PUFAs and NSAIDs inhibit lung cancer metastatic process through Rho GTPase signaling

Lauren E. Perry[1], Hailey M. York[1], Rosemary A. Poku[2], Jamie K. Alan[3], and Felix Amissah[1,*]

[1]Ferris State University, College of Pharmacy, Big Rapids, MI 49307, USA; perryl16@ferris.edu; yorkh1@ferris.edu; amissaf@ferris.edu; [2]College of Medicine, Central Michigan University, Mt. Pleasant, MI, 48859, USA; poku1r@cmich.edu; [3]Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824, USA; alanjami@msu.edu * Correspondence: amissaf@ferris.edu; Tel.: (+1-231-591-3790 (F.A.)

ABSTRACT

Objective

Ras-related C3 botulinum toxin substrate (Rac1) and Cell division control protein 42 (Cdc42) GTPases play critical roles in cancer cell metastasis. Omega-3 polyunsaturated fatty acids (PUFAs) and non-steroidal anti-inflammatory drugs (NSAIDs) show anticancer activities through diverse molecular mechanisms. We have already demonstrated that combining NSAIDs with PUFAs was more effective at altering the expression of critical proteins in the RAS/MEK/ERK and PI3K/Akt pathways. We studied the ability of selected NSAIDs and omega-3 PUFAs combination to inhibit lung cancer cell invasion and determine their impact on Rho GTPase signaling in the metastatic process.

Methods

We conducted western blotting to detect Rho GTPases (RhoA, Rac1, and CDC42) in A549, NCI-H1299, and NCI-H1975 cells after exposure to PUFAs and NSAIDs. We further examined the effect of co-treatment on NSCLC cell migration and invasion. Western blot analysis detected the expression of marker proteins in EMT.

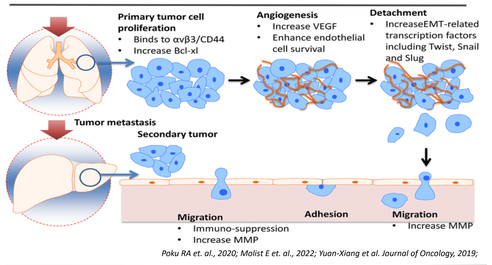
Results

Exposure of A549, H1975, and H1299 cells to DHA, EPA or E-EPA, and diclofenac or ketorolac resulted in suppression of both the distance of migration, the number of cells that migrated into the wounded and cell invasion. Furthermore, co-treatment with DHA and diclofenac disrupted actin filament assembly and inhibited cell migration and invasion. In addition, the co-treatment resulted in altered expression of Rho GTPases and the marker proteins involved in EMT by increasing expression of the epithelial marker, E-cadherin, while downregulating the expressions of mesenchymal markers, N-cadherin, Fibronectin and Vimentin, ZEB1, and β-catenin.

Conclusions

The data from this study demonstrate that co-treatment with PUFAs and low-dose NSAIDs suppressed NSCLC cell motility and invasion, is associated with inhibition of the Rho GTPase activity and provides more significant anticancer potential in preventing or controlling NSCLC metastasis.

BACKGROUND



Poku RA et. al., 2020; Molist E et. al., 2022; Yuan-Xiang et al. Journal of Oncology, 2019;

Rho-family GTPases

RhoA
Rac1
Cdc42

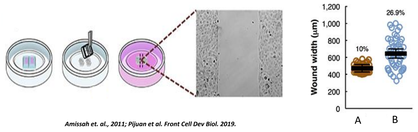
- Uncontrolled cell proliferation
- Invasion & metastasis
- Epithelial-mesenchymal transition
- Angiogenesis
- Reduced apoptosis
- Abnormal metabolism

Tumorigenesis

Poku RA et. al., 2020; Poku R. et. al., 2023; Molist E et. al., 2022; Porter 2017

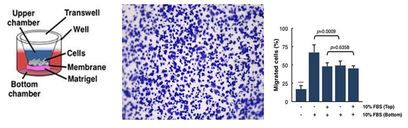
METHODS

- **Migration assay:** Wound healing assay was used to assess the inhibitory potential of PUFAs and NSAIDs on lung cancer cell migration.

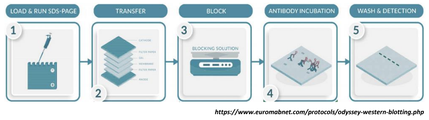


Amissah et. al., 2011; Pijuan et al. Front Cell Dev Biol. 2019.

- **Matrigel invasion assay:** was used to assess the inhibitory potential of PUFAs and NSAIDs lung cancer cell invasion.

