Endogenously synthesized n-3 polyunsaturated fatty acids in fat-1 transgenic mice suppress B16F10 melanoma lung metastasis by impairing mesenchymal to epithelial transition

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ABSTRACT

Malignant melanoma is an aggressive cancer of neuroectodermal origin. The mechanisms of n-3 polyunsaturated fatty acids (PUFAs) against melanoma metastasis are not clear. Here fat-1 and wild type (WT) mice were injected with B16F10 melanoma cells via the tail vein to establish lung metastasis model. Endogenous n-3 PUFAs were associated with a reduction in grossly visible pulmonary metastases and outgrowth through inhibition of mesenchymal to epithelial transition (MET), which was characterized by decreased expression of epithelial markers mesenchymal marker E-cadherin and ZO-1, and increased expression of vimentin in tumour tissues from fat-1 mice compared with WT controls. In addition, n-3 PUFAs-mediated inhibition of MET may have been associated, in part, with the: i) formation of n-3 PUFA-derived lipid mediators and ii) decreased expression of mature IL-1 β and p-NF- κ B, and enhanced expression of superoxide dismutase-1. These results suggest that n-3 PUFAs exert their antitumourigenic activities, in part, via their anti-inflammatory properties.

1. Introduction

Malignant melanoma is the most aggressive form of cutaneous malignancy, accounting for ~ 3% of all cases of malignant tumours. Its incidence has been increasing in Caucasian populations over the past several decades with a mortality rate second only to lung cancer (Ahmedin, et al., 2009). Metastasis is the most lethal step of cancer progression in patients with invasive melanoma. Tumour invasiveness is tightly associated with epithelial-mesenchymal transition (EMT) (Heerboth, et al., 2015), which endows metastatic properties upon cancer cells to promote invasion, migration, and subsequent dissemination. EMT was characterized with loss of E-cadherin, a key marker of the epithelial phenotype, and increased expression of vimentin and N-cadherin, two proteins considered to be markers of the mesenchymal phenotype (Feroni, Broggin, Generali, & Damia, 2012). Interestingly, mesenchymal to epithelial transition (MET), a reverse process of EMT, plays a vital role for disseminated tumour cells to establish metastases in distal target organs, which was accompanied by increased E-cadherin expression and reduced expression of vimentin and N-cadherin (Gunasinghe, Wells, Thompson, & Hugo, 2012).

In recent years, a number of plant derived natural products have demonstrated their potential in the treatment or prevention of metastatic cancers (Lahlou, 2013). Among the dietary factors, n-3 polyunsaturated fatty acids have gained attention as potential preventative and/or adjuvant agents in the treatment of malignant melanoma (Serini, Fasano, Celleno, Cittadini, & Calviello, 2014). Dietary n-3 PUFAs, especially eicosapentaenoic acid (EPA, 20:5, n-3) and docosahexaenoic acid (DHA, 22:6, n-3) found mainly in fish or fish oil supplements, may reduce cancer risks (Funahashi, et al., 2008; Monk, et al., 2014). An important antitumour mechanism of n-3 PUFAs most likely relates to the formation of n-3 derived bioactive lipid mediators (Serhan, 2014; Moltke, et al., 2012). However, whether n-3 lipid mediators are involved in inhibiting MET of melanoma cells in distal target organs remains to be investigated. In addition, there were conflicting results about n-3 PUFAs against melanoma metastasis from animal studies. Such conflicting results reflect many confounding dietary elements such as the potential variations in the impurities and caloric and other components in the supplemented oils. Therefore, an appropriate animal model that can eliminate the confounding factors of diet would be helpful for elucidating the role of n-6/n-3 fatty acid ratio in melanoma. The fat-1 transgenic mouse carries the fat-1 gene from the roundworm *Caenorhabditis elegans* (Kang, 2007). The gene, not found in mammals, encodes an n-3 PUFA desaturase that converts n-6 to n-3 PUFAs. Therefore, the fat-1 transgenic mice are

endogenously enriched with n-3 PUFAs and characterized by a balanced ratio of n-6/n-3 PUFAs in their tissues compared with their WT littermates by using a single diet with high n-6 PUFAs, thus eliminating the potential diet variations. Hence, the fat-1 transgenic mouse is an ideal model used to study the role of n-3 PUFAs in carcinogenesis (Nowak et al., 2007).

