Exercise, Physical Activity and Breast Cancer: The Role of Tumor-Associated Macrophages

Jorming Goh[1, 2], Elizabeth A. Kirk[1], Shu Xian Lee[2], Warren C. Ladiges[2, *]

[1] Interdisciplinary Program in Nutritional Sciences, University of Washington, WA Seattle, USA.
[2] Department of Comparative Medicine, University of Washington, WA Seattle, USA.

ABSTRACT

Regular exercise and physical activity provide many health benefits and are encouraged by medical professionals for the primary prevention of, and adjuvant treatment of breast cancer. Current consensus in the discipline of exercise oncology is that both regular physical activity and exercise training exert some protective effect against breast cancer risk, and may reduce morbidity in some advanced cases. While there is growing interest in the role of exercise and physical activity in breast cancer prevention, it is currently unclear how exercise may modulate tumor behavior. The tumor microenvironment is populated by stromal cells such as fibroblasts and adipocytes, as well as macrophages. Termed tumor-associated macrophages (TAMs), these immune cells are highly plastic and respond to different signals from the cancer microenvironment, causing them to either display tumor-promoting or tumor-suppressing phenotypes. Because of such plasticity, there has been considerable interest by immunologists to develop immunotherapies based on skewing the behavior of TAMs to become cancer-suppressive. Previous studies have indirectly shown the ability of exercise training to induce an anti-tumor effect of macrophages, although the studies did not address this in the tumor microenvironment. Nevertheless, this opens up the possibility that regular exercise training may exert a protective innate immune effect against breast cancer, potentially by inducing a cancer-suppressing phenotype of TAMs. This review will describe potential mechanisms through which exercise may modulate the behavior of TAMs.

Key words: Exercise, physical activity, breast cancer, microenvironment, tumor-associated macrophages.

[*] *Address for correspondence:*
Warren C. Ladiges DVM, MS. Professor, Department of Comparative Medicine, University of Washington. Email: wladiges@uw.edu

INTRODUCTION

Breast cancer is the primary type of cancer afflicting women in the United States of America (51). The American Cancer Society estimated up to 226,000 American women to be newly diagnosed with breast cancer in 2012 (51). Importantly, this disease is the second leading cause of deaths among different cancer types in American women, with an expected 40,000 deaths in 2012 (51). Breast cancer is a disease of the mammary gland. The normal mammary gland is comprised of branching mammary milk ducts, containing ductal epithelial cells, that terminate in the lobule with luminal epithelial cells forming an inner lining in the lobular lumen (Figure 1). Surrounding these cells are the extracellular matrix and stromal cells (fibroblasts, endothelial cells, leukocytes, adipocytes) of the microenvironment. Similar to other types of cancer, the progression of breast cancer follows a sequential series of events: initiation, promotion and progression (35). Initiation occurs when DNA in mammary epithelial cells encounters some form of deleterious interaction with a carcinogen. A DNA adduct is formed and results in the erroneous insertion of the complementary nucleotide during DNA transcription. At this stage, without a supportive microenvironment, the initiated epithelial cells remain latent and will not develop into tumors. In the promotion stage, initiated epithelial cells are exposed to promoters that increase their proliferation. The proliferation of these epithelial cells is not permanent, as removal of the promoters would reverse this process. In the progression state, initiated cells become tumors when a second genetic event allows the initiated cells to become permanently altered. Some of these cells acquire a selective growth advantage and become malignant. Malignant cells proliferate uncontrollably and in advanced stages, spread to distant organs (metastasis), resulting in death. In recent years, the role of the tumor microenvironment in cancer biology has been better understood. It is apparent that tumor cells communicate with stromal cells in the microenvironment in a complicated, bi-directional crosstalk. The outcome of this crosstalk then influences the response of the tumor cells.

