Clinical Research

Effect of dietary omega-3 fatty acids on castrate-resistant prostate cancer and tumor-associated macrophages

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Abstract
Background M2-like macrophages are associated with the pathogenesis of castrate-resistant prostate cancer (CRPC). We sought to determine if dietary omega-3 fatty acids (ω-3 FAs) delay the development and progression of CRPC and inhibit tumor-associated M2-like macrophages.

Methods MycCap cells were grown subcutaneously in immunocompetent FVB mice. Mice were castrated when tumors reached 300 mm². To study effects of dietary ω-3 FAs on development of CRPC, ω-3 or ω-6 diets were started 2 days after castration and mice sacrificed after early regrowth of tumors. To study ω-3 FA effects on progression of CRPC, tumors were allowed to regrow after castration before starting the diets. M2 (CD206+) macrophages were isolated from allografts to examine ω-3 FA effects on macrophage function. Omega-3 fatty acid effects on androgen-deprived RAW264.7 M2 macrophages were studied by RT-qPCR and a migration/ invasion assay.

Results The ω-3 diet combined with castration lead to greater MycCap tumor regression (tumor volume reduction: 182.2 ± 33.6 mm³) than the ω-6 diet (tumor volume reduction: 148.3 ± 35.2; p = 0.003) and significantly delayed the time to CRPC (p = 0.006). Likewise, the ω-3 diet significantly delayed progression of established castrate-resistant MycCaP tumors (p = 0.003). The ω-3 diet (as compared to the ω-6 diet) significantly reduced tumor-associated M2-like macrophage expression of CSF-1R in the CRPC development model, and matrix metallopeptidase-9 (MMP-9) and vascular endothelial growth factor (VEGF) in the CRPC progression model. Migration of androgen-depleted RAW264.7 M2 macrophages towards MycCaP cells was reversed by addition of docosahexaenoic acid (ω-3).

Conclusions Dietary omega-3 FAs (as compared to omega-6 FAs) decreased the development and progression of CRPC in an immunocompetent mouse model, and had inhibitory effects on M2-like macrophage function. Clinical trials are warranted evaluating if a fish oil-based diet can delay the time to castration resistance in men on androgen deprivation therapy, whereas further preclinical studies are warranted evaluating fish oil for more advanced CRPC.

Introduction
Effective therapies are now available for castrate-resistant prostate cancer (CRPC), but eventually the disease progresses necessitating the need for additional therapies. Whereas epidemiologic studies are inconclusive for the association between fish oil intake and risk of overall and advanced prostate cancer [1–6], preclinical studies suggest a clinical benefit for androgen-sensitive and castrate-resistant disease [7, 8]. Wang et al. reported that fish oil (as compared to corn oil) decreased growth of phosphatase and tensin homolog (PTEN) knockout (KO) allografts in castrated nude mice and decreased growth of native prostate tumors in castrated PTEN KO mice [9]. Tumors in the fish oil group had decreased androgen receptor levels and decreased leucocyte common

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antigen (CD45+) lymphocyte infiltration [9]. Likewise, Gevariya et al. recently reported that dietary fish oil delayed progression of androgen-deprived TRAMP C-2 tumors in immunocompetent mice and was associated with an increased local inflammatory response [10].

Using an androgen-sensitive immunocompetent allograft model, we reported that fish oil delayed prostate cancer progression and inhibited the number and function of M2-like macrophages in tumor tissue [11]. Macrophages are highly plastic cells and respond to surrounding stimuli to develop tissue-specific functions [12]. Distinct states of polarized TAMs include the “classically” activated (M1) macrophages that have tumoricidal activity while the “alternatively” activated (M2) macrophages promote tissue repair and angiogenesis, and favor tumor progression [13]. M2 tumor-associated macrophages (TAMs) make up the majority of TAMs in prostate cancer [12]. M2 polarized TAMs are associated with poor prognosis and high tumor grade in patients with prostate cancer [12–14]. Zarif et al. reviewed potential treatment strategies for targeting M2-like macrophages in the tumor microenvironment to treat advanced prostate cancer [15]. Since TAMs represent a functionally diverse group of macrophages with variable activation states, the terminology “M1-like” and “M2-like” is often used for in vivo studies in recognition of the variation in functional states [16].