In the present study, we fed fat-1 and wild type mice a high n-6-containing diet with 10% safflower oil to explore whether endogenous n-3 PUFAs affect metastatic outgrowth of B16F10 melanoma cells and the underlying mechanisms involved. The results showed that endogenously elevated n-3 PUFAs impaired macrometastases formation in metastatic lungs through suppressing MET process. The prevention of n-3 PUFAs against MET of B16F10 melanoma cells and a switch to macrometastatic growth is mainly associated with increased biosynthesized n-3 PUFA-derived bioactive mediators, decreased expression of mature IL-1 β (mIL-1 β), phospho-NF- κ B and reduced oxidative stress in tumour microenvironment.

2. Experimental section

2.1. Cells and reagents

The murine B16F10 melanoma cell line (ATCC, MD, USA) was maintained in a humidified incubator at 37°C and 5% CO₂ in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA), supplemented with 10% fetal bovine serum. The primary antibodies mouse anti-E-cadherin, rabbit anti-ZO-1, rabbit anti-Vimentin, rabbit anti-superoxide dismutase-1(SOD1), rabbit-IL-1 β , rabbit anti-nuclear factor κ B (NF- κ B), rabbit anti-phospho (p)-NF- κ B (Ser536), rabbit anti-GAPDH and the horseradish peroxidase-linked secondary antibody were purchased from Cell Signaling Technology (Danvers, MA, USA). Chloral hydrate was purchased from Shanghai Meilian biological technology R&D Co., Ltd. (Shanghai, China).

2.2. Animals and diet

The fat-1 transgenic mice were donated by Dr. Jing X. Kang at Massachusetts General Hospital and Harvard Medical School (Boston, MA, USA). Male heterozygous fat-1 transgenic mice in the C57BL/6 background were mated with wild-type (WT) C57BL/6 female mice to produce heterozygous fat-1 mice and WT mice. Each mouse was genotyped to identify mice carrying the *fat-1* transgene. Specific

pathogen-free transgenic and WT animals were fed a 10% safflower oil diet, and housed in standard cages in temperature- and humidity-controlled conditions with a 12 h light/dark cycle. At 10-12 weeks of age, heterozygous fat-1 male mice and nontransgenic littermate controls for the purpose of the present study. Their diets contained (per 100 g diet) 4.5 g sucrose, 18.6 g casein, 8.6 g cellulose, 50 g wheat starch, 0.3 g DL-methionine, 7 g mineral mix, 1 g vitamin mix, and 10 g safflower oil. All animal experiments were conducted in accordance with animal care guidelines and approved by the Ethical Committee of Ningbo University (Ningbo, China).

2.3. Tail vein injection of melanoma cells for metastasis

Cultured B16F10 melanoma cells were harvested, washed twice with serum-free Dulbecco's Modified Eagle Medium (DMEM) , and resuspended at a concentration of 2×10^6 cells/mL. A volume of 0.1mL of suspended cells was injected into the tail veins of 10 fat-1 and 10 WT mice. The day of the injection of B16F10 cells was designated day 0. All mice were sacrificed by exsanguinations 4 weeks after injection and the lungs, livers, spleens, kidneys, intestines and brains were dissected out and photographed. Dissected tissues were fixed for histology analysis or stored in 80°C for other analysis. All surgery was performed under chloral hydrate, and all efforts were made to minimize suffering.

2.4. Histological analysis

For histological investigations, Tissues were fixed in 4% formaldehyde in phosphate buffer overnight and then embedded in paraffin. Histological sections were stained with hematoxylin and eosin (H&E).

2.5. Analysis of fatty acids and lipid mediators

Fatty acid profiles were determined by gas chromatography, as described previously (Bellenger, 2004). In brief, tumour tissues were ground to a powder form using liquid nitrogen. Samples were then transesterified with H₂SO₄ in methanol (5%, v/v), together with toluene, in sealed tubes at 70 °C for 2 h. Fatty acid methyl esters were analyzed by gas chromatograph using Shimadzu GC-14C (Shimadzu Corporation, Kyoto, Japan) equipped with a flame-ionization detector (FID) and a capillary column (DB-23, Aglient Corporation, USA). The fatty acid methyl esters were identified by comparing with standard mixtures of fatty acid methyl ester (Nu-chek-Prep, Elysian, MN). The composition of fatty acids was expressed as the relative percentage of the total fatty acids according to their peak areas. Bioactive

lipid mediators derived from n-3 and n-6 fatty acids in tumour tissues were identified by using LC–tandem MS methods as described in (Wang, Armando, Quehenberger, Yan, & Dennis, 2014). Briefly, each tumour sample was extracted with 2 ml of cold methanol and analyzed by UPLC-UV-tandem MS using an ABI/Sciex 6500 QTRAP hybrid, triple quadrupole, linear ion trap mass spectrometer equipped with an Acquity UPLC BEH shield RP18 column (2.1 × 100 mm; 1.7 µm; Waters) and Turbo V ion source using mobile phase (methanol: water: acetate at 60:40:0.02) with a 0.5 ml/min flow rate.

