Divergent Effects of Ultra-High Dose Arachidonic Acid versus Docosahexaenoic Acid on *MYCN*-Driven Neuroblastoma Progression in a Syngeneic Mouse Model

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Abstract

Neuroblastoma (NB) represents the most common extracranial solid tumor in children, with high-risk cases showing particularly poor prognosis. We used a syngeneic mouse model to investigate the effects of ultra-high dose highly unsaturated fatty acids (HUFA) on NB progression. Following tumor establishment, mice were randomized to receive daily oral gavage with omega-6 (ω6) arachidonic acid (ARA) at 4.7 g/d human equivalent (hEq), or omega-3 (ω3) docosahexaenoic acid (DHA) at 24 g/d hEq, and controls did not receive gavage. We observed strikingly divergent effects: ARA significantly promoted tumor growth, resulting in 100% tumor survival and 4-fold larger tumors compared to controls, with enhanced vascularization and invasive morphology. In contrast, DHA administration reduced tumor survival (40% versus 92% in controls) and significantly suppressed the progression of remaining tumors, with remaining DHA-treated tumors approximately 4.5-fold smaller than controls and 18-fold smaller than ARA-treated tumors. DHA-treated tumors appeared well-circumscribed with standard surrounding tissue architecture. No significant differences in body weight were observed among treatment groups, indicating the treatments were well-tolerated. These findings build upon our previous research demonstrating that ultra-high dose ω3 supplementation can completely block tumor formation in *MYCN*-driven NB. The profound tumor-suppressive effects of DHA suggest that dietary modulation of ω3 and ω6 HUFA intake may offer a complementary, low-toxicity approach to high-risk NB standard of care (SoC). Our findings suggest that dietary intervention may be an effective adjunctive strategy in pediatric oncology, allowing for reduced SoC dosing while improving outcomes and reducing survivorship concerns.

Introduction

Neuroblastoma is an aggressive pediatric cancer originating from neural crest-derived progenitor cells, representing the most common extracranial solid tumor in children with a disproportionate mortality rate(1). Clinically, it exhibits remarkable heterogeneity, from spontaneously regressing tumors to rapidly progressing, treatment-resistant forms. Inflammation has emerged as a critical component in tumor biology, playing key roles in initiation, progression, and immune evasion (2).

The tumor microenvironment, characterized by its inflammatory mediators, cytokines, and bioactive lipids, significantly influences tumor behavior. Polyunsaturated fatty acids (PUFAs), particularly those belonging to the omega-6 and omega-3 families, play opposing roles in cancer biology.(3) Arachidonic acid (ARA), a primary omega-6 PUFA in animal-derived phospholipids, generates pro-inflammatory eicosanoids that promote angiogenesis, suppress anti-tumor immunity, and enhance metastasis.(4) Higher levels of ARA metabolites correlate with aggressive tumor phenotypes and poorer outcomes across

multiple cancers, including neuroblastoma. In contrast, omega-3 fatty acids, primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), exhibit anti-inflammatory properties and offer numerous health benefits. These essential fatty acids compete with ARA in cell membrane incorporation and enzymatic pathways, reducing pro-inflammatory eicosanoids and possibly increasing anti-inflammatory mediators like resolvins and protectins (5). Clinical applications have shown that ultra-high dose omega-3 therapy (up to 20 g/day) is well-tolerated and effective in conditions including traumatic brain injury and hypertriglyceridemia (6).

Preclinical evidence suggests that omega-3 fatty acids inhibit tumor cell proliferation, induce apoptosis, and modulate immune responses (7–9). Notably, our recent publication demonstrated that ultra-high doses of oral omega-3 fatty acids (EPA, DHA, or oxidation-resistant deuterated DHA) at human-equivalent doses of 12-14 g/day completely blocked tumorigenesis in a MYCN-driven neuroblastoma model(10). Conversely, omega-6 ARA supplementation enhanced tumor formation and reduced tumor latency, highlighting the opposing impacts of these lipid families (11)

We report here an investigation of the therapeutic potential of DHA in a syngeneic mouse model of MYCN-driven neuroblastoma, examining its effects on tumor growth kinetics to provide insights for potential dietary interventions in pediatric cancer management.