Androgen ablation therapy has been shown to increase the expression of M2-like macrophage markers and the number of M2-like macrophages in the tumor environment. For example, prostate cancer cells in a tumor cell-macrophage co-culture system undergoing androgen deprivation exhibited enhanced gene expression of M2-expressing cytokines VEGF-A, MMP-9, and Arg-1, and a reduction in proinflammatory M1 cytokines [17]. In addition, following androgen ablation therapy, a significant increase in M2-like (CD163+) macrophages was observed in prostate tumor tissues from hormone ablation-treated patients compared to hormone-naive tissues [18]. Zarif et al. also found M2 macrophage infiltration in human mCRPC tissues from rapid autopsies [19].

In the present study, our objective was to investigate if omega-3 FAs delay the development and progression of CRPC. Based on our previous findings in androgen-sensitive prostate cancer, we hypothesized that dietary omega-3 FAs will decrease the number and function of M2-like macrophages leading to a decrease in tumor growth, angiogenesis, migration, invasion, and suppression of immune cell function.

Materials and methods

Chemicals, reagents and diets

Docosahexaenoic acid (DHA) was obtained from Cayman Chemical (Ann Harbor, MI, USA), RPMI and DMEM media and fetal bovine serum (FBS) from Invitrogen (Carlsbad, CA, USA), and mouse interleukin 4 (IL-4) from Sigma Chemical (St Louis, MO, USA). Mouse diets were purchased from DYETS, Inc. (Bethlehem, PA). For the ω-6 diet, 30% of energy (134 g/kg) was provided by corn oil, and the ω-6 to ω-3 ratio was 18:1. For the ω-3 diet 30% of energy was provided by menhaden oil (134 g/kg) and the ω-6 to ω-3 ratio was 1:8 as previously described [11].

Allograft tumor models

All animal experiments were approved by the Animal Research Committee of the University of California, Los Angeles (UCLA, Los Angeles, CA). For the development of CRPC model, 40 male FVB mice (6–8-week-old) were acclimated for 7 days on a standard AIN-93 G diet (DYETS, Bethlehem, PA). 5 × 10^5 MycCap cells, derived from the FVB genetic background (provided by Dr. L. Wu, UCLA), were injected subcutaneously into the rear flank. MycCap cells were derived in the Sawyers laboratory at UCLA from Hi-Myc mouse prostate tumors [20, 21]. MycCap cells have an amplified androgen receptor gene and show androgen-dependent growth in soft agar [21]. For cell line authentication c-myc gene expression was quantified by RT-qPCR. MycCap cells are tested annually for mycoplasma using the mycoplasma qPCR detection kit (Sigma-Aldrich, St. Louis, MO). Castration was performed when tumors reached 300 mm^3 and the mice were assigned to the ω-3 or ω-6 diet 2 days after castration based on matching tumor volumes at the time of castration (Supplementary Fig. 1). Investigators were not blinded to the group assignment. When mouse tumors in the ω-6 group regrew to 300 mm^3, mice in the ω-6 group and time-matched ω-3 group mice were sacrificed. To examine the effect of fish oil on the progression of CRPC, 28 male FVB mice were acclimated for 7 days on a standard AIN-93G diet (DYETS, Bethlehem, PA) and subcutaneously injected with 5 × 10^5 MycCap cells. When tumors reached a volume of 300 mm^3, mice were castrated and tumors were allowed to regrow until they reached 300 mm^3 at which time mice were assigned to two groups of 14 receiving either the ω-3 or ω-6 diet (Supplementary Fig. 1). Mice were assigned to diet groups based on matching tumor volumes at the time of castration. Eleven days after the diet change mice were sacrificed in both groups. For both experiments, mice were housed individually to measure food consumption. Body weights and tumor volumes were measured twice per week. At sacrifice 100 mg of the tumor tissue was snap-frozen in liquid nitrogen, and the remaining tissue used for flow cytometry and CD206 + macrophage isolation.
mRNA isolation and quantitative PCR

