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Inhibitory Effect of Eicosapentaenoic Acid on the Migration of the Esophageal Squamous Cell Carcinoma Cell Line TE-1

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Abstract. *Background/Aim: Eicosapentaenoic acid (EPA) inhibits NF-κB activation and IL-6 production in TE-1 esophageal cancer cells. NF-κB is related to cancer cell migration. The aim of this study is to evaluate whether EPA has a metastasis suppressing effect. Herein, we investigated EPA-treated TE-1 cell migration using TAXIScan. Materials and Methods: EZ-TAXIScan® was used to verify whether EPA inhibits cancer cell chemotaxis. Results: Using 50% fetal bovine serum (chemoattractant) without EPA (positive control), average velocity was 0.306±0.084 μm/min compared to 0.162±0.067 μm/min without chemoattraction (negative control). Directionalities of positive and negative controls were 1.039±0.152 and 0.488±0.251 radians, respectively, indicating a significant increase in migration of the positive control compared to that of the negative control. Average velocities were 0.306±0.084 (no EPA), 0.288±0.078 (100 μM EPA), and 0.240±0.054 200 μM (EPA) μm/min, indicating that EPA reduced velocity dose-dependently. Average directionalities were 1.039±0.152 (no EPA), 0.967±0.164 (100 μM EPA), and 0.901±0.146 (200 μM EPA) radians, indicating that EPA also inhibited directionality dose-dependently. Conclusion: EPA suppresses directional migration of TE-1 cells.*

Eicosapentaenoic acid (EPA) is an omega-3 polyunsaturated fatty acid (PUFA) with anti-inflammatory effects (1-3). Previously, we reported that EPA inhibits nuclear factor-κB (NF-κB) activation and IL-6 production in TE-1 esophageal cancer cells by inducing apoptosis (3). PUFAs have many effects on cancer cells. In terms of cancer cell migration, PUFAs have inhibitory effects (4-6) mediated through up-regulation of the DNA-binding activity of PPAR-γ and down-regulation of NF-κB p65 protein expression (4), as well as blocking of the Nav1.5 channel (6). However, a report showed a promoting effect of PUFAs on cell proliferation and migration at 24 h of culture (7), which caused controversy. In these reports, the method to observe cell migration was the double chamber method that only measures the number of cells that migrate to the bottom chamber at the endpoint. Recently, an image-based cell migration (chemotaxis) analysis method for solid tumor cells was reported, namely EZ-TAXIScan (ECI, Inc., Kawasaki, Japan), which provides considerably more information regarding cellular dynamics with small amounts of samples (8). Therefore, it is of great interest to investigate the migratory ability of TE-1 cells using the EZ-TAXIScan system. Herein, we report the suppressive effect of EPA on both the directionality and velocity of TE-1 cell migration.

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Materials and Methods

Materials. TE-1 cells derived from an esophageal squamous cell carcinoma were purchased from the RIKEN Bioresource Center Cell Bank (Tsukaba, Japan). The cells were cultured in RPMI-1640 medium containing 0.1% fatty acid-free BSA in a humidified atmosphere with 5% CO_2 at 37˚C. 5, 8, 11, 14, 17-Eicosapentaenoic acid sodium salt was purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as EPA. EPA was dissolved at 100 and 200 μM in phosphate-buffered saline to prepare stock solutions.

Chemotaxis assay of TE-1 cells. A chemotaxis assay to analyze cell migration was performed using EZ-TAXIScan® (8-11). Images of migrating TE-1 cells were recorded with the CCD camera of the system at 15-min intervals for 24 h. The images were analyzed by the TAXIScan Analyzer 2 software. The trajectories of cells in the image were traced manually. The velocity and directionality of each cell were calculated using the traced trajectories (12). Thirty cells were counted in one channel, and the experiment was repeated five

times. First, we confirmed TE-1 cell chemotaxis without EPA. We used silicon chips with an 8-μm depth terrace. After filling with assay buffer in the common space at the top of the EZ-TAXIScan holder, 1 μl of TE-1 cells (2×10^6 cells/ml) was placed into the holes of the cell injection side. After obtaining the first series of images, the chemoattractant solutions were added to the holes of the ligand injection side to initiate migration. As the negative control, RPMI-1640 medium containing 0.1% bovine serum albumin (BSA) was used. As the positive control, RPMI-1640 medium containing 0.1% BSA with 50% fetal bovine serum (FBS) was used.