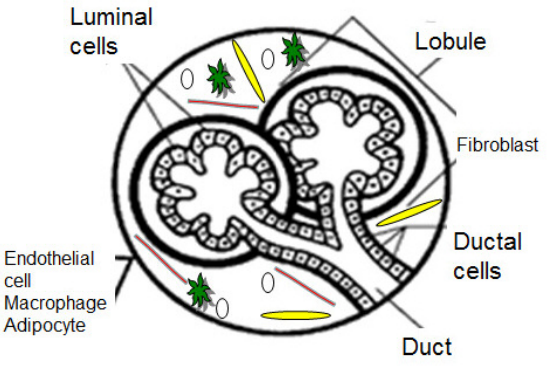

Figure 1. The normal mammary gland is comprised of a branching duct, containing ductal epithelial cells, that leads to the lobule. In the lumen of the lobule are the luminal epithelial cells. Different stromal cells reside in the mammary gland microenvironment, such as fibroblasts (yellow), macrophages (green), endothelial cells (red) and adipocytes (white). During tumorigenesis, the interaction of these stromal cells with the epithelial cells influences the progression of the disease.

The terms tumor and cancer have been used interchangeably, but it is important to differentiate the two. A tumor is an amalgamation of cell mass, and can be

benign or malignant. A benign tumor grows slowly, seldom divides and has morphological characteristics similar to the tissue it arose from (35). In contrast, cancer is a malignant tumor that has lost the regulatory control mechanisms for cell proliferation and division (35). In this review, the term tumor will be used when describing a cancer phenotype and the tumor microenvironment. This is pertinent when describing i) the primary tumor, localized to the site of origin, and ii) the secondary tumor, when the primary tumor has breached the basement membrane and seeded individual tumor cells into the blood circulation that metastasize to distant locations. Metastasis is the final stage in the development of breast cancer and ends with the death of the host.

The risk factors of breast cancer have been identified and are attributed to i) genetic heritability; carriers of mutated BRCA1 and BRCA2 gene have increased risk of breast cancer (46) and ii) environmental influences, such as diet and physical activity (53). Thus, the combination of both susceptible genes and poor lifestyle behavior can contribute to increased breast cancer risk, suggesting that lifestyle behavior is one modifiable risk factor for cancer. Physical activity is one such modifiable risk factor. It is defined as skeletal muscle contraction that results in increased energy expenditure above basal levels (4). It includes activity that is related to home maintenance (e.g. gardening), occupation (e.g. construction), commuting (e.g. biking to work) or recreation (e.g. sports, dance etc) (4). Exercise is a subset of physical activity, where it is planned, repetitive and structured, with the goal of improving or maintaining physical fitness (4). Exercise can be further categorized as “acute” or “chronic”, where the former typically refers to one bout of activity, and the latter refers to regular, periodic bouts of activity.

Throughout this text, the distinction between physical activity and exercise are made when referring to the pertinent studies. This distinction is important for several reasons. First, in epidemiological studies, physical activity is an independent variable that is observed but not manipulated, whereas exercise is an independent variable that is manipulated in randomized controlled trials or other forms of interventional studies. This suggests that the degree of experimental control is different, with the "dose" (frequency, intensity, time, type) of physical activity more variable amongst subjects in physical activity studies, compared with exercise studies. Second, outcomes from observational studies depict correlations between physical activity and disease outcome. Experimentally, it is difficult to elucidate the mechanistic effects of physical activity on cancer outcomes because physical activity usually spans a broad definition and the amount of physical activity performed is neither uniform nor controlled, leading to inter-subject variation. Finally, manipulation of the independent variable (exercise) is necessary in order to determine cause and effect.

Exercise training or physical activity could be protective against cancer by regulating the behavior of macrophages in the tumor microenvironment. Research has shown that exercise exerts modulatory effects on macrophage metabolism, phagocytosis, chemotaxis, and anti-tumor activity (66). Therefore, it is relevant to understand how their beneficial effects against breast cancer can be harnessed with exercise training or regular physical activity. A paradox in breast cancer and tumor-associated macrophages (TAMs) exists, whereby the presence of the TAMs in the breast microenvironment is usually correlated with poor prognosis. Yet, experimental models have often shown that macrophages are capable of destroy-

ing tumors (22). It may be possible that the paradox depends on the phenotype of the macrophages present, which will be the focus of this review. It is acknowledged that other cellular mechanisms such as anti-oxidative effects and metabolic alteration on tumor cells may contribute to the exercise-induced effects on carcinogenesis and metastasis, but they are beyond the scope of this review.