Methods

Cell Culture and MYCN Transfection

Murine neuroblastoma Neuro-2a cells transduced with a human *MYCN* transgene were initially obtained from the American Type Culture Collection (ATCC; CCL-131; details available at ATCC) and cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (HI-FBS). Cells were grown at 37 °C in a water-saturated atmosphere of 95% air and 5% CO_2. Cells were tested monthly and consistently found to be negative for mycoplasma contamination. Cells were stably transfected with human MYCN using Lipofectamine 3000. Transfected cells were selected from GFP-expressing cells using a fluorescence-activated cell sorter.

Animal Model and Treatment

All procedures were IACUC-approved. Six-week-old male wild-type strain A/J mice (n = 42) were purchased from The Jackson Laboratory (Jaxmice strain #000646, Sacramento, CA, USA) and allowed to acclimate for one week under standard conditions. Mice were fed customary mouse chow with a composition approximating AIN-93G. A model was established by subcutaneous injection of 3×10^6 MYCN-expressing Neuro-2a cells into the right flank of each mouse. When tumors became palpable (approximately day 6), mice were randomized into three treatment groups: Control group (n=12), ARA group (n=15) receiving daily oral gavage with 75 µL ARA oil (44% ARA, 4.6 g/d human equivalent (hEq) dose), and DHA group (n=15) receiving daily oral gavage with 250 µL DHA oil (82% DHA, 24 g/d hEq). All dose and chow fatty acids were as presented previously (10). Oral gavage was performed daily throughout the study. Tumor dimensions were measured every two days using digital calipers, with volume calculated as: Tumor volume = (length × width^2) × 0.5. Body weight and health status were continuously monitored. The study concluded when the control tumors reached approximately 1,000 mm^3. Mice were euthanized by CO_2 inhalation followed by cervical dislocation. Tumors were excised, photographed, weighed, and processed for analysis (flash-frozen, fixed in formalin, or processed immediately).

Statistical Analysis

Data are presented as mean ± SEM. Analyses were performed using GraphPad Prism (version 3.1). Comparisons between groups used one-way ANOVA with Tukey's post hoc test. For tumor growth kinetics, two-way repeated measures ANOVA were used. P-values < 0.05 were considered significant.

Results

DHA suppresses tumor growth in a MYCN-driven neuroblastoma model

To evaluate the therapeutic potential of omega-3 fatty acid (DHA) in neuroblastoma, we employed a syngeneic xenograft model using MYCN-transfected Neuro2a cells. Mice (n=42) were injected subcutaneously with Neuro-2a cells. At 6 days, the 30 mice with palpable tumors were randomly assigned to one of three groups so that each group had mice with 10 tumors. The remaining mice were assigned randomly to groups to make the initial treatment groups as follows (***Figure 1***)—Control (n=12), ARA (omega-6, n=15), and DHA (omega-3, n=15). At day 8, more mice had developed palpable tumors to make the final count of tumors per group as Control (11/15), ARA (11/15), DHA (15/15) when daily oral gavage started on day 8 post-injection. One cage containing several mice were euthanized due to wounds acquired from fighting over the course of 29 d of gavage (Control, 1; DHA, 3; ARA, 3), leaving at study termination the following numbers of mice that ever had tumors: Control 10, ARA 12; DHA 12. At the day 37 study termination, tumor counts were control, 10/10, ARA 12/12, DHA 6/12; in the DHA group, 6 mice that had tumors were tumor-free by palpation.

a.

Neuro 2a				
Groups	Mice Injected	Oil gavage (µL)	Tumors at d8	Tumors at d37
Control	12	-	11/12	10/12
ARA	15	75	11/15	12/12
DHA	15	250	15/15	6/12