EPA concentrations. We have reported that the IC50 of EPA for inhibition of TE-1 cell proliferation is 260 μM (3). In this study, 200 μM EPA did not show an inhibitory effect on cell proliferation, and 300 μM EPA had a remarkable inhibitory effect on cell proliferation. Therefore, EPA concentrations of 0, 100, and 200 μM were used to assess cell migration.

Statistical analysis. All analyses were performed using EZR and GraphPad InStat 3. Statistical analysis was performed using the Mann–Whitney test in the case of the negative control versus the positive control or the non-parametric Kruskal–Wallis test followed by Dunnett's multiple comparisons test in the case of comparing three groups (0, 100, and 200 mM EPA). A p-value of <0.05 was considered significantly different.

Results

Migration of TE-1 cells. Cell migration was imaged every 15 min for 24 h with a CCD camera using the EZ-TAXIScan system. The images at 0, 600, and 1,200 min are shown in Figure 1A. Based on the migratory pathway data, the migration velocity and directionality of each cell was calculated. When the chemoattractant accelerated chemotaxis, the velocity and directionality of cells were increased. The average velocity of the positive control was 0.306±0.084 μm/min, and that of the negative control was 0.162±0.067 μm/min ($p<0.0001$). The directionality of the positive control was 1.039±0.152 radians, and that of the negative control was 0.488±0.251 radians ($p<0.0001$). These results revealed a significant increase of the migratory ability of the positive control compared with that of the negative control (Figure 1B). Therefore, we determined that 50% FBS was appropriate to elicit migration of TE-1 cells.

Effect of EPA on the migratory ability. To observe the effect of EPA on TE-1 cell migration, 100 and 200 μM EPA were used (Figure 2). The average velocity was 0.306±0.084 μm/min with 0 μM EPA, 0.288±0.078 μm/min with 100 μM EPA, and 0.240±0.054 μm/min with 200 μM EPA. Therefore, EPA inhibited the velocity of TE-1 cell migration in a dose-dependent manner (Figure 3). The average directionality of TE-1 cell migration was 1.039±0.152 radians with 0 μM EPA, 0.967±0.164 radians with 100 μM EPA, and 0.901±0.146 radians with 200 μM EPA. Therefore, as the EPA concentration increased, less directionality was observed and the decreases were significant (Figure 3).

Discussion

Surgical treatment for esophageal cancer can be safely performed, but the survival rate of patients due to surgery alone is still unsatisfactory. Therefore, various adjuvant therapies have been introduced. Currently, neoadjuvant chemotherapy using cisplatin (CDDP) and 5-fluorouracil (5-FU) is recommended as the standard-of-care in Japan. For a more effective outcome, the use of triplet chemotherapy agents CDDP, 5-FU, and docetaxel, namely DCF therapy, was introduced. DCF therapy has a high response rate, but also has a high rate of adverse events (12). The ideal method to enhance anti-cancer effects is to exert positive effects without any increase of adverse events. We reported the anti-cancer effect of EPA on TE-1 cells, a cell-line derived from esophageal squamous cell cancer (ESCC) (3). Moreover, we reported the synergistic anti-proliferative effect of EPA and anti-cancer drugs on TE-1 cells. This effect was mediated through NF-κB inactivation by blocking nuclear translocation (13). NF-κB has many biological actions on the esophageal cancer cell line (14). Some factors increase (15-17) or suppress (18, 19) the migration of ESCC cells through the NF-κB pathway. These reports revealed the importance of the NF-κB pathway in the control of cell migration. However, a report showed a promoting effect of EPA and DHA on the proliferation and migration of osteosarcoma cells (7). As mentioned above, we confirmed that EPA inhibits NF-κB activation and IL-6 production in TE-1 esophageal cancer cells to induce apoptosis (3); indicating that EPA has an anti-inflammatory effect and induces apoptosis on cancer cells. However, the relationship between EPA and metastasis is still unclear. Therefore, in this study we focused on the effect of EPA on cancer cell migration and investigated the inhibition of TE-1 cell migration by EPA.