Physical Activity Attenuates Breast Cancer Risk and Improves Survival in Human Epidemiology Studies

In order to define the "dose" of physical activity in epidemiological studies, scientists typically report the weekly caloric expenditure of their subjects. Caloric expenditure in this case, is measured in terms of metabolic equivalents (MET)s, which is the oxygen cost of a physical activity expressed as a ratio to oxygen cost at rest. These MET values are used widely and obtained from the compendium of physical activity (1). It has been well described that regular physical activity is associated with decreased incidence of some cancers. A five-year prospective follow up of a cohort of post-menopausal women showed, after controlling for confounding factors, that women with the highest baseline levels of physical activity had a 29% lower incidence of breast cancer compared to women who were least physically active (38). The most physically active women expended 42 metabolic equivalents (MET) hours per week, whereas the most sedentary women expended between 1-7 MET hours per week (38). In a systematic literature review (15), a total of 87 cohort studies and case-control studies specific to different types of physical activity (recreational, occupational, transport, household) and breast cancer were retrieved and studied. The overall finding was a 25% risk reduction for cancer risk amongst women in the most physically active group, compared with the least physically active women. In addition, the authors reported a dose-response relationship, where participation in vigorous intensity physical activity was associated with a greater decrease in breast cancer risk, compared with moderate intensity physical activity (mean decrease of 26% versus 22%). Agreeing with these findings, another study showed that American women between the ages of 35 and 64 years, who participated in recreational physical activity throughout their lifetime, had a 35% reduced risk of developing invasive breast carcinoma, compared with women that were sedentary (39).

In July 2010, an expert panel from the American College of Sports Medicine reviewed current studies of exercise training and cancer survivorship and released a roundtable consensus statement, concluding that exercise training is "safe during and after cancer treatments and results in improvements in physical functioning, quality of life, and cancer-related fatigue" (49). The panel also stated that exercise training before and after breast cancer diagnosis is associated with a decrease in the risk of recurrence and/ or death from breast cancer. In this regard, Schmidt (48) reported that four of six cohort studies have shown a protective effect of pre-diagnosis physical activity on breast cancer survivorship, whereas two studies did not. In another prospective cohort study that recruited women diagnosed with either *in situ* or regional cancer (27), participation in physical activity after breast cancer diagnosis had a stronger protective effect compared with pre-diagnostic physical activity. In this study, compared with inactive women, physically active women who expended a minimum of 9 METs per week prior to diagnosis, had a hazard ratio for total deaths of 0.69 (95% CI, 0.45 to

1.06, P=0.045), compared with a hazard ratio for total deaths of 0.33 (95%CI, 0.15 to 0.73, P=0.046), for women that were physically active 2 years after diagnosis. These results have been corroborated by similar findings by other studies (24, 25). In the Holick study (24), women between the ages of 20 and 79 years and diagnosed with invasive breast cancer were recruited into a prospective study and followed for an average of 6 years. The authors reported that compared with women that were sedentary, women expending 21 or more MET hours per week had a lower risk of breast cancer mortality (hazard ratio, 0.51; 95% CI: 0.29-0.89; P for trend =0.05). Finally, in the Nurses Health Study (25), women aged 30-55 years and diagnosed with breast cancer (stages I-III) were enrolled in a prospective observational study. During the follow up period, it was observed that postmenopausal women that participated in moderate physical activity (greater than 9 METS hours per week) had a reduced risk of breast cancer mortality (relative risk, 0.73; 95% CI: 0.54, 0.98), compared with women that expended less than 9 METS hours per week. In addition, the hormonal levels of breast cancer also appeared to be influenced by physical activity. Moderate physical activity was shown to exert a more protective effect in women that were physically active and had estrogen receptor (ER)- positive and progesterone receptor (PR)-positive breast cancers than women with ER-negative and PR-negative breast cancers (odds ratio 0.50; 95% CI: 0.34-0.74 versus odds ratio 0.91; 95% CI: 0.43-1.96).

