelines. Written informed consent was obtained from all subjects. Immunohistochemical (IHC) staining for PPAR γ was performed by Wistar Histotechnology Facility. For the paired breast cancer samples, 3–8 images, depended on the tissue size, were obtain from each sample for PPAR γ staining score.

Statistical analysis

Statistical analysis was performed using GraphPad software (Prism) and Student's t-test (two-tailed). P values <0.05 were considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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STATEMENT OF SIGNIFICANCE

Brain-tropic cancer cells take advantage of the lipid-rich brain microenvironment to facilitate their proliferation by activating PPAR γ signaling. This pro-tumor effect of PPAR γ in advanced brain metastases is in contrast to its anti-tumor function in carcinogenesis and early metastatic steps, indicating that PPAR γ has diverse functions at different stages of cancer development.

Figure 1. Astrocytes facilitate the growth of brain metastatic cancer cells.

A. Schematic illustration of *in vivo* selection of brain tropic melanoma cells from patient-derived xenograft (PDX) model and transgenic mouse model. **B.** Confocal microscopy showing interactions between WM4265.2-BrM1 cells (with GFP staining in green) and activated astrocytes (with GFAP staining in red) in the brain metastatic lesions from the experimental mouse. DAPI: nuclear staining in blue. Scale bar, 200 μ m. **C.** Representative image of activated astrocytes (with GFAP staining in brown) in surgically removed brain metastatic lesions from melanoma patients. Scale bar, 100 μ m. **D-G.** Astrocytes promote the growth of BrM cancer cells under 2-dimensional (2D) co-culture condition. **D.** Schematic illustration of 2D experimental setup. **E.** Representative fluorescent images showing increased GFP⁺ WM4265.2-BrM1 cells after astrocyte co-culture. Scale bar, 10mm. **F.** Representative bioluminescent images (BLI) showing increased luciferase signals from

WM4265.2-BrM1 cells after astrocyte co-culture. **G.** Quantification of BLI of luciferase signals from BrM cells. $N = 3$ biologically independent experiments. **H-K.** Astrocytes promote the growth of BrM cancer cells under 3-dimensional (3D) co-culture condition. **H.** Schematic illustration of 3D experimental setup. **I.** Representative confocal image of WM4265.2-BrM1 cells (staining with GFP in green) and astrocytes (stained with GFAP in red) in 3D spheroid. DAPI: nuclear staining in blue. Scale bar, 100 μ m. **J.** Representative BLI showing increased luciferase signals from WM4265.2-BrM1 cells after astrocyte co-culture. **K.** Quantification of BLI of luciferase signals from BrM cells. $n = 3$ biologically independent experiments.

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Figure 2. Shared activated pathways in high brain metastatic WM4265.2-BrM1 cells and by astrocyte coculture.

A,B. Two BrM cells isolated from WM4265.2 cells, WM4265.2-BrM1 and WM4265.2-BrM2, have different abilities to form brain metastases in the experimental mice 28 days after the inoculation of BrM cells. $n = 2$ biologically independent experiments. **A.** Quantification of brain lesions by bioluminescent imaging (BLI). **B.** Numbers of brain lesions detected by IVIS-microCT (signals over 600 counts). **C.** WM4265.2 and two BrM cells have similar ability to migrate across blood brain barrier to enter brain parenchyma in

the experimental mice 7 days after the inoculation of BrM cells. Data are from 2 independent experiments, **D-F**. WM4265.2 and two BrM cells response to cisplatin-induced cell death at similar level. **D**. Experimental design. **E**. IC50 of cisplatin. **F**. Western blot result of cisplatin-induced apoptosis detected by caspase 3 cleavage. $n = 2$ biologically independent experiments. **G**. WM4265.2-BrM2 cells grow slower than WM4265 and WM4265.2-BrM1 cells. Alamar Blue proliferation assays. Data are mean value from 10 technical replicates. $n = 3$ biologically independent experiments. **H**. Effects of astrocytes on the growth of WM4265.2 and two BrM cells. Data are the quantifications of BLI of luciferase signals from BrM cells. $n = 3$ biologically independent experiments. **I**. RNA sequencing experiment to compare high brain metastatic WM4265.2-BrM1 and low brain metastatic WM4265.2-BrM1 cells. Z-scores from Ingenuity Pathway analysis (IPA). **J**. RNA sequencing experiment to compare astrocyte-induced changes between WM4265.2-BrM1 and WM4265.2-BrM2 cells. Z-scores from IPA.