We reported a new method to observe cancer cell migration using the EZ-TAXIScan system (8). The pancreatic cancer cell line BxPC3 was used in the previous study (8). The EZ-TAXIScan system is superior compared to Boyden chamber method to observe cell migration, because it observes the moving activity of each cell under the direct vision and measures the velocity and directionality of migrating cells with small amounts of samples (about 100 cells in one channel), whilst the Boyden chamber method only provides migrated cell numbers at endpoints and requires larger amounts of samples (>100,000 cells per well). The results showed quantification of various parameters such as velocity, directionality and cell morphology which were not available by the Boyden chamber method. We, therefore, investigated the effect of EPA on TE-1 cell migration using the EZ-TAXIScan system to obtain the details of how EPA inhibits TE-1 cell migration. Of note, this study is different from the previous report using BxPC3 cells. In the previous report, we used lysophosphatic acid (LPA) as a potent chemoattractant. In this study, on the other hand, FBS

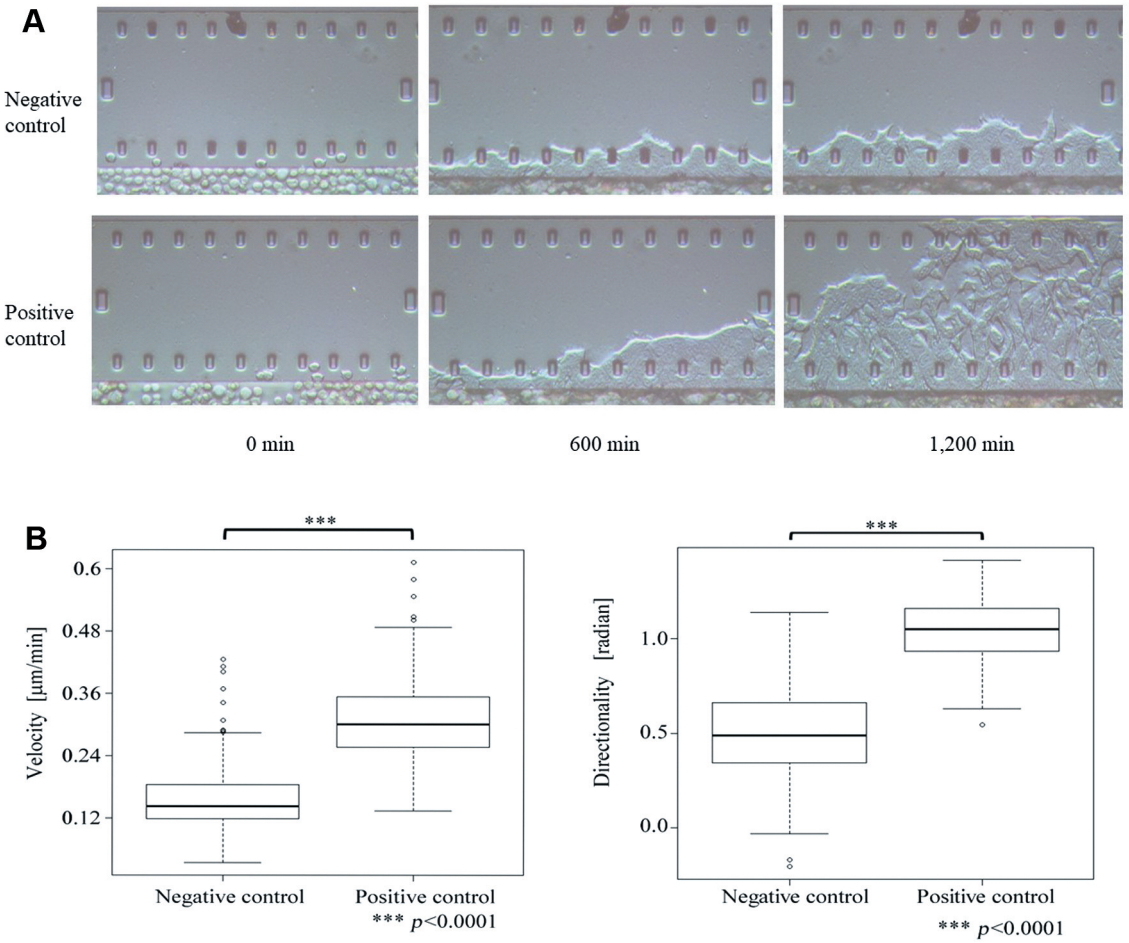

Figure 1. *(A) Images of chemotaxis in negative and positive controls. (B) Comparison of velocity and direction in positive and negative controls.*

was used as the chemoattractant. The reasons why we used FBS are the following; Serum is essential in the human body. (ii) Serum contains LPA, and (iii) FBS is one of the strongest chemoattractants based on our preliminary experiments using various kinds of chemoattractants such as chemokines, lipid-mediators, and small molecular-weight compounds (unpublished observation).