Total RNA was isolated from tumors and from isolated CD206+ macrophages using RNeasy Mini kit (Qiagen, Valencia, CA) according to the manufacturer’s protocol. The reverse-transcriptional PCR and quantitative real-time PCR were performed as previously described [11]. Briefly, first-strand cDNA was synthesized using MLV-Reverse Transcriptase and random hexamers (Promega, Madison, WI, USA). Quantitative PCR was performed using a Universal SYBR Green mastermix (Applied Biosystems, Grand Island, NY, USA) on CFX96 Real-time PCR system (Bio-Rad, Hercules, CA, USA). Gene expression was calculated after normalization to GAPDH using the ΔΔCT method and expressed as relative mRNA level compared to control.

Magnetic cell sorting of M2 (CD206+) macrophage cells

CD206+ macrophages were isolated from single-cell suspensions from fresh tumor tissue using CD206-APC primary antibody and anti-APC magnetic beads with miniMACS (MiltenyiBiotec, Auburn, CA, USA) according to the manufacturer’s protocol. The CD206+ macrophage fraction contained >95% macrophages, as confirmed by flow cytometry.

Flow cytometry analysis of immune cells

To prepare single-cell suspensions for flow cytometry, fresh tumor tissue was dissected into approximately 1–3 mm³ fragments and digested with 80 U/ml collagenase (Invitrogen) in DMEM containing 10% FBS for 1 h at 37 °C while shaking. After red blood cell (RBC) lysis, single-cell suspensions were filtered and incubated for 20 min on ice with the following antibodies (1:100): CD45-PE (eBioscience, San Diego, CA), F4/80-PE-Cy7 (eBioscience, San Diego, CA), CD11b-FITC (BD Biosciences San Jose, CA), CD206-APC (Biolegend, San Diego, CA), CD68-PerCP-Cy5.5 for macrophages (Biolegend, San Diego, CA). Cells were washed with phosphate-buffered saline before analysis on the BD LSR-II flow cytometer (Beckman Coulter).

M2 macrophage culture and invasion assay

RAW264.7 cells were purchased from ATCC (Manassas, VA). RAW264.7 cells are monocyte/macrophages derived from Abelson murine leukemia virus-induced tumor. To generate M2 macrophages, RAW264.7 cells were treated with 20 ng/µl of murine IL-4 (Sigma, St. Louis, MO) for 24 h as previously described [22]. M-2 type cells were grown in RPMI medium with 10% FBS medium or with 10% charcoal stripped serum (CSS medium) (Life Technologies, Grand Island, NY). Cells were treated with 1 pmol/l to 1 µmol/l of dihydrotestosterone (DHT) (Sigma, St Louis, MO) for 48 h. At the end of incubation, mRNA expression was determined as described above. Twenty-four-well plates with matrigel-coated 8 mm pore size inserts (BD Biosciences, Bedford, MA, USA) were used for the invasion assays. 1 × 10⁵ M2 polarized RAW264.7 cells were seeded in the upper compartment in 500 µl FBS medium. 1 × 10⁵ MycCap cells were seeded in the lower compartment in 700 µl FBS medium. After cell attachment, medium was replaced with serum-free PRMI1640 in both upper and lower compartments. After 48 h incubation at 37 °C with 5% CO₂, cells on the upper insert surface of the membrane were removed with a cotton swab. The invasive cells that grew through the membrane to the lower insert surface were fixed with 4% paraformaldehyde and quantified by staining with 0.5% crystal violet in 2% methanol and photographed with the digital microscope (Nikon).