Figure 3. PPAR γ pathway is activated in high brain metastatic WM4265.2-BrM1 cells and further enhanced by astrocyte co-culture.

A. PPAR transcription factor binding assays for PPAR α , PPAR β/δ and PPAR γ , comparing WM 4265.2 and two BrM cells. All values are mean $\pm$ S.E.M. from 4 technical replicates. $n = 2$ biologically independent experiments. **B.** Western blot detecting the expression of nd PPAR β/δ in total, cytosol and nucleus fractions of WM 4265.2 and two BrM cells. β -actin and nuclear envelope protein Lamin A/C are used as loading control. $n = 3$ biologically independent experiments. **C,D.** Effect of PPAR γ and PPAR β/δ antagonists on WM4265.2-

BrM1 cell growth in culture alone (**C**) and astrocyte-coculture (**D**) experiments. Data are the quantifications of bioluminescent images (BLI) of luciferase signals from BrM cells. $n = 3$ biologically independent experiments. **E.** Effect of PPAR γ agonist, Rosiglitazone, on the growth of WM4265.2-BrM1 and WM4265.2-BrM2 cells. Data are the quantifications of BLI of luciferase signals from BrM cells. $n = 3$ biologically independent experiments. **F.** Depleting PPAR γ expression in WM4265-2-BrM1 by doxycycline-inducible shRNAs. PPAR γ protein was detected by western blot. $n = 2$ biologically independent experiments. **G.** Depleting PPAR γ expression significantly decreases the growth in WM4265.2-BrM1, but not WM4265.2-BrM2 cells. Alamar Blue proliferation assays. Data are mean value from 10 technical replicates. $n = 3$ biologically independent experiments. **H,I.** Depleting PPAR γ expression in WM4265-2-BrM1 significantly decreases astrocyte-induced growth (**H**) and brain metastatic outgrowth in the experimental mice (**I**). **H.** Quantification of BLI luciferase signals from BrM cells. $n = 3$ biologically independent experiments. **I.** Quantification of brain lesions by BLI. $n = 2$ biologically independent experiments.

Figure 4. PPAR γ pathway promotes BrM cell proliferation.

A. Effect of PPAR γ antagonist on BrM cell proliferation. BrM cells are labelled with CellTrace dye to track and quantified their proliferation. Histograms are the raw data of CellTrace dye in GFP⁺ BrM cells, detected by flow cytometry. Bar graphs are mean $\pm$ S.E.M. from 3 technical replicates. $n = 3$ biologically independent experiments. **B,C.** PPAR γ signaling does not affect BrM cell death. **B.** BrM cells are treated with PPAR γ antagonist and the apoptotic and necrotic cells are stained by Annexin V and DAPI and quantified by flow cytometry. Data are mean $\pm$ S.E.M. from 3 technical replicates. $n = 2$

biologically independent experiments. **C.** BrM cell are treated with cell death inducer, in combination with PPAR γ agonist or antagonist. Apoptosis are detected by caspase 3 cleavage. $n = 2$ biologically independent experiments. **D.** Effect of PPAR γ antagonist on astrocyte-induced BrM cell proliferation. BrM cells are labelled with CellTrace dye to track and quantified their proliferation. Histograms are the raw data of CellTrace dye in GFP $^{+}$ BrM cells, detected by flow cytometry. Bar graphs are mean $\pm$ S.E.M. from 3 technical replicates. $n = 3$ biologically independent experiments. **E.** PPAR γ antagonist does not change the protected effect of astrocyte on MDA231-BrM cell apoptosis induced by TRAIL (250 ng/ml). The apoptotic and necrotic GFP $^{+}$ BrM cells are stained by Annexin V and DAPI and quantified by flow cytometry. Data are mean $\pm$ S.E.M. from 3 technical replicates. $n = 2$ biologically independent experiments.

