Life Sciences 155 (2016) 1–9

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

# DHA-mediated regulation of lung cancer cell migration is not directly associated with Gelsolin or Vimentin expression

Mehboob Ali [a,*], Kathryn Heyob [a], Lynette K. Rogers [a,b]

[a] Center for Perinatal Research, The Research Institute at Nationwide Children's Hospital, Columbus, OH, USA
[b] Department of Pediatrics, The Ohio State University, Columbus, OH, USA

ARTICLE INFO

*Article history:*
Received 8 March 2016
Received in revised form 25 April 2016
Accepted 4 May 2016
Available online 5 May 2016

*Keywords:*
Lung cancer
Migration
Docosahexaenoic acid
Actin binding proteins
Metastasis and actin dynamics

ABSTRACT

*Aims:* Deaths associated with cancer metastasis have steadily increased making the need for newer, anti-metastatic therapeutics imparative. Gelsolin and vimentin, actin binding proteins expressed in metastatic tumors, participate in actin remodelling and regulate cell migration. Docosahexaenoic acid (DHA) limits cancer cell proliferation and adhesion but the mechanisms involved in reducing metastatic phenotypes are unknown. We aimed to investigate the effects of DHA on gelsolin and vimentin expression, and ultimately cell migration and proliferation, in this context.

*Main methods:* Non-invasive lung epithelial cells (MLE12) and invasive lung cancer cells (A549) were treated with DHA (30 μmol/ml) or/and 8 bromo-cyclic adenosine monophosphate (8 Br-cAMP) (300 μmol/ml) for 6 or 24 h either before (pre-treatment) or after (post-treatment) plating in transwells. Migration was assessed by the number of cells that progressed through the transwell. Gelsolin and vimentin expression were measured by Western blot and confocal microscopy in cells, and by immunohistochemistry in human lung cancer biopsy samples.

*Key findings:* A significant decrease in cell migration was detected for A549 cells treated with DHA verses control but this same decrease was not seen in MLE12 cells. DHA and 8 Br-cAMP altered gelsolin and vimentin expression but no clear pattern of change was observed. Immunofluorescence staining indicated slightly higher vimentin expression in human lung tissue that was malignant compared to control.

*Significance:* Collectively, our data indicate that DHA inhibits cancer cell migration and further suggests that vimentin and gelsolin may play secondary roles in cancer cell migration and proliferation, but are not the primary regulators.



## 1. Introduction

Cancer cases resulting in high mortality is reflected by tumor growth, invasion, and metastasis to distant organs [1–3]. Moreover, the lack of curative chemotherapeutic approaches and short median survival (~14 months) indicate the inefficiency of current adjuvant chemotherapy or other interventions such as radiation, against cancer cells possessing the complete malignant phenotype [4–6]. Currently patients with recurring cancer experience high-frequencies of residual micrometastases and relapse [7] with ~50% of surgically treated patients suffering recurrent disease [2,8–13]. Statistics demonstrate that cancer accounts for 585,720 Americans deaths each year, corresponding to almost 1600 deaths per day. Even after a 20% decline in the death rate from 1991 to 2009 there still exists an intolerable socio-economical toll for this disease [14,15]. Overall these data indicate the need to explore new anti-cancer treatments such as dietary/nutritional supplements which have few or no effects on normal cell growth, as a novel approach for limiting cancer growth and reoccurrence.

Docosahexaenoic acid (DHA), a polyunsaturated fatty acid long approved as dietary supplement, has been shown to offer a broad range of health benefits. DHA has been established as a viable therapeutic for diseases including hypertension, arthritis, atherosclerosis, depression, adult-onset diabetes mellitus, myocardial infarction, thrombosis, and some cancers [16–18]. In relation to our current study, DHA has previously been shown to inhibit cancer cell proliferation and survival [19,20] but its involvement in cancer metastasis and associated mechanisms are not known. Therefore, we chose to explore the effect of DHA on cancer cell migration, a potent reflector of metastasis while also evaluating non-cancerous cells which should show minimal to no detrimental effects. We chose the lung adenocarcinoma cell line, A549, as they express the invasive metastatic phenotype, and the transformed lung epithelial cell line, MLE12, to represent non-invasive cells.