Statistical analysis

Group size was estimated based on previous allograft studies performed in our laboratory [23]. Quantitative measures were compared between the two groups (ω-3 and ω-6 diet) using two-tailed Student’s t test calculated by GraphPad Prism6.0 software (GraphPad Software, La Jolla CA). Tumor volume over time was compared between groups using Generalized Estimating Equations (GEE) models in SAS V9.4 (Cary, NC). For the CRPC development model, the GEE model was constructed using terms for diet, time (in days), the interaction term, and a repeated mouse effect. For the CRPC progression model, the GEE model was constructed similarly and the slopes (tumor volume increase per day) were compared between groups from the interaction term. The relevant differences between groups were estimated from the models and presented with 95% confidence intervals. For each mouse, tumor regression was computed by taking the difference between their starting tumor volume and their lowest tumor volume observed and compared between groups using the two-tailed Student’s t test. Time to development of CRPC was compared between the ω-3 and ω-6 groups using the log-rank test, and survival estimates (e.g. median survival by group) over time were estimated using Kaplan–Meier curves (Fig. 1c). For this analysis, CRPC was defined as either two consecutive increases in the tumor volume or an increase above the tumor volume prior to castration. Mice who did not reach CRPC were considered censored at the time of sacrifice. The data are presented as standard error of the mean (SEM) unless otherwise noted. In vitro experiments were performed in triplicate. A p value < 0.05 was considered statistically significant.
Fig. 1 Dietary ω-3 fatty acids (as compared to ω-6) delayed development of CRPC. a Tumor growth. Precastration tumor volume not shown. The numbers at each time point indicate the number of surviving mice in the two groups combined. The numbers at time points decreased as mice were sacrificed during the experiment as ω-6 group tumors reached 300 mm^3. Tumor volume over time was compared between groups (Figs. 1a and 3a) using Generalized Estimating Equations (GEE) models. b Kaplan–Meier curve showing time to development of CRPC. Data are means ± SEM (n = 20 ω-3 diet, n = 20 ω-6 diet); *p < 0.05, **p < 0.001.

Results

Effect of dietary ω-3 FAs on the development of CRPC and tumor infiltrating immune cells

We investigated the effect of dietary ω-3 FAs on the development of CRPC by initiating the ω-3 or ω-6 diets 2 days after castration. Using the GEE model incorporating all data points, tumor volumes were significantly lower in the ω-3 vs ω-6 diet group by an average of 120 mm^3 (95% CI 77.2–164.5; p < 0.001) (Fig. 1a). Following castration, MycCaP allograft tumor volumes decreased in all mice, but there was a greater reduction in tumor volumes in mice fed the ω-3 (tumor volume reduction: 182.3 ± 33.6 mm^3) as compared to the ω-6 diet (tumor volume reduction: 148.3 ± 35.2 mm^3; p = 0.003) (Fig. 1a). Mean tumor weights at sacrifice were also significantly lower in mice fed the ω-3 (0.26 ± 0.03 g) vs. the ω-6 diet (0.68 ± 0.08 g; p = 0.01) (Fig. 1b). To compare the effects of the ω-3 and ω-6 diet on time to development of CRPC, when tumors in the ω-6 group regrew to approximately 300 mm^3, mice in the ω-6 group and time-matched ω-3 group mice were sacrificed. The development of CRPC in mice fed the ω-3 diet was significantly delayed (median time to CRPC 25 days) compared to mice fed the ω-6 diet (median time to CRPC 18 days; p = 0.006) (Fig. 1c). There was no significant difference in caloric intake or mouse weights between the groups (data not shown).

There was a significant decrease in the number of F4/80^+ CD68^+-M1 polarized macrophages and CD4^+ T cells in tumors from mice fed the ω-3 compared to ω-6 diet (Fig. 2a, b). There was no significant difference in other tumor infiltrating immune cells (F4/80^+ CD11b^+ total macrophages, CD11b^+Gr1^+ myeloid-derived suppressor cells (MDSCs), F4/80^+ CD11b^+Gr1^+ neutrophils, CD8^+ T cells and B220^+ B cells) between the groups (Fig. 2b). There was a significant decrease in gene expression of colony stimulating factor 1 receptor (CSF-1R) in CD206^+ (M2) macrophages isolated from the ω-3 compared to ω-6 group tumors (Fig. 2c).