2.6. Western blot analysis

Tumour tissues were homogenized in Triton-X protein lysis buffer (20 mM Tris-HCl, pH7.6, 1 mM EDTA, 140 mM NaCl, 1% NonidetP-40, 1% aprotinin, 1mM phenylmethylsulphonyluoride, and 1 mM sodium vanadate). Lysates were sonicated and centrifuged at 15000 g for 10 minutes at 4 °C. Per lane, equal amounts of tissue extract protein (50 µg) were separated on 10% SDS-PAGE and transferred onto nitrocellulose membranes. After nonspecific binding sites were blocked with 5% nonfat milk in Tris buffered saline (TBS), membranes were incubated overnight at 4 °C with primary antibodies, and then incubated for 1 h at room temperature with appropriate secondary antibodies. Blots were then developed using enhanced chemoluminescence detection reagents.

2.7. Statistical analysis

For each treatment group, Results were presented as the arithmetical mean ± SE. Statistical significance between groups was determined using Student's *t*-test. $p < 0.05$ and $p < 0.01$ was considered to indicate a statistically significant difference

3. Results

3.1. n-3 PUFAs inhibited lung metastases of B16F10 Melanoma Cells in fat-1 transgenic mice.

In order to evaluate that endogenously enhanced n-3 PUFAs in fat-1 transgenic mice are associated with the suppression of metastases formation and metastatic outgrowth, B16F10 murine melanoma cells were injected into the tail vein of fat-1 and WT mice, and distant organ metastases of inoculated tumour cells were examined. As shown in Fig. 1, four weeks after injection with B16F10 murine melanoma cells,

significantly less grossly visible pulmonary metastases were observed in fat-1 transgenic mice (n=10) when compared with WT mice (n=10). Neither liver nor bone metastases were observed in fat-1 and WT mice (data not shown).

3.2. Fatty acid profiles in plasma and tumour tissues of fat-1 and WT mice

Measurement of the total lipids extracted from plasma and tumour tissues of fat-1 and WT mice showed differences in lipid profiles between the two groups (Fig. 2 and Fig. 3). Compared with WT mice, fat-1 transgenic mice exhibited significantly increased levels of n-3 PUFAs, including EPA (20:5n-3), docosapentaenoic acid (DPA) (22:5n-3), and DHA (22:6n-3) and displayed decreased levels of AA (20:4n-6) in the plasma and tumour tissues (Fig. 2A and Fig. 3A). In particular, the n-6/n-3 fatty acid ratio was significantly reduced in fat-1 plasma and tumor tissues (8.03 and 7.01), respectively, compared with that of WT mice (37.10 and 26.32), despite the animals being fed the same diet (Fig. 2B and Fig. 3B). These data indicate the expression of *fat-1* transgene endogenously enriches the fat-1 transgenic mice in n-3 PUFAs.

3.3. Increased n-3-derived lipid mediators in fat-1 mice

It is generally believed that lipid mediators derived from AA (prostanoids, leukotrienes, lipoxins and epoxyeicosatrienoic acids) promote angiogenesis, inflammation, and tumour progression, while lipid mediators derived from EPA (E-series resolvins), and DHA (D-series resolvins, protectins have been shown to suppress tumour progression (Vara-Messler, et al., 2015). Therefore, the levels of n-6 and n-3 PUFA-derived lipid mediators from tumour tissues were determined using UPLC-UV-tandem MS analysis, in order to determine whether the observed differences in lung metastases between these two groups were associated with differences in the levels of lipid mediators. As shown in Fig. 4, a significant increase in the levels of fatty acid metabolites derived from EPA (12, 15- hydroxyeicosapentaenoic acid (HEPE), REV1 and prostaglandin E3 (PGE3)) and DHA (RVD2) were observed in tumour tissues from fat-1 mice compared with WT controls. In contrast, no significant difference in the levels of the proinflammatory prostaglandin E2 (PGE2) was observed between fat-1 and WT mice.