As previously reported, a high concentration of EPA suppressed cell proliferation. The IC_{50} of EPA for inhibition of TE-1 cell proliferation is 260 μM (3). The high concentration EPA suppresses cell migration, but this result reflects cell viability itself. Two hundred micromolar EPA did not suppress cell proliferation. Therefore, we used 100 and 200 μm EPA. Both concentrations of EPA suppressed the velocity and directionality of cell migration in a dose-dependent manner. These results indicate suppression of the cell migratory ability itself by EPA and not through inhibition of proliferation or viability.

Our data revealed the major role of EPA in the metastatic mechanism of ESCC.

Migration of cancer cells correlates with distant metastasis and local invasion (8).

Several randomized, controlled trials and meta-analyses have demonstrated that perioperative use of an enteral formula containing arginine, glutamine, and omega-3 fatty acids has a beneficial effect on surgical outcomes in both well-nourished and malnourished patients (20-23). A better surgical outcome might result in better survival of cancer patients. We reported this hypothesis as surgical oncotaxis (24). Most importantly, adverse events of EPA treatment are minimal. Therefore, perioperative use of EPA might be meaningful.

This study was *in vitro*, and the molecular mechanism was not investigated. Therefore, the precise mechanism of the suppressive effect of EPA on cell migration, including the NF-κB pathway, should be clarified in a future study.