Effect of dietary ω-3 FAs on the progression of CRPC and tumor infiltrating M2 macrophages

We investigated the effect of dietary ω-3 FAs on the progression of CRPC by initiating the ω-3 or ω-6 diets, when tumors reached precastration volumes after castration. The ω-3 diet (as compared to ω-6 diet) significantly delayed the progression of established CRPC MycCap allografts with the estimated tumor volume slope significantly lower in the ω-3 group as compared to the ω-6 group (24.4 vs. 79.8 mm^3/d, respectively; 95%CI 24.6–86.1; p = 0.003) (Fig. 3a). Likewise, mean final tumor weight was significantly lower in mice fed the ω-3 (0.6 ± 0.09 g) vs. the ω-6 diet (1.5 ± 0.2 g; p = 0.001) (Fig. 3b). No significant difference in mean caloric intake or mouse body weight was observed between the groups (data not shown). Gene expression of matrix metalloproteinase-9 (MMP9) and vascular endothelial growth factor (VEGF) was significantly decreased in CD206^+ (M2) cells isolated from allografts from ω-3 fed mice compared to ω-6 fed mice (Fig. 3c).
Effect of androgen and DHA (ω-3) on M2 macrophages in vitro

Hormone deprivation by using CCS medium significantly increased the activity of M2 polarized macrophages derived from Raw264.7 cells in vitro as measured by Arg1 and CD206 gene expression (Supplementary Fig. 2), and this was partially reversed by addition of DHT. M2 macrophage gene expression of arginase 1 (Arg1), CSF-1R, MMP9, VEGF, and interleukin 10 (IL-10) was significantly reduced by DHA in both standard and charcoal stripped conditions (Fig. 4).

DHA inhibited M2 polarized macrophage migration towards prostate cancer cells

In charcoal-stripped conditions, M2 polarized macrophages (derived from Raw264.7 cells) migrated towards MycCaP cells in an established co-culture assay (Fig. 5). In charcoal-stripped conditions, DHA treatment of M2 polarized macrophages significantly inhibited migration (Fig. 5).

Discussion

Omega-3 fatty acid diets have previously been reported to delay progression of castrate-resistant prostate cancer in preclinical models [7, 9, 10]. Novel in our investigation was examining ω-3 fatty acid effects on the development of CRPC, and examining ω-3 effects on M2 macrophages in early vs. established castrate-resistant tumors. To examine the impact on CRPC development we started the ω-3 (or ω-6) diets immediately after castration and compared the regrowth of the tumors. Using a fully immunocompetent MycCaP allograft model, we found that dietary ω-3 (as compared to ω-6 diet) significantly increased tumor regression in the castrated mice and delayed the time to CRPC. The model we used mirrors the clinical scenario in which patients start androgen deprivation therapy for rising prostate-specific antigen (PSA). We believe our positive findings warrant translation to clinical trials in patients initiating androgen deprivation therapy to evaluate compliance and feasibility with a long-term randomized intervention. Given that the control group in our preclinical studies received a corn oil (ω-6) diet, and reduction of ω-6 intake was previously found to slow prostate cancer progression in preclinical models, it would also be rational, in clinical trials, to combine lowering ω-6 intake along with increasing ω-3 intake in the intervention group [24, 25].

In our present investigation, we also found that the ω-3 diet (compared to an ω-6 diet) decreased tumor volume of established CRPC-MycCaP allografts in immunocompetent mice. For this experimental design, after MycCaP allografts were established we performed orchietomy and allowed tumors to regrow prior to starting the fish oil and corn oil diets. Effective therapies now exist for nonmetastatic CRPC with a PSA doubling time of 10 months or less, and for metastatic CRPC [26]. Possibly a fish oil-based intervention would be appropriate for clinical trials in nonmetastatic
CRPC patients with longer PSA doubling times that elect not to take the second-generation androgen receptor blocking agents. For patients with more aggressive CRPC, preclinical studies evaluating synergy between dietary fish oil and established therapies are warranted prior to initiating clinical trials.

Tumor-associated macrophages, specifically M2-like macrophages, play a key role in prostate cancer progression and metastasis through a number of mechanisms including angiogenesis, immunosuppression, and migration/invasion [18, 19, 27, 28]. As previously reported, and confirmed in the present report, reducing androgen levels in tissue culture media promotes M2 activity [18, 29]. We found that M2 macrophage gene expression of CSF-1R, MMP9, VEGF, and IL-10 were significantly reduced by DHA in both standard and charcoal-stripped conditions in vitro. Likewise, DHA inhibited migration of androgen-depleted M2 macrophages in a migration assay. We found

**Fig. 3** Dietary ω-3 fatty acids (as compared to ω-6) decreased tumor growth in established CRPC and decreased expression of VEGF and MMP-9 in tumor infiltrating M2-like macrophages. 