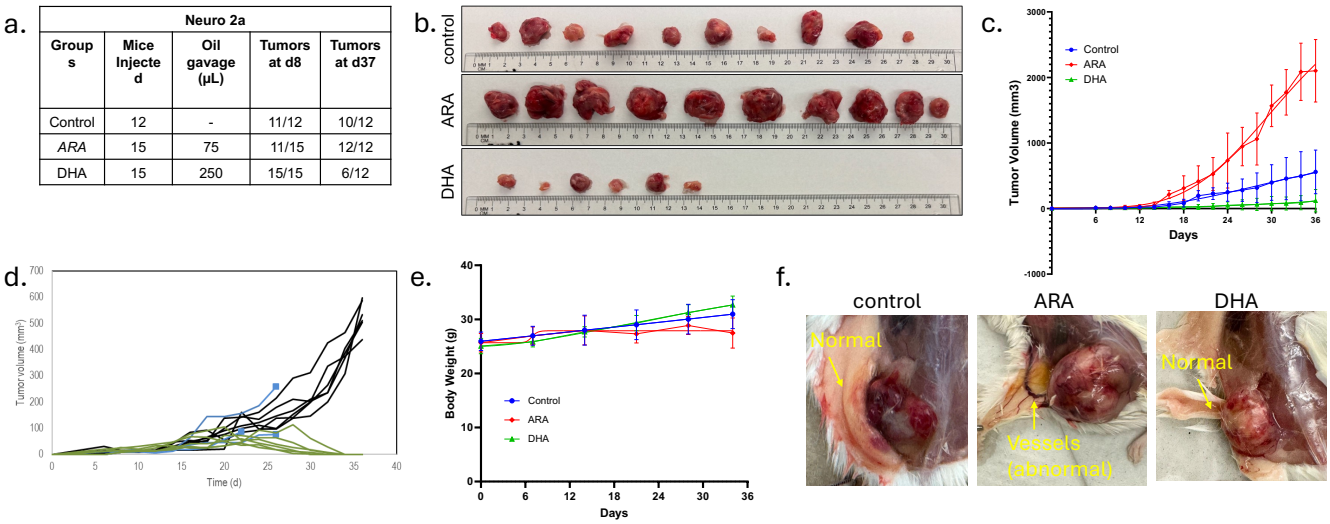

Figure 1. **Ultra-high-dose DHA inhibits tumor growth in a MYCN-driven neuroblastoma model. a)** Schematic representation of the experimental design. Mice were subcutaneously injected with MYCN- transfected cells 3.0*10^6 cells. Treatment was administered via oral gavage daily, starting on day 8 post-injection. b) Representative images of tumors harvested from each group at the end of the study (day 36). Tumors were aligned along a metric scale to illustrate size differences between treatment groups. **c)** Tumor growth curves over time. Tumor volume (mm^3) was measured every 2 days starting from day 8. Data represent mean ± SEM; n = 12–15 mice per group. **d)** Individual DHA treated tumor growth curves over time. **e)** Body weight of mice during treatment period. No significant differences in body weight were observed among the groups, indicating minimal. **f)** Gross morphology of subcutaneous tumors at endpoint highlighting differences in vascularization and surrounding tissue architecture.

At the endpoint (day 37), tumors were collected and aligned for visual comparison (***Figure 1b***). The DHA group displayed drastically smaller and fewer tumors, whereas the ARA group exhibited the most significant tumor burden. Longitudinal monitoring of tumor progression (***Figure 1c***) revealed that DHA treatment nearly abolished tumor growth, while ARA significantly accelerated tumor progression compared to controls. Mice receiving ARA exhibited dramatically accelerated tumor growth compared to the control group, with tumor volumes approximately 4-fold higher by Day 37 (p<0.01). In contrast, DHA

treatment substantially inhibited tumor progression, with mean tumor volumes approximately 4.5 times smaller than those of the control group ($p < 0.01$) and 18 times smaller than those of the ARA group ($p < 0.001$) by the endpoint. Individual tumor growth was broadly suppressed by DHA, including six tumors that regressed completely (***Figure 2d***).

a.

Neuro 2a			
Groups	Tumor Length (mm)	Tumor width (mm)	Tumor weight (g)
ARA	16	20	2.1
	22	22	4.71
	21	21	4.8
	24	17	3.25
	23	16	3.55
	20	17	4.07
	16	20	3.55
	19	21	3.9
	21	15	2.71
	11	11	1.22
	7	10	0.5
	7	10	0.57

Neuro 2a (Experiment 2)			
Groups	Tumor Length (mm)	Tumor width (mm)	Tumor weight (g)
Control	11	10	0.37
	15	13	1.7
	10	9	0.63
	14	17	1.76
	7	7	0.49
	15	13	1.64
	8	4	0.35
	20	11	1.81
	14	12	2.23
	7	4	0.11
DHA	7	9	0.95
	4	4	0.08
	9	10	0.72
	9	7	0.21
	9	11	0.73
	12	4	0.21