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Figure 5. The high content of polyunsaturated fatty acids from astrocytes activate PPAR γ pathway in BrM cancer cells.

A,B. Quantification of fatty acids in human astrocytes and BrM cells. $n = 2$ biologically independent experiments. **A.** Heat map of fatty acid contents in astrocytes and BrM cancer cells. Data are mean from 3 technical replicates. **B.** Normalized arachidonic acid (AA), mead acid and docosahexaenoic acid (DHA) content in astrocytes, WM4265.2-BrM1 and MDA231-BrM cells. **C.** Exogenous AA (5 μ M) and mead acid (2.5 μ M), but not DHA (10 μ M), promote the growth of BrM cells. Data are the quantifications of bioluminescent

imaging (BLI) of luciferase signals from BrM cells. $n = 3$ biologically independent experiments. **D.** PPAR γ antagonist inhibits AA- and mead acid-enhanced BrM cell growth. Data are the quantifications of BLI of luciferase signals from BrM cells. $n = 2$ biologically independent experiments.

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Figure 6. Astrocytes secrete polyunsaturated fatty acids to activate PPAR γ pathway in BrM cancer cells.

A. Secreted AA, mead acid and DHA are measured in the conditioned media of human astrocytes and BrM cells. $n = 2$ biologically independent experiments. **B.** Astrocyte conditioned media (CM) activate PPAR γ in BrM cancer cells. Data are the results of PPAR γ transcription factor binding assays. $n = 2$ biologically independent experiments. **C.** Exogenous arachidonic acid (5 μ M) activates PPAR γ in BrM cancer cells. Data are the results of PPAR γ transcription factor binding assays. $n = 2$ biologically independent experiments. **D.** Astrocyte CM enhances the growth of BrM cells, and this effect is blocked by PPAR γ antagonist. Data are the quantifications of BLI of luciferase signals from BrM cells. $n = 2$ biologically independent experiments.

Figure 7. PPAR γ as a therapeutic target for brain metastasis.

A,B. Immunohistochemical staining of PPAR γ in clinical samples of melanoma (A) and breast cancer (B) patients. **A.** Images are from the PPAR γ staining in the representative melanoma samples. Scale bar, 400 μ m (left) and 50 μ m (right). Pie charts are the percentages and numbers of positively (+) and negatively (-) stained cases. **B.** Images are from the PPAR γ staining in one paired primary and brain metastatic breast cancer samples from the same patient. Scale bar, 100 μ m. Data are mean value of PPAR γ staining scores in paired primary and brain metastatic samples, n = staining scores from 3–8 images per sample. **C,D.**

PPAR γ antagonist is systemically applied in the female athymic mice on day 0 (**C**) or day 7(**D**) after BrM cell inoculation. Brain metastases are quantified by bioluminescent imaging (BLI). $n = 2$ biologically independent experiments. **E.** PPAR γ antagonist is systemically applied in the experimental mice on day 0 after subcutaneous injection of BrM cell. Tumor growth is quantified by BLI. $n = 2$ biologically independent experiments. **F.** PPAR γ antagonist is systemically applied in the female experimental mice on day 0 after injecting MDA231-BrM cell via tail vein. Lung metastases are quantified by BLI. $n = 2$ biologically independent experiments. **G.** Weekly body weight changes of the female experimental mice. $n = 4$ biologically independent experiments. **H.** Schematic summary of the pro-proliferation effect of astrocyte on invaded brain metastasis cancer cell, the activation of PPAR γ signaling in cancer cells by astrocyte-released fatty acid (i.e., AA and mead acid), and PPAR γ as a therapeutic target to control brain metastatic outgrowth.