It is concluded that epidemiological studies generally support the use of physical activity and exercise training after diagnosis of breast cancer, suggesting that this type of life style change may slow the progression of breast cancer and perhaps also reduce the risk of recurrence and hence improve survivorship. However, unresolved questions remain regarding the effect on immunity. The only clinical studies that investigated the role of the immune system in cancer and exercise intervention in human subjects, have thus far have involved NK cells (11) and lymphocytes (26) in the blood circulation. We speculate that TAMs represent an under-studied cell population in the tumor microenvironment, particularly as it relates to exercise oncology. It is unknown whether exercise training or physical activity modulates the immune response in the tumor microenvironment, and if so, what mechanisms are involved. Elucidating these mechanisms can identify how macrophages and their secreted factors can play a role in reduced metastasis and explain the improved survivorship for physically active women with breast cancer.

Macrophages in the Tumor Microenvironment Modulate Tumor Behavior

The "seed and soil" hypothesis suggests that for tumor cells ("seeds") to propagate and advance to malignancy, the tumor microenvironment ("soil") has to be permissive and supportive of their growth (38). In other words, stromal cells secrete factors and cross-talk with tumor cells to display the phenotypic hallmarks of cancer, such as self-sufficiency in growth and increased invasiveness and metastatic potential. Macrophages in the tumor microenvironment, referred to as TAMs, are stromal cells that can influence tumor behavior. Macrophages are recruited into the tumor microenvironment where they differentiate to become TAMs. In general, the presence of TAMs is associated with poor prognosis in cancer survivors (42). This clinical outcome is likely attributed to TAMs' role in supporting tumor progression (increased tumor proliferation, vascularization, tissue

invasion and metastasis). In paraffin embedded, archived samples of human mammary carcinoma, a higher count of macrophages in random high powered fields, shown by positive cluster of differentiation (CD) immunostaining was correlated with less than 5 years of survival, compared with samples that stained for a lower count of macrophages (19).

The role of macrophages in malignancy was well characterized in a murine model of cancer, where knock out of the gene encoding the macrophage growth factor, colony-stimulating factor (CSF)-1, resulted in the growth of benign mammary cancers with a reduction in pulmonary metastasis (31). In breast cancer, CSF-1 expressed by epithelial carcinomas promotes the recruitment of macrophages to the tumor microenvironment (42). Once these macrophages arrive, they produce epithelial growth factor (EGF) that in turn, enhances the migration and invasion capabilities of mammary carcinomas in a CSF-1-dependent manner (20). Furthermore, primary tumors induce the upregulation of inflammatory chemokines, S100A8 and S100A9, which recruit macrophage antigen (MAC)-1 myeloid cells in the pre-metastatic tumor microenvironment (23). In this study, administration of S100A8 and S100A9 antibodies prevented the development of pseudopodia in the primary tumor cells, as well as the migration of primary tumor cells and MAC-1 myeloid cells to the pre-metastatic sites, suggesting that certain sub-populations of macrophages are responsible for promoting tumor metastasis.

Even though increased populations of TAMs in the tumor microenvironment have been associated with a poor clinical prognosis, it must be noted that TAMs are phenotypically diverse, reflecting their plasticity within different tissue microenvironments. Two different sub-populations of activated macrophages have been described, namely, "classically activated," or M1 macrophages, or "alternatively activated," or M2 macrophages (47). This nomenclature is a simplistic view of the complicated functions and behavior of macrophages, but is used to functionally distinguish the cytokine signals that induce their differential polarization. The main phenotypic characteristics of M1- and M2 tumor-associated macrophages are listed in Figure 2.