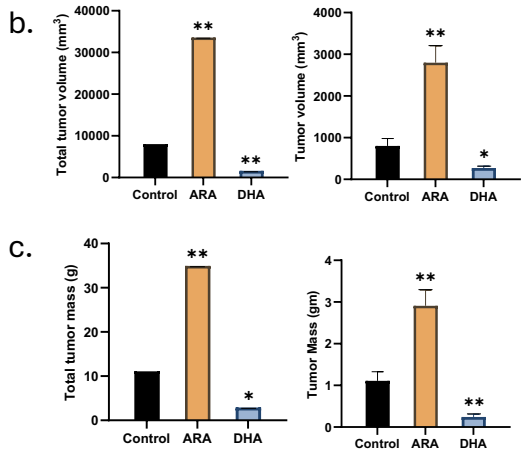

Figure 2 : Syngeneic cell-derived xenograft model of MYCN-driven neuroblastoma. a) ARA and DHA were administered orally every day to the mice after 8 days (tumor palpable days) after cell injection. 3 *10^6 MYCN expressing Neuro-2a cells were injected subcutaneously into the mice. Summary of tumor size and mass with ARA, DHA, and control. **b,c)** A bar graph depicts the change in average tumor volume (b) or mass (c) across different experimental groups, showing the highest tumor volume in the ARA-treated group in both experiment rounds. Significance was calculated using a t-test: *p<0.05 and **p<0.01.

Importantly, no significant differences in body weight were observed among the treatment groups (***Figure 1e***), indicating that the treatments were well tolerated, with some evidence of divergence at the end of the study. The gross site of tumors was inspected (***Figure 1f***), revealing that ARA-treated tumors appeared more vascularized and invasive, with irregular borders and evidence of abnormal vessel formation. In contrast, tumors from DHA-treated mice were generally well-circumscribed, paler in appearance, and showed standard surrounding tissue architecture. Control tumors exhibited intermediate characteristics. DHA reduces tumor volume and mass across experimental replicates.

Detailed analysis of tumor dimensions and mass (***Figure 2a***) further confirmed the differential effects of ARA and DHA treatments. This analysis included comprehensive measurements of individual tumors from each treatment group, revealing consistent patterns. The mean tumor length and width in the ARA group (20.00 mm × 17.83 mm) significantly exceeded those in both the control (16.00 mm × 20.00 mm) and DHA groups (7.00 mm × 10.00 mm). Quantitative analysis of the tumor metrics revealed that ARA treatment resulted in the highest total tumor volume and mass, whereas DHA treatment yielded a significant reduction in both parameters. Bar graphs summarizing the tumor volume (***Figure 2b***) and tumor mass (***Figure 2c***) confirmed that DHA significantly suppressed tumor growth ($p < 0.05$ vs. control, $p < 0.01$ vs. ARA). At the same time, ARA treatment resulted in significantly larger tumors ($p < 0.01$ vs. control). The total tumor mass in the ARA group was approximately three times higher than in the controls and six times higher than in the DHA group.

Together, these data demonstrate that DHA causes regression or markedly reduces tumor progression in *MYCN*-driven neuroblastoma, while ARA promotes tumor growth and abnormal tissue morphology.

Importantly, DHA achieved these anti-tumor effects without causing systemic toxicity, suggesting its potential as a safe therapeutic approach for neuroblastoma treatment.

Discussion

Neuroblastoma, particularly in high-risk *MYCN*-amplified cases, remains a therapeutic challenge due to its aggressive progression and treatment resistance. Our study reveals striking divergent effects of ultra-high omega-6 and omega-3 fatty acids on neuroblastoma progression: ARA significantly promoted tumor growth, while DHA consistently suppressed it. The tumor-promoting effect of ARA aligns with its role as a precursor to pro-inflammatory eicosanoids that enhance angiogenesis, immune evasion, and metastasis (12). The cyclooxygenase-2 (COX-2) pathway, which metabolizes ARA to prostaglandins, is frequently upregulated in various cancers, including neuroblastoma, with pathway inhibition showing anti-tumor effects (13).