**a** Tumor growth. Precastration tumor volume not shown (ω-3 diet, n = 14; ω-6 diet, n = 14). Tumor volume over time was compared between groups (Figs. 1a and 3a) using Generalized Estimating Equations (GEE) models.

**b** Tumor weight at sacrifice (ω-3 diet, n = 14; ω-6 diet). Gene expression in M2-like macrophages isolated from tumor tissue (ω-3 diet, n = 10; ω-6 diet). Data are means ± SEM; *p < 0.05, **p < 0.01, ***p < 0.003

**Fig. 4** DHA inhibited gene expression in M2 macrophages in FBS medium and charcoal-stripped serum (CSS) medium in vitro. Murine macrophages Raw264.7 were polarized to M2-type macrophages and cultured in androgen-deprived conditions in CSS medium or nonandrogen-deprived conditions in FBS medium and treated with 50 μmol/l of DHA. Mean ± SD, *p < 0.05, **p < 0.01
similar inhibitory effects of an ω-3 diet (compared to an ω-6 diet) on M2-like macrophages in castrate-resistant allografts. In the MycCaP model used for our present studies, M2-like macrophages make up 55% of the immune cells infiltrating the allografts [23]. For our experiment in which the ω-3 or ω-6 diet was started 2 days after castration and tumors were harvested after early tumor regrowth, M2 macrophages in the tumor were 16.6% lower in the fish oil vs. corn oil group as measured by flow cytometry. There was also a significant decrease in gene expression of CSF-1R in M2-like macrophages isolated from the allografts and a decrease in allograft CD4+ T cells. CSF-1/CSF-1R regulates the formation, differentiation, and function of M2 macrophages, which in turn supports tumor growth and metastasis [30]. M2-like macrophages isolated from established castrate-resistant MycCaP allografts had decreased expression of MMP-9 and VEGF in the ω-3 vs. ω-6 diet groups. These data all point to significant anti-M2 effects of dietary fish oil and the
potential for inhibition of prostate cancer progression in patients. Establishing a causal link between dietary fish oil, M2-like macrophages, and antiprostate cancer effects will require additional experiments utilizing novel animal models.

A number of challenges and unanswered questions remain regarding the role of dietary fish oil as a potential treatment for patients with prostate cancer. In our present series of experiments we found that an ω-3 diet (as compared to an ω-6 diet) inhibited CSF-1R expression in M2-like macrophages isolated from tumors in the early CRPC model, whereas VEGF and MMP-9 were inhibited in M2-like macrophages isolated from more advanced castrate-resistant tumors (Fig. 6). Further research is required to explain the differing effects of fish oil in the early vs. late stages of CRPC. Another complexity of applying fish oil therapies in clinical trials is the multiple antiprostate cancer mechanisms of fish oil. Berquin et al. reviewed a number of mechanisms whereby ω-3 fatty acids inhibit prostate cancer progression in preclinical studies [31, 32]. In this review a number of other potential antiprostate cancer mechanisms have been reported including effects on androgen receptor levels, AKT signaling, the local antitumor inflammatory response, and dependence on g-protein-coupled receptor 120 [9, 10, 23, 33]. Multiple anticancer mechanisms creates a high level of complexity for devising synergistic combination therapies with existing agents. However, hitting multiple targets may also be beneficial given tumor resistance mechanisms to targeted therapy. Although there are a number of trials in clinicaltrials.gov evaluating fish oil for advanced or CRPC.