M1 macrophages are activated in response to bacterial lipopolysaccaride (LPS) and interferon (IFN)-γ. In turn, they secrete tumor necrosis factor (TNF)-α, interleukin (IL)-12, reactive oxygen species (ROS) and reactive nitrogen species, as evidenced by the up-regulation of inducible nitric oxide synthase (iNOS) (47). Secretory products such as TNF-α and ROS can destroy cancers (47) while iNOS has been demonstrated to enhance the anti-tumor effects of doxorubicin (8). As well, IL-12, a heterodimeric cytokine is secreted by macrophages to activate natural killer (NK) cells (21) and also activate T-helper 1 (Th1) cells to elicit anti-tumor immune responses (10). Nuclear Factor-kappa B (NF-κB) activation by the binding of the p50 and p65 subunits is also another characteristic of M1 macrophage activation (50). Although tumor cells down-regulate major histocompatibility complex (MHC)-I molecules to escape immune detection, dying primary tumor cells express extracellular damage-associated molecular patterns (DAMPs), such as high mobility group box protein (HMGB)-1 and heat shock proteins (HSPs) (16). These are detectable by macrophages *via* toll-like receptors (TLRs) (16). Purified HSP70 from mice with Dalton's Lymphoma was able to reverse the immunosuppressive macrophage phenotype induced by the tumor,

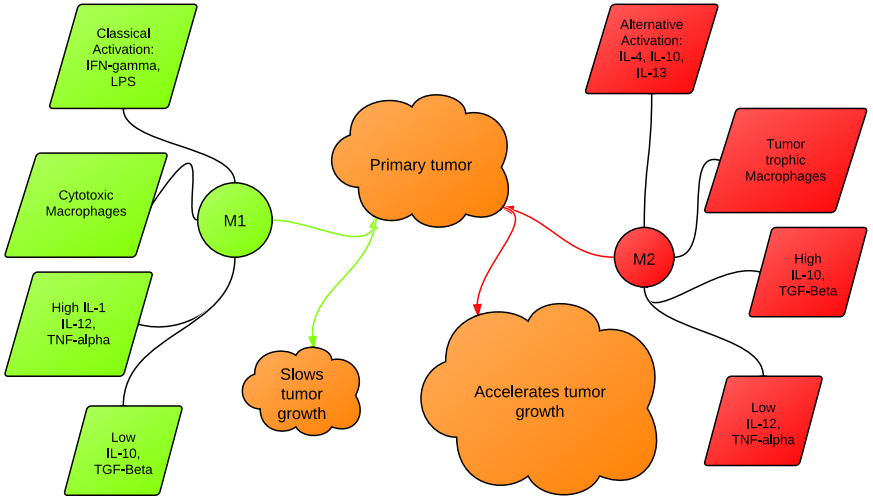

Figure 2: Characteristics of M1 and M2 macrophages. M1 macrophages produce high amounts of TNF-alpha, IL-1, IL-12 and low amounts of IL-10 and TGF-beta. Conversely M2 macrophages produce high amounts of IL-10 and TGF-beta, and low amounts of TNF-alpha, IL-1 and IL-12. M1 macrophages are cytotoxic and pro-inflammatory, whereas M2 macrophages support tumor growth and are associated with wound repair and tissue remodeling.

suggesting that HSP70 can change the polarization status of M2 macrophages to that of M1 (30).

There is practical rationale for investigating the effects of M1 macrophages in breast cancer because they play a role in tumor regression. These anti-tumor abilities of macrophages were reported in a study conducted by Hicks and colleagues (22). The authors generated a line of mice that displayed resistance against experimental tumor induction. These mice were named spontaneous regression/ complete resistance (SR/ CR) mice because they were able to either completely eradicate injected cancers, or to prevent the cancers from growing. Intriguingly, when the macrophages from these mice were injected into wild-type mice, the latter also developed resistance to the experimental cancer. This study suggests that macrophages are capable of recognizing and destroying certain cancers and hence useful for clinical immunotherapy. Although the polarization state of the macrophages was not investigated in that study, it is probable that they may share characteristics of M1 macrophages.