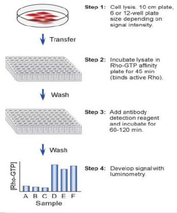


- **Western blotting analysis** was conducted on lung cancer cells exposed to PUFAs and NSAIDs



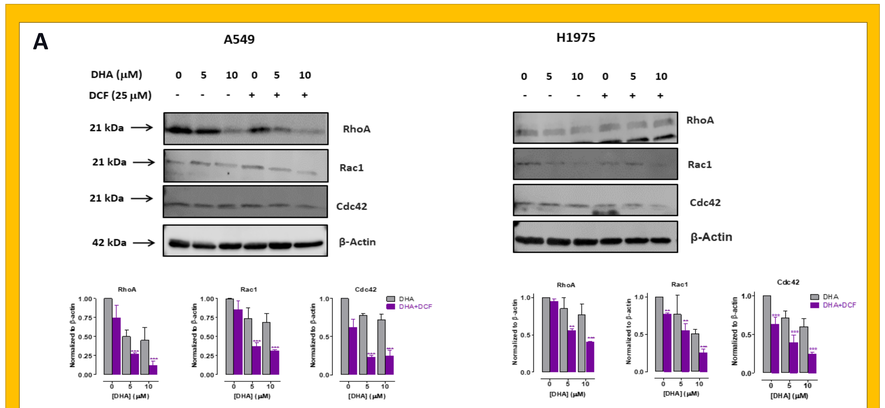
https://www.euromabnet.com/protocols/odyssey-western-blotting.php

- **G-LISA™ GTPase Activation Assay** was conducted on lung cancer cells exposed to PUFAs and NSAIDs.
- **Immunofluorescence:** Treated A549 cells were fixed, permeabilized and stained with DAPI and fluorescein-phalloidin or anti- Rac1 or RhoA antibody. The fluorescent images obtained with Nikon Eclipse C*i* microscpe coupled to QImagingQIClick™ digital camera.



https://www.cytoskeleton.com/bk128

RESULTS



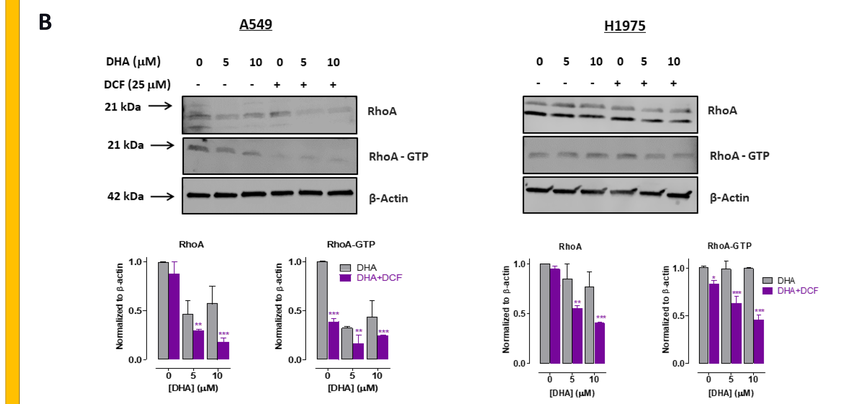


Figure 1. PUFAs and NSAIDs decreased the (**A**) expression and (**B**) activation of RhoA, Rac1, and Cdc42 in A549 and H1975.

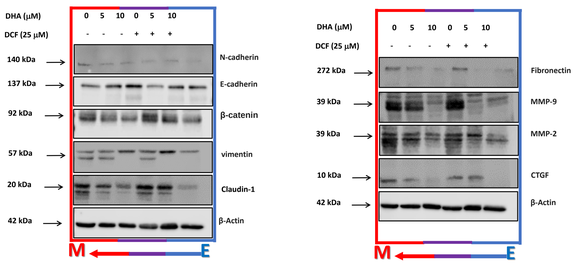


Figure 3. PUFAs and NSAIDs suppress epithelial-to-mesenchymal transition in A549 cells.

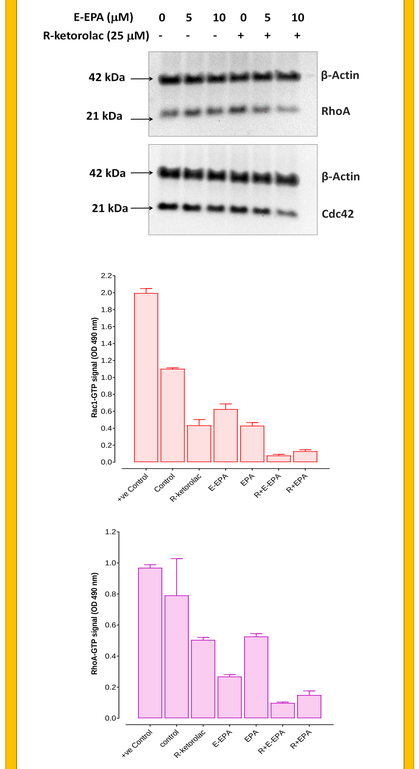


Figure 2. PUFAs and NSAIDs decrease RhoA, Rac1, and Cdc42 activity. A GTPase effector-binding assay was used to measure Rho GTPase activities in A549 cell lysates.