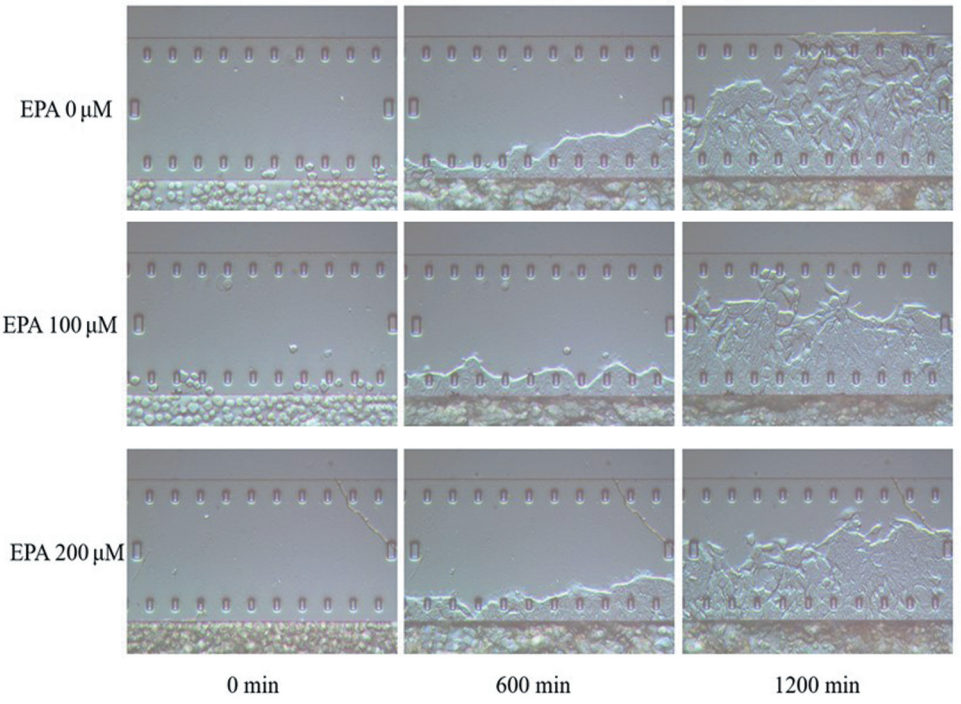

Figure 2. *Images of chemotaxis in each EPA concentration.*

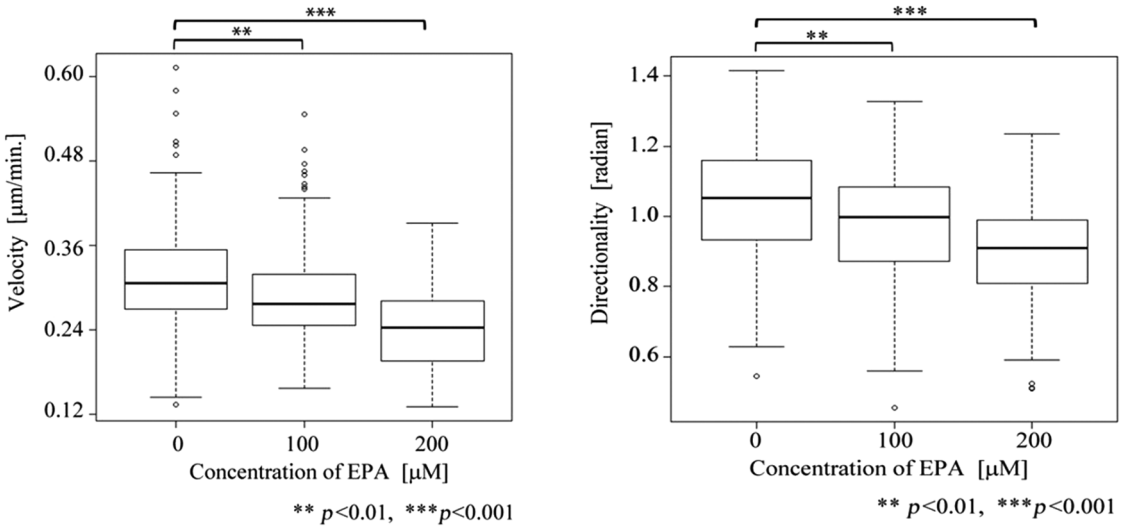

Figure 3. *Comparison of velocity and directionality in each EPA concentration.*

Conclusion

We investigated the migration of TE-1 cells, a human squamous cell carcinoma cell line, using the EZ-TAXIScan system. EPA suppressed the migratory ability of TE-1 cells in a dose-dependent manner. Based on this result, EPA has not only nutritional advantages such as an anti-inflammatory effect and an apoptosis inducing effect, but also the possibility to suppress metastasis of cancer cells.

Conflicts of Interest

The Authors have no conflicts of interest to declare.

Authors' Contributions

H.K., A.O., A.Y., and T.H. designed and conducted the study. H.K. and T.H. wrote the manuscript. Y.A. contributed to statistical analysis. H.M. helped with the study. T.H., and T.U. critically revised the manuscript. All Authors approved the final manuscript.

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