Mechanistically, actin binding protein (ABP)-mediated cytoskeletal remodelling at the cellular membrane edges facilitates cancer progression and metastasis by inducing the formation of invasive organelles like lamellipodia, filopodia and invadopodia [21–25]. cAMP induces actin polymerization through protein kinase A mediated regulation of ABP. Understanding the role of ABPs in migration processes could provide

* Corresponding author at: Center for Perinatal Research, The Research Institute at Nationwide Children's Hospital, 575 Children's Cross Road, Columbus, OH, Department of Pediatrics, The Ohio State University, USA.
*E-mail addresses:* mehboob.ali@nationwidechildrens.org, mehtox@hotmail.com (M. Ali).

http://dx.doi.org/10.1016/j.lfs.2016.05.008
0024-3205/

novel therapeutic targets for anti-metastatic drugs. Our preliminary data indicated that DHA supplementation may modulate the expression and localization of ABPs; therefore, we investigated the effects of DHA supplementation on the ABPs gelsolin and vimentin.

The expression and function of gelsolin and vimentin are well defined in the context of actin dynamics, cancer progression, metastasis, and apoptosis [26–29]. Gelsolin belongs to the actin-severing gelsolin/villin superfamily and plays a key role in actin filament disassembly by severing larger actin filaments into smaller ones [30,31]. As a viral actin regulator, gelsolin is involved in the formation of cancer cell invasive structures in coordination with other proteins like Arp3, cortactin, and Rho GTPases [30,32]. Vimentin interacts with tubulin-based microtubules and actin-based microfilaments to comprise the cytoskeleton and thus maintain cellular integrity [33,34]. Vimentin facilitates the elongation of filamentous structures along with actin in the formation of cell protrusions which are required by cancer cells for migration [35,36].

These studies tested the hypothesis that the anti-metastatic properties of DHA are through modulation of ABPs, specifically gelsolin and vimentin. Our findings indicate that DHA inhibits cAMP-induced cell migration in A549 cancer cells and has little to no effect on MLE12 control cells. Expression of both gelsolin and vimentin were altered with cAMP treatment and DHA supplementation, but no clear patterns were observed. Collectively, these data indicate that DHA suppresses cell migration induced by cAMP in cancerous cells but the ABPs gelsolin and vimentin may not be involved directly in DHA-mediated inhibition.

## 2. Materials and methods

### 2.1. Cell culture and treatment

Non-invasive lung epithelial cells (MLE12) and invasive lung cancer cells (A549) (American Type Culture Collection, Manassas, VA) were cultured at 37 °C in a humidified atmosphere containing 5% $CO_2$. MLE12 cells were maintained in HITES media previously published by our laboratory [37]. A549 cells were grown in DMEM 1× with 10% FBS supplemented with 4.5 g/L-glucose. Cells were treated when ~80% confluent (for 6 h or 24 h) with either PBS (the vehicle control), cell permeant analogs of cAMP (8Br-cAMP, 300 μM) (Sigma Chemical Co., St. Louis, MO), DHA (30 μM), or both cAMP and DHA prior to plating (pre-treatment) or post plating (post-treatment).

### 2.2. Transwell analysis

As a measurement of metastatic phenotype, the cell migration rate was studied using transwell apparatus. Six well plates were set-up using Falcon's Transparent PET Membrane, 8.0 μm pore size, $1 \times 10^5$ pores/cm$^2$ inserts, according to the manufacturer's instructions. Cells were treated with 8Br-cAMP and/or DHA either just prior to plating (pre-treatment) or immediately after (post-treatment) to mimic the pre- and post-metastasis treatment of cancers. The cAMP analogue, 8Br-cAMP, was used to induce a proliferative state in our cells. A549 and MLE12 cells were plated at $1 \times 10^5$ cells per well and were incubated in a humidified tissue culture incubator at 37 °C with 5% $CO_2$ atmosphere. After 24 h of growth, cells that migrated to the lower compartment were counted using a Neubauer cell counting chamber and the percentage of cells that migrated were calculated.