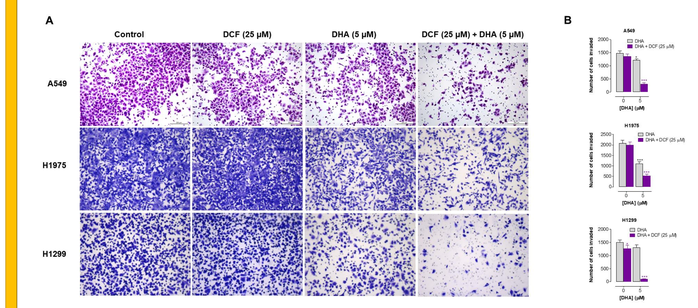


Figure 4. PUFAs and NSAIDs inhibit the invasion of multiple lung cancer cell lines. Co-treatment of DHA with diclofenac inhibited NSCLC cell invasion of A549, H1299, and H1975 cells

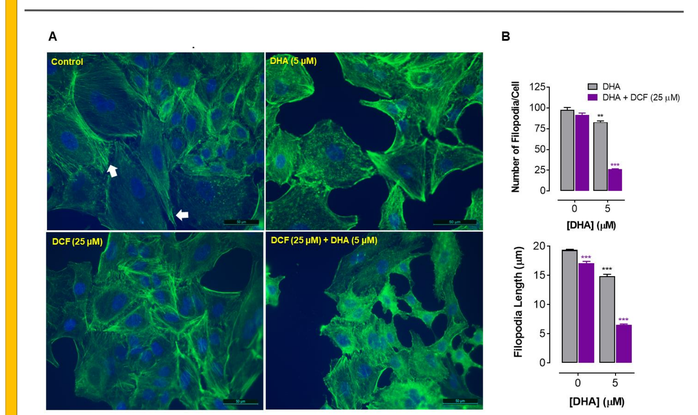


Figure 5. PUFAs and NSAIDs disrupt F-actin organization in A549 cells. Co-treatment with DHA and diclofenac disrupts F-actin organization in A549 cells.

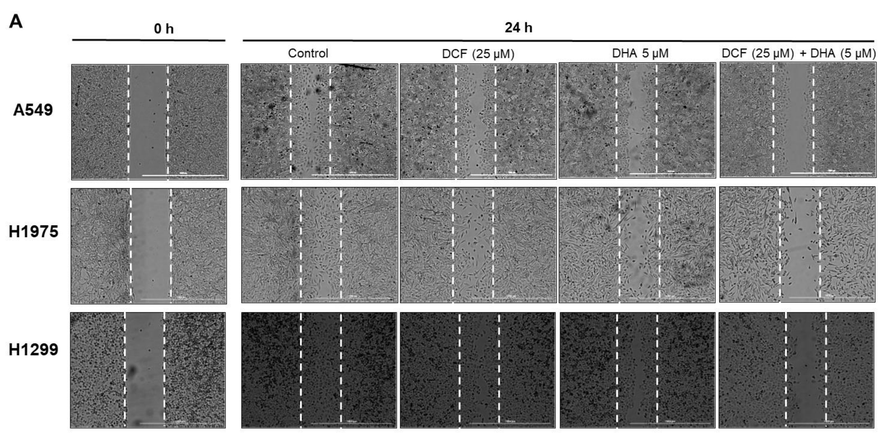
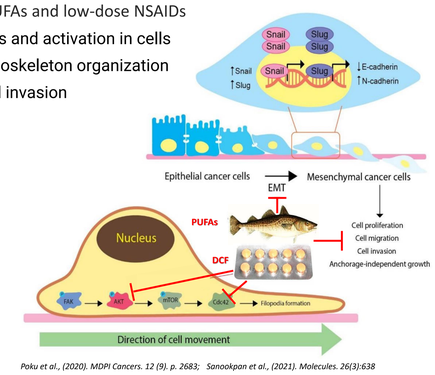


Figure 4. PUFAs and NSAIDs inhibit migration of multiple cell lines. Co-treatment of DHA with diclofenac (DCF) inhibited the migration of A549, H1975, and H1299 cells.

CONCLUSIONS

Co-treatment with PUFAs and low-dose NSAIDs

- ↓Rho GTPase levels and activation in cells
- disrupting actin cytoskeleton organization
- ↓cell migration and invasion
- ↓EMT



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