In summary, an ω-3 diet (compared to an ω-6 diet) increased tumor regression in castrated mice, delayed the development and progression of CRPC, and had inhibitory effects on M2-like macrophage function. Clinical trials are warranted evaluating if a fish oil-based diet can delay the time to castration-resistant prostate cancer in men on androgen deprivation therapy, whereas further preclinical combination therapy studies are warranted evaluating fish oil for more advanced CRPC.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Augustsson K, Michaud DS, Rimm EB, Leitzmann MF, Stampfer MJ, Willett WC, et al. A prospective study of intake of fish and marine fatty acids and prostate cancer. Cancer Epidemiol Biomarkers Prev. 2003;12:64–67.
2. Brasky TM, Darke AK, Song X, Tangen CM, Goodman PJ, Thompson JM, et al. Plasma phospholipid fatty acids and prostate cancer risk in the SELECT trial. J Natl Cancer Inst. 2013;105:1132–41.
3. Brasky TM, Till C, White E, Neuhouser ML, Song X, Goodman P, et al. Serum phospholipid fatty acids and prostate cancer risk: results from the Prostate Cancer Prevention Trial. Am J Epidemiol. 2011;173:1429–39.
4. Leitzmann MF, Stampfer MJ, Michaud DS, Augustsson K, Collitz GC, Willett WC, et al. Dietary intake of n-3 and n-6 fatty acids and the risk of prostate cancer. Am J Clin Nutr. 2004;80:204–16.
5. Lovegrove C, Ahmed K, Challacombe B, Khan MS, Popert R, Dasgupta P. Systematic review of prostate cancer risk and association with consumption of fish and fish-oils: analysis of 495,321 participants. Int J Clin Pract. 2013;69:87–105.
6. Norrish AE, Skeaf CM, Arrbis GL, Sharpe SJ, Jackson RT. Prostate cancer risk and consumption of fish oils: a dietary biomarker-based case-control study. Br J Cancer. 1999;81:1238–42.
7. Friedrichs W, Ruparel SB, Marciniak RA, de Graffenried L. Omega-3 fatty acid inhibition of prostate cancer progression to hormone independence is associated with suppression of mTOR signaling and androgen receptor expression. Nutr Cancer. 2011;63:771–7.
8. Lloyd JC, Masko EM, Wu C, Keenan MM, Pilla DM, Aronson WJ, et al. Fish oil slows prostate cancer xenograft growth relative to other dietary fats and is associated with decreased mitochondrial and insulin pathway gene expression. Prostate Cancer Prostatic Dis. 2013;16:285–91.
9. Wang S, Wu J, Saburru J, Gu Z, Cai J, Axanova LS, et al. Effect of dietary polyunsaturated fatty acids on castration-resistant Pten-null prostate cancer. Carcinogenesis. 2012;33:404–12.
10. Gevariya N, Besancon M, Robitaille K, Picard V, Diabate L, Alesawi A, et al. Omega-3 fatty acids decrease prostate cancer progression associated with an anti-tumor immune response in eugonadal and castrated mice. Prostate. 2019;79:9–20.
11. Liang P, Henning SM, Schokpurp S, Wu L, Doan N, Said J, et al. Effect of dietary omega-3 fatty acids on tumor-associated macrophages and prostate cancer progression. Prostate. 2016;76:1293–302.
12. Lundholm M, Hagglof C, Wikberg ML, Stattin P, Egevad L, Bergh A, et al. Secreted factors from colorectal and prostate cancer cells skew the immune response in opposite directions. Sci Rep. 2015;5:15651.
13. Comito G, Giannoni E, Segura CP, Barcellos-de-Souza P, Raspollini MR, Baroni G, et al. Cancer-associated fibroblasts and M2-polarized macrophages synergize during prostate carcinoma progression. Oncogene. 2014;33:2423–31.
14. Lanciotti M, Masieri L, Raspolini MR, Minervini A, Mari A, Comito G, et al. The role of M1 and M2 macrophages in prostate cancer in relation to extracapsular tumor extension and biochemical recurrence after radical prostatectomy. BioMed Res Int. 2014;2014:486798.

15. Zarif JC, Taichman RS, Pienta KJ. TAM macrophages promote growth and metastasis within the cancer ecosystem. Oncoimmunology. 2014;3:e941734.

16. Roszer T. Understanding the mysterious M2 macrophage through activation markers and effector mechanisms. Mediators Inflamm. 2015;2015:816460.

17. Silva JAF, Bruni-Cardoso A, Augusto TM, Damas-Souza DM, Barbosa GO, Felisbino SL, et al. Macrophage roles in the clearance of apoptotic cells and control of inflammation in the prostate gland after castration. Prostate. 2018;78:95–103.