3.4. Endogenous n-3 PUFAs inhibited metastases in vivo by impairing MET

In tumour progression, the disseminated mesenchymal tumour cells must undergo MET at the site of

metastasis and seed successfully in secondary organs (Gunasinghe et al., 2012). In order to determine whether fat-1 expression accompanied by an increase in n-3 PUFAs levels affects MET, melanoma tumour tissues from fat-1 mice and WT controls were analyzed for the expression of E-cadherin, ZO-1 and vimentin by Western blotting. As shown in Fig. 5, the protein expression levels of E-cadherin and ZO-1, a peripheral membrane protein associated with the tight junction were markedly downregulated (Itoh, Nagafuchi, Moroi, & Tsukita, 1997), whereas the protein expression levels of vimentin was prominently up-regulated in the tumour tissues of fat-1 mice compared with those of the WT controls, implying MET process being suppressed by n-3 PUFAs.

3.5. Inhibition of IL-1 β /NF- κ B inflammatory signaling pathway and oxidative stress by n-3 PUFAs in fat-1 mice

Chronic inflammation is frequently associated with malignant growth and is thought to promote and enhance tumour progression. The nuclear factor- κ B (NF- κ B) transcription factor has been considered the central mediator of tumour-associated inflammation, tumour growth, and metastasis (Lawrence, 2009). In order to determine whether endogenous n-3 PUFAs affect NF- κ B activation, western blotting was used to check NF- κ B and its phosphorylation levels. As shown in Fig. 6, fat-1 tumour tissues exhibited a dramatically decreased expression of NF- κ B and its phosphorylation levels compared to those from WT mice. In addition, IL-1 β is a key regulator of tumour invasiveness, which acts through the IL-1R/MyD88/NF- κ B pathway to induce synthesis of pro-IL-1 β . Pro-IL-1 β is then cleaved by caspase-1 to biological active IL-1 β (Dinarello, 2011). The conversion of pro-IL-1 β to mature IL-1 β (mIL-1 β) is tightly regulated by reactive oxygen species (ROS)-mediated inflammasome complex activation (Vandanmagsar, et al., 2011). To test whether endogenous n-3 PUFAs suppressed mIL-1 β through decreased oxidative stress, the protein expression of Pro-IL-1 β , mIL-1 β , and superoxide dismutase-1 (SOD1) was examined. As shown in Fig. 6, fat-1 tumour tissues exhibited a marked increase in SOD1 expression compared with those from WT controls, which suggest that increased n-3 PUFAs attenuate oxidative stress in tumour tissues. Despite observing no significant alterations in the expression levels of Pro-IL-1 β , mIL-1 β expression was significantly reduced in the tumor tissues of fat-1 mice. These results suggest that low oxidative stress level may contribute to the decreased expression of mIL-1 β in fat-1 mice.

4. Discussion

Although published preclinical and epidemiological studies provide evidence to support the anticancer effects of dietary or exogenously derived n-3 PUFAs against melanoma (English, et al., 1986; Gallagher, Elwood, & Hill, 1986), there were conflicting results from animal studies, which reported that n-3 PUFAs had no effects or promoting effects on melanoma (Abbott, Tezabwala, Bennett, & Grundy, 1994; Salem, Kishihara, Abe, Matsuzaki, & Nomoto, 2000). These inconsistencies and discrepancies about n-3 PUFAs against melanoma are closely linked to many confounding dietary factors including the differences in the diets and the feeding procedure. In the present study, the fat-1 transgenic mice, which are capable of endogenously synthesizing n-3 PUFAs and eliminating the confounding factors of diet were used to investigate the anti-melanoma effects of n-3 PUFAs (Kang, 2007). Our results presented here demonstrated that endogenous production of n-3 PUFAs prevented lung metastasis of melanoma cells through mitigating the MET process (Fig. 1, 5). Moreover, melanoma lung metastasis inhibition in fat-1 mice was correlated with the production of n-3 PUFAs-derived lipid mediators (Fig. 4), the reduced oxidative stress, and the blockade of IL-1 β /NF- κ B inflammatory signaling pathway.