### 2.3. Western blot

Lysis buffer (Nupage®LDS sample buffer) containing PMSF (0.3 nmol/ml), okadoic acid (0.01 nmol/ml), leupeptin (0.1 nmol/ml) and aprotonin (0.005–0.01 TIU/ml) was used to prepare total cell lysates. Proteins were separated by SDS-PAGE, transferred to nitrocellulose membranes, and probed with anti-human rabbit monoclonal antibodies targeting gelsolin (dilution, 1:1000) (Cell Signaling Technology, Inc., Danvers, MA), anti-human mouse monoclonal antibodies targeting Vimentin (dilution, 1:1000) (Sigma Chemical Co., St. Louis, MO), and mouse monoclonal antibodies targeting β-actin (1:10,000) (Abcam, Cambridge, MA). Finally, membranes were probed with horseradish peroxidase-conjugated secondary antibody (dilution, 1:1000) (BD Pharminogen®, Franklin lakes, NJ) for 1 h at room temperature and specific bands were visualized employing Amersham™ECL™ Prime Western Blotting Detection Reagent (GE Healthcare, Buckinghamshire, UK). The band intensity was measured by densitometry.

### 2.4. Immunofluorescence (IF) microscopy

Cells were plated in 6-well plates containing cover slips (Corning Incorporated Corning, New York, NY) at $1 \times 10^5$ cells/well and treated with 8Br-cAMP and/or DHA as previously described. A wound was induced across the cell monolayer, and incubated for an additional 24 h. The cover slips were then removed and the cells were stained with anti-gelsolin (1:500) or anti-vimentin (1:500) followed by the secondary antibody, Alexa 488 labeled IgG (1:1000). Nuclei were stained with DAPI (Invitrogen, Carlsbad, CA). Images were acquired by confocal microscopy (LSM510, Carl Zeiss, Jena, Germany) in a blinded fashion using identical settings for all images (200×). Percent change in protein intensity was quantified using NIH Image J analysis.

Human control ($n = 4$) and malignant ($n = 4$) lung tissue slides were obtained from Lung Cancer Biospecimen Research Network (LCBRN)(University of Virginia, Charlottesville, VA). Tissues were processed for immunofluorescence labelling using standard protocols [38]. Tissue sections were stained with primary anti-human rabbit monoclonal antibodies for gelsolin (1:100) or anti-human mouse monoclonal antibodies for vimentin (1:100) followed by secondary antibody Alexa 488 labeled IgG (1:1000) and nuclei were stained with DAPI (Invitrogen, Carlsbad, CA). Four images from each slide were obtained using Carl Zeiss's Axio Scope A1 Polarized Light Fluorescent Microscope (Carl Zeiss, Jena, Germany) with 200× magnification and identical settings. The intensity of image color was quantified using NIH image J software.

## 3. Results

### 3.1. DHA inhibits cAMP-induced cell migration in A549 cells

MLE 12 and A549 cells were cultured and treated with 8Br-cAMP and/or DHA for 6 or 24 h either prior to or after plating into the transwell apparatus. In MLE12 cells, no changes in cell migration were observed due to treatments with the exception of a minor increase in the cAMP/DHA pre-treated cells at 24 h (Figs. 1, a and b). In A549 cells, a significant increase in cell migration was observed with cAMP and cAMP/DHA treatment at 6 and 24 h in both pre- and post-treatment groups. DHA supplementation alone decreased cell migration and attenuated the increase in the cAMP/DHA treated cells at both time points and with pre- and post-treatment (Figs. 1, c and d). These data indicate that DHA supplementation, both before (pre-) or immediately after (post-) induction is able to suppress the pro-migratory effects of cAMP.

### 3.2. DHA modulates the expression of gelsolin and vimentin

To determine whether DHA supplementation suppressed the expression of gelsolin or vimentin, Western blot analyses were performed on whole cell lysates obtained at 6 or 24 h after treatments. cAMP increased the expression of gelsolin in both MLE12 and A549 cell at 6 h and in MLE12 cells at 24 h; the increase in MLE12 cells was substantially greater than A549 cells at 24 h (Figs. 2, a and c). DHA was able to attenuate the cAMP-induced increases in gelsolin in A549 cells at 6 h. Vimentin expression was unaffected by any treatment in MLE12 cells (Fig. 2, b). In A549 cells, a modest decrease in vimentin expression was observed in the