Unlike M1 macrophages, M2 macrophages are activated by the cytokines IL-4, IL-10, and IL-13 as well as glucocorticoids, while secreting factors and cytokines such as vascular endothelial growth factor A (VEGFA) (pro-angiogenic), IL-10 (inhibits dendritic cell maturation and promotes Th2 response) and matrix metalloproteinases (MMPs-2, -7, -9, -12) (47). Additionally, the balance of L-arginine metabolism in macrophages is also indicative of the direction of polarization to either M1 or M2 macrophages in the tumor microenvironment. M1

macrophages catalyze L-arginine to synthesize nitric oxide and L-citrulline, whereas M2 macrophages catalyze the hydrolysis of L-arginine to form L-ornithine and urea (50). A depletion of L-arginine in the tumor microenvironment can then inhibit T lymphocyte function and induce immunotolerance (47).

A certain sub-population of immature myeloid cells, termed myeloid-derived suppressor cells (MDSCs), further influences macrophage polarization in the tumor microenvironment (37). The presence of MDSCs in the tumor microenvironment has been reported in many cancers, including breast tumor and evidence suggests that MDSCs suppress immunosurveillance, and promote cancer progression and metastasis (54). MDSCs express the surface receptors CD11b and granulocyte differentiation antigen (Gr)-1 (57), originate from the bone marrow and are found in the tumor microenvironment. This is where they cross-talk with macrophages via cell-to-cell contact to induce the M2 phenotype, with an increase in IL-10 production that cause a corresponding decrease in IL-12 production by macrophages. The reduced macrophage production of IL-12 is particularly significant, since it dampens natural killer (NK) cell activity and also polarizes M1 macrophages toward the M2 phenotype (52). As well, increased MDSC production of IL-10 skews $CD4^+$ and $CD8^+$ T cells toward a cancer-promoting program and also inhibits dendritic cell (DC) maturation (52). Thus, macrophage polarization in the tumor microenvironment is influenced by complex cross-talk with MDSCs.

The macrophage phenotype is typically M2 in the tumor microenvironment. However, recent research also suggests that the phenotype of TAMs might not simply be M2, but a more progressive transition from M1 to M2, as the tumor becomes malignant and induces a different array of molecular signaling (47). Thus, it is conceivable that M1 macrophages are first polarized within an initiated tumor. With progressive growth and acquisition of malignancy, M1 macrophages might then be polarized to differentiate towards M2 macrophages, which then become pro-tumor and become the "tumor-educated" macrophages that Pollard hypothesized (43).

Physical Activity or Exercise Modulates Macrophage Anti-tumor Activity

Exercise or physical activity has a profound effect on macrophage physiology, including phagocytosis, chemotaxis, metabolism and anti-tumor activity (66). In murine models of acute exercise, peritoneal macrophage phagocytosis (12) was increased *in vitro*, relative to sedentary conditions. In young, healthy humans subjected to strenuous interval training (running and cycling), an exercise-induced decrease in monocyte chemotactic protein (MCP)-1 induced monocyte chemotaxis was observed (7). This contrasts with the increase in macrophage recruitment in the murine models described above. However, it must be noted that the murine studies utilized acute bouts of exercise, whereas the human study utilized a three-week exercise training protocol. It would be interesting to compare the effects of macrophage recruitment in murine models after exercise training, compared with acute exercise. The physiological implication of this study suggests that exercise training may be "anti-inflammatory", in that there may be a decrease in monocytes being recruited into a pre-malignant tumor microenvironment. Perhaps one anti-tumor mechanism induced by exercise could involve a reduction in macrophage presence in the tumor microenvironment. What is clearly needed is to determine whether polarized phenotypes are different with exercise training.

Woods and colleagues reported other macrophage functions that were modulated with acute exercise (61, 62, 63, 64, 65). In one of their studies, male C3H/HeN mice pre-assigned to either three days of moderate-intensity or exhaustive treadmill running were injected subcutaneously with SCA-1 adenocarcinomas. Subsequently, the mice were exercised for an additional 14 days (64). Moderate-intensity exercise resulted in greater numbers of highly phagocytic cancer-infiltrating macrophages compared with either controls or exhaustive exercise. Tumor incidence, defined as the onset of palpable cancers, was delayed in the control group on the 7th day after implant compared with either of the exercise groups. However, final tumor weights were not different between groups. This suggests that short-term exercise training in C3H/HeN mice slowed the early onset of tumor growth, but was ineffective in reducing the final tumor burden. This lack of a robust effect may be due to the "dose" of the exercise given, which was a few days of treadmill running. A long-term exercise protocol greater than two weeks may be needed to stimulate a stronger anti-tumor effect.