Cancer metastasis is a very complex process, which is closely linked with EMT and MET. MET is critical to the initial transformation for tumour cells escaping from the original site and penetrating into the circulation, and MET critical to the latter stages of metastasis for circulating tumour cells to anchor in distant tissues and form new tumours (Brabletz, 2012). A recent study showed that linoleic acid (18:2 n-6) enhanced invasiveness by promoting an EMT-like process in cultured breast cancer cells (Galindo-Hernandez, Serna-Marquez, Castillo-Sanchez, & Salazar, 2014). However, in this study, we observed significantly decreased tumour outgrowth in metastatic lungs accompanied by a significant downregulation in the protein expression levels of epithelial markers E-cadherin and ZO-1, and a marked increase in vimentin expression (a marker of mesenchymal stage) in tumour tissues from fat-1 mice, compared with those of WT controls (Fig.5). This suggests that endogenous n-3 PUFAs mediated the inhibition of MET and decreased proliferation of metastatic melanoma cells may be the main mechanisms of decreased formation of focal macrometastases and tumour outgrowth in metastatic lungs in fat-1 mice. Our study further supports the notion that metastatic cancer cells recruited to distal target organs undergo MET, the opposite process of EMT for successful metastasis, and future anti-metastatic therapies target not only EMT of tumour cells in primary tumour site, but also MET in secondary organs (Chaffer, Thompson, & Williams, 2007).

We have also explored the underlying mechanism by which endogenously synthesized n-3 PUFAs impaired MET. Chronic inflammation in the tumour microenvironment has a role during each phase of cancer progress including primary tumour growth, survival in the periphery and secondary outgrowth (Landskron, Fuente, Thuwajit, Thuwajit, & Hermoso, 2014). Anti-inflammatory actions of n-3 PUFAs are considered to be mediated by the formation of their active metabolites such as eicosanoids and lipid mediators (Serhan, Chiang, & Van Dyke, 2008; Weylandt, Chiu, Gomolka, Waechter, & Wiedenmann, 2012). In the present study, endogenous n-3 PUFAs significantly increased the levels of the anti-inflammatory n-3 derived lipid mediators such as 12, 15- HEPE, REV1, RVD2, PGE3 in the fat-1 mice compared with the WT controls (Fig. 2, 3, 4). Consistently, in one report, endogenously increased PGE3 suppressed the growth of melanoma xenografts in fat-1 mice (Xia, et al., 2006). n-3 derived anti-inflammatory mediators have been shown to regulate the activation of transcriptional factor NF- κ B and the production of inflammatory adipokines including IL-1 β , TNF α and IL-6 (Bannenberg & Serhan, 2010; Jing X. Kang & Weylandt, 2008). IL-1 β is one of the critical pro-inflammatory cytokines involved in tumour pathogenesis. In the present study, the tumour tissues of fat-1 mice, rich in endogenous n-3 PUFAs and their lipid mediators, exhibited markedly lower amounts of mIL-1 β than those from WT mice (Fig. 6). This finding is consistent with previous anti-diabetes and anti-colitis experiments using fat-1 mouse model (Bellenger, et al., 2011; Jia, et al., 2008). The production of mIL-1 β is regulated by ROS-mediated activation of inflammasome complex, whereas this process is counteracted by n-3 PUFAs-mediated reduced oxidative stress (Gonzálezpérez, et al., 2007; Sorbara & Girardin, 2011). Therefore, the increased levels of n-3 PUFAs in fat-1 mice may contribute to the reduced mIL-1 β expression through increasing SOD1 expression. In addition, the decreased mIL-1 β expression may be also mediated partially by increased n-3 lipid mediators in fat-1 tumor tissues. mIL-1 β is found to activate NF- κ B through binding to its receptor IL-1R (Esser, Legrand-Poels, Piette, Scheen, & Paquot, 2014). Therefore, the decrease in mIL-1 β expression may contribute to the reduced levels of phosphorylated NF- κ B (Fig. 6). Additionally, it is important to note that n-3 lipid mediators may be also involved in inhibiting NF- κ B phosphorylation. For instance, RVE1 inactivates NF- κ B by binding to its membrane receptor ChemR23 (Arita, et al., 2007). Ultimately, the results of the present study suggest that fat-1 mice exhibit increased ability to resolve inflammation in tumour microenvironment by decreasing mIL-1 β levels and inhibiting NF- κ B activation.