Our data provides compelling in vivo evidence of ARA's tumorigenic activity in neuroblastoma. The dramatic tumor growth enhancement observed with daily ARA administration (75 μL/day, 44% ARA) resulted in 4-fold larger tumors compared to controls and 100% tumor incidence. These results suggest that even modest increases in dietary omega-6 intake may significantly accelerate disease progression. Notably, ARA-treated tumors displayed abnormal vascularization and invasive morphology, consistent with enhanced angiogenesis and metastatic potential. This finding is corroborated by our previous study, which demonstrated that ARA supplementation (4.6-6.0 g/day, human dose equivalent) enhanced tumor formation, increased vascularization, and reduced tumor latency from 10 to 5.5 days in a similar model (6). These results have important implications for dietary recommendations in pediatric cancer patients, especially given the high omega-6 content in Western diets. Conversely, DHA (250 μL/day, 100% DHA) exerted a powerful tumor-suppressive effect, significantly reducing both tumor incidence (40% in DHA vs. 92% in controls) and tumor progression. Tumors that did develop in DHA-treated mice were approximately 4.5-fold smaller than controls and 18-fold smaller than ARA-treated tumors. Notably, DHA treatment was associated with well-circumscribed tumors characterized by standard surrounding tissue architecture, suggesting effects extending beyond simple growth inhibition. These findings align with multiple preclinical studies reporting anti-proliferative, pro-apoptotic, and immune-modulating effects of omega-3 fatty acids. Also, several mechanisms may contribute to DHA's anti-tumor effects: (1) competitive inhibition of ARA metabolism, reducing pro-inflammatory eicosanoid production; (2) alteration of membrane fluidity and lipid raft composition; (3) direct modulation of gene expression through nuclear receptors; and (4) potential production of specialized pro-resolving mediators that actively resolve inflammation. (8,9,14,15) The opposing effects of ARA and DHA are particularly relevant in *MYCN*-driven neuroblastoma, where *MYCN* amplification is associated with poor prognosis and accelerated tumor growth. *MYCN* reprograms cellular metabolism, including lipid metabolism, to support rapid proliferation (16). Our findings suggest that dietary fatty acids interact with MYCN-driven metabolic alterations, either exacerbating (ARA) or mitigating (DHA) their effects on tumor progression.

From a translational perspective, our results suggest dietary modulation of PUFA intake—specifically, reducing ARA and increasing DHA—may offer a complementary, low-toxicity approach to conventional neuroblastoma therapies. The substantial tumor suppression observed with DHA treatment suggests that omega-3 supplementation might be valuable in neuroblastoma management. This is particularly promising given that high-dose omega-3 supplementation is well-tolerated in humans for extended periods with only mild side effects (10).

Study limitations include the model's incomplete representation of the human neuroblastoma's complex tumor microenvironment, the use of fixed doses rather than titration, and the lack of detailed analysis of tumor-infiltrating immune cells and lipid mediator profiles. Future research should include comprehensive lipidomic profiling, detailed immunophenotyping, exploration of potential synergies with standard therapies, investigation of ROS-independent mechanisms, and evaluation in patient-derived xenograft models.

Conclusion

This study demonstrates that arachidonic acid promotes, while docosahexaenoic acid suppresses tumor progression in a MYCN-driven neuroblastoma model. Daily oral administration of ARA (75 μL/day) significantly accelerated tumor growth, while DHA (200 μL/day) substantially inhibited tumor development compared to control treatment. These findings align with our recent findings, where we demonstrated that ultra-high-dose omega-3 supplementation (EPA, DHA, or oxidation-resistant deuterated DHA) at human-equivalent doses of 12-14 g/day completely blocked tumor formation in a similar model. These results highlight the critical influence of dietary fatty acid composition on neuroblastoma behavior and underscore the therapeutic potential of omega-3 supplementation in neuroblastoma management. Given that ultra-high-dose omega-3 therapy has been successfully used and well-tolerated in humans for other conditions, our findings provide a compelling foundation for further investigation into dietary interventions as complementary approaches in pediatric oncology. Modulation of PUFA intake may represent a promising, low-toxicity strategy to influence disease outcomes in neuroblastoma patients, potentially allowing for reduced dosages of conventional genotoxic therapies and improved quality of life for survivors.

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