To address the mechanistic effects of acute exercise on macrophage activation (64) and inflammatory macrophage response (65) against cancers, male C3H/HeN mice were injected intraperitoneally with thioglycollate (64, 65) or propionibacterium acnes (64) to induce peritoneal inflammation and macrophage influx. The mice were then subjected to acute moderate-intensity treadmill running or exhaustive treadmill running for three consecutive days post-injection before they were sacrificed. The significant finding from both studies was that compared with controls, moderate-intensity and exhaustive treadmill running resulted in enhanced macrophage cytotoxicity against spinocerebellar ataxia (SCA)-1 cancer cells *in vitro*, as measured by the reduced [^{3}H] Thymidine incorporation by the cancer cells, a marker of cell proliferation. Acute exercise had neither effect on the percentages of macrophages in peritoneal cells nor the number of macrophages that adhered to culture dishes, suggesting that quantitative changes in macrophage numbers may not be responsible for the phenotypes observed with acute exercise. These two studies also suggest that peritoneal macrophage anti-cancer cytotoxicity may be modulated with acute exercise *in vitro,* but do not give any indication of TAM function nor the types of macrophages (M1 or M2) that are recruited into the cancer microenvironment. As discussed earlier, TAMs either inhibit or stimulate cancer growth and metastasis, depending on their polarized phenotype. Zielinksi and colleagues (67) reported that in female BALB/c mice that ran on treadmills for two weeks after implantation of allogeneic lymphoid cancers, macrophage infiltration into the cancers were significantly lower than control sedentary mice. Whether such an effect is seen in other cancer models, strains of mice, or the phenotype of macrophages that were reduced is unclear, but is an important issue to address.

Not all acute or short-term exercise-induced changes in macrophage functions are necessarily beneficial. Antigen presentation by macrophages may be down-regulated. To illustrate this, male BALB/c mice were injected with thioglycollate and then subjected to moderate-intensity or exhaustive treadmill running for four consecutive days (5). Upon sacrifice, peritoneal exudate cells were harvested, washed to remove non-adherent cells, and incubated with T-hybridoma cells and chicken ovalbumin. Chicken ovalbumin was used as an antigen for macrophage antigen presentation to the T-hybridoma cells, and the resultant pro-

duction of IL-2 by the hybridoma cells was a direct measure of macrophage antigen presentation. Exercised mice showed decreased IL-2 concentrations, as measured by an enzyme linked immunosorbent assay (ELISA) kit at different concentrations of ovalbumin, thus suggesting a suppression of macrophage antigen presentation, allowing the cancer to escape immune detection and cytolysis. An exercise training study that was of longer duration was also conducted. It involved young (6 months) and old (22 months) BALB/c male mice made to run on treadmills for four months (32), the investigators observed that compared with sedentary controls, exercise training increased macrophage cytolysis of P815 cancer cell lines, although the effect was stronger in the young mice. In addition, macrophage production of nitric oxide was also increased in exercised mice, with an increased gene expression of iNOS in the young exercised mice, but not old exercised mice, suggesting that the cytotoxic effects may not be mediated *via* iNOS.

The conclusion drawn from these studies is that exercise training in mice generally enhances the anti-tumor effect of macrophages *in vitro.* Discrepancies in the findings from the various studies may be due to differences in exercise duration and/or intensity, length of exercise training, diet protocol, dosage or timing of tumor cells or carcinogens injected and strain of rodents studied. In some cases, discrepant results may stem from the fact that some unidentified subsets of dendritic cells, which play a bigger role in antigen presentation than macrophages, may influence the immune response in cooperation with, or independent of macrophages after exercise.

Can Physical Activity or Exercise Training Shift Macrophage Polarization?