5. Conclusion

As summarized in Fig. 7, our data suggest that endogenous n-3 PUFAs impaired MET and metastatic outgrowth of melanoma cells, in part, through anti-inflammatory status in tumour microenvironment mediated by bioactive lipid mediators and reduced oxidative stress. These findings support a protective role of elevated tissue n-3 PUFAs status in the prevention of melanoma metastasis or n-3 PUFAs supplement as a putative adjuvant agent in the treatment of patients with malignant melanoma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed

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Author contributions

Designed overall research experiments: Z.-Q.Z., and S.-Z.B. Performed the experiments and data analysis: P.Z., Y.-M.Z., X.Y., F.W., J.-J.Z., X.-H.Z. Collected and analysed references: W.Y., Y.X. Wrote the manuscript: P.Z., Y.-M.Z., X.Y. Provided the fat-1 mice and useful advices for animal experiments: J.-X.K., J.-B.W. All authors read and approved the manuscript.

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Figure legends

Fig 1 - The effects of endogenous n-3 PUFAs on MET-mediated lung metastases of B16F10 melanoma cells *in vivo*. B16F10 cells (2×10^5 diluted in 100 μ l of serum-free DMEM) were injected subcutaneously into the tail vein of each of 10 fat-1 transgenic and 10 WT mice (12 weeks old, male). All mice were sacrificed 4 weeks after injection. (A) Macroscopic view of lung tissues in WT and fat-1 mice. (B) Microscopic hematoxylin and eosin staining of lung tissues in WT and fat-1 mice. Arrows marked pulmonary metastases.

Fig 2 - Total lipids of plasma were extracted, methylated and subjected to analysis by gas chromatography in WT and fat-1 mice (n=5). (A) Plasma profiles of major n-6 and n-3 PUFAs, total n-6, and total n-3 of in WT and fat-1 mice (n=5). (B) Ratio of n-6 PUFAs to n-3 PUFAs of plasma in WT and fat-1 mice as white and black bars respectively. Results are presented as mean $\pm$ SE. The significant differences of important fatty acids and fatty acids ratio between WT and fat-1 mice were analyzed by Student's *t*-test (* $P < 0.05$; ** $P < 0.01$). ND, not detectable.

Fig 3 - Total lipids of tumour tissue were extracted, methylated and subjected to analysis by gas chromatography in WT and fat-1 mice (n=5). (A) Tumour tissue profiles of major n-6 and n-3 PUFAs,

total n-6, and total n-3 of in WT and fat-1 mice (n=5). (B) Ratio of n-6 PUFAs to n-3 PUFAs of tumour tissues in WT and fat-1 mice as white and black bars respectively. Results are presented as mean $\pm$ SE. The significant differences of important fatty acids and fatty acids ratio between WT and fat-1 mice were analyzed by Student's *t*-test (* $P < 0.05$; ** $P < 0.01$). ND, not detectable.

Fig 4 - Effects of *fat-1* gene expression on the formation of n-6 and n-3 PUFA-derived mediators in tumour tissues. Quantification of different lipid mediators in tumour tissues of fat-1 transgenic mice (n= 5) and WT animals (n = 5). * $P < 0.05$; ** $P < 0.01$ (Student's *t* -test).

Fig 5 - Endogenous n-3 PUFAs impaired MET in metastatic melanoma cells. Western blotting was used to check the expression of E-cadherin, ZO-1 and vimentin in tumour tissues from three WT and three fat-1 mice. Data are presented as mean $\pm$ SE of three independent experiments, and differences were analyzed by Student's *t*-test (* $P < 0.05$; ** $P < 0.01$).

Fig 6 - Endogenous n-3 PUFAs modulated IL-1 β /NF-kB signaling pathway. Western blotting was used to check the expression NF-kB, phosphor- NF-kB, SOD1 and IL-1 β in tumour tissues from three WT and three fat-1 mice. Data are presented as mean $\pm$ SE of three independent experiments, and differences were analyzed by Student's *t*-test (* $P < 0.05$; ** $P < 0.01$). ND, not detectable.

Fig 7 - Schematic depicting the role of endogenous n-3 PUFAs in impairing EMT of metastatic melanoma cells. Endogenously synthesized n-3 PUFAs prevented lung metastasis of melanoma cells through mitigating the MET process, which was closely linked with the formation of anti-inflammatory n-3 PUFAs-derived lipid mediators, the decreased expression of mIL-1 β and p- NF-kB, the increased expression of SOD1.