18. Escamilla J, Schokrpur S, Liu C, Priceman SJ, Moughon D, Jiang Z, et al. CSF1 receptor targeting in prostate cancer reverses macrophage-mediated resistance to androgen blockade therapy. Cancer Res. 2015;75:950–62.

19. Zarif JC, Yang W, Hernandez JR, Zhang H, Pienta KJ. The identification of macrophage-enriched glycoproteins using glycoproteomics. Mol Cell Proteom. 2017;16:1029–37.

20. Ellwood-Yen K, Graeber TG, Wongvipat J, Iruela-Arispe ML, Zhang J, Matusik R, et al. Myc-driven murine prostate cancer shares molecular features with human prostate tumors. Cancer Cell. 2003;4:223–38.

21. Watson CS, Bialek P, Anzo M, Khosravi J, Yee SP, Han VK. Elevated circulating insulin-like growth factor binding protein-1 is sufficient to cause fetal growth restriction. Endocrinology. 2006;147:1175–86.

22. Zhang C, Wang Y, Wang F, Wang Z, Lu Y, Xu Y, et al. Quantitative profiling of glycerophospholipids during mouse and human macrophage differentiation using targeted mass spectrometry. Sci Rep. 2017;7:412.

23. Liang P, Henning SM, Guan J, Grogan T, Elashoff D, Olefsky JM, et al. Role of host GPR120 in mediating dietary omega-3 fatty acid inhibition of prostate cancer. J Natl Cancer Inst. 2019;111:52–59.

24. Ngo TH, Barnard RJ, Anton T, Tran C, Elashoff D, Heber D, et al. Effect of isocaloric low-fat diet on prostate cancer xenograft progression to androgen independence. Cancer Res. 2004;64:1252–4.

25. Ngo TH, Barnard RJ, Cohen P, Freedland S, Tran C, deGregorio F, et al. Effect of isocaloric low-fat diet on human LAPC-4 prostate cancer xenografts in severe combined immunodeficient mice and the insulin-like growth factor axis. Clin Cancer Res. 2003;9:2734–43.

26. Mateo J, Fizazi K, Gillessen S, Heidenreich A, Perez-Lopez R, Oyen WJG, et al. Managing nonmetastatic castration-resistant prostate cancer. Eur Urol. 2019;75:285–93.

27. Ammirante M, Luo J, Grivennikov S, Nedospasov S, Karin M. B-cell-derived lymphotaxis promotes castration-resistant prostate cancer. Nature. 2010;464:302–5.

28. Lin TH, Iizumi K, Lee SO, Lin WJ, Yeh S, Chang C. Anti-androgen receptor ASC-J9 versus anti-androgens MDV3100 (Enzalutamide) or Casodex (Bicalutamide) leads to opposite effects on prostate cancer metastasis via differential modulation of macrophage infiltration and STAT3-CCL2 signaling. Cell Death Dis. 2013;4:e764.

29. Ren X, Fu X, Zhang X, Chen S, Huang S, Yao L, et al. Testosterone regulates 3T3-L1 pre-adipocyte differentiation and epididymal fat accumulation in mice through modulating macrophage polarization. Biochem Pharmacol. 2017;140:73–88.

30. Zhang P, Zhao S, Wu C, Li J, Li Z, Wen C, et al. Effects of CSF1R-targeted chimeric antigen receptor-modified NK92MI & T cells on tumor-associated macrophages. Immunotherapy. 2018;10:935–49.

31. Berquin IM, Edwards IJ, Kridel SJ, Chen YQ. Polysaturated fatty acid metabolism in prostate cancer. Cancer Metast Rev. 2011;30:295–309.

32. Aucoin M, Cooley K, Knee C, Fritz H, Balneaves LG, Breau R, et al. Fish-derived omega-3 fatty acids and prostate cancer: a systematic review. Integr Cancer Ther. 2017;16:32–62.

33. Gu Z, Wu J, Wang S, Suburu J, Chen H, Thomas MJ, et al. Polysaturated fatty acids affect the localization and signaling of PIP3/AKT in prostate cancer cells. Carcinogenesis. 2013;34:1968–75.