Exercise training in mice appears to shift macrophage polarization, at least as extrapolated indirectly from the cytokine milieu of three animal studies. In the first study (58), 10 days of treadmill running in male BALB/c mice transplanted intraperitoneally with Dalton's lymphoma resulted in reduced vascularization around the peritoneal region, compared with sedentary control mice. This observation was accompanied by the reduction of VEGF expression, decrease in the number of erythrocytes in peritoneal fluid, and increase in oxygen concentration in Dalton's lymphoma cell-free ascitic fluid. Finally, the authors reported that the peritoneal fluid from exercised mice had a higher concentration of Th1 cytokines, compared with Th2 cytokines, such that there was an increase in IFN-γ and a decrease in IL-4 and IL-10. In the second study (29), three weeks of treadmill running increased LPS-stimulated NO, IFN-γ and TNF-α production in peritoneal macrophages of male BALB/c mice, compared with control, sedentary mice. On the other hand, the production of IL-10, a cytokine that is commonly associated with M2 macrophages, was lower in trained mice versus control mice. Finally, in the third study (32), exercise training increased macrophage production of nitric oxide, concomitant with increased iNOS gene expression. This effect was however, attenuated in old mice.

The first study suggests that exercise training in cancer-bearing BALB/c male mice may shift the cytokine balance from a Th2 to a Th1 phenotype, at least in the cancer microenvironment. The second study indirectly corroborates the first, and suggests that biomarkers of M1 macrophages appeared to be increased in peritoneal macrophages of healthy, exercise-trained BALB/c male mice. Although the first study was conducted using Dalton's lymphoma, there is a pos-

sibility that exercise training or physical activity may result in a similar outcome in mammary carcinoma. The exercise-induced phenotypes in BALB/c mice from both studies suggest a shift in macrophage polarization, although whether these phenotypes extend to mice of other strains is unclear. For example, bronchoalveolar macrophages obtained from C57BL/6 mice and BALB/c mice were reported to respond differently to acute treadmill running. C57BL/6 mice are prototypical Th1 strains, whereas BALB/c mice are Th2 strains. In this study, unlike M2 bronchoalveolar macrophages from BALB/c mice, M1 bronchoalveolar macrophages from C57BL/6 mice did not increase phagocytosis of unopsonized particles after an acute bout of treadmill exercise, nor did they increase expression of macrophage receptor with collagenous structure (MARCO). The studies cited above suggest that exercise training in mouse models may shift the cytokine milieu to be representative of M1 macrophages.

Exercise-Induced Macrophage Signaling Triggers Specific Anti-Tumor Mechanisms

It is known that macrophages and MDSCs cross-talk in the cancer microenvironment. It is possible that cytokines specific to both cell types, and that are responsive to acute or chronic bouts of exercise, may represent an "immune" signature for exercise-induced immunomodulation in the cancer microenvironment. That is, the balance of these cytokines may indirectly reflect changes in the macrophage phenotype in the tumor microenvironment. For example, it was reported that acute exercise increases serum IL-12 in elite female soccer players, when blood was drawn 15-20 minutes after a soccer match (2). It appears that to elicit increases in this cytokine, the exercise must be done at an intensity that could be considered vigorous. Increases in serum IL-12 were observed 24 hours after cycle ergometry was performed at a high intensity (70% of VO_2max), but were not observed when exercise was performed at moderate intensity (55% of VO_2max) (17). Therefore, these human studies suggest that vigorous exercise may elicit an increase in serum IL-12. While the source of this cytokine is unknown, it is possible that it may be produced by macrophages. While speculative, regular exercise training may induce IL-12 production in the tumor microenvironment, which enhances the release of IL-15 in TAMs and subsequently, recruits NK and $CD8^+$ cells to aid in cancer regression (59).

Exercise also increases the release of extracellular HSP70 from liver into the circulation (45). The secretion of this molecular chaperone has immunomodulatory implication, for it is known that HSP70 binds to human monocytes and upregulates the expression of TNF-α, IL-1 and IL-6 (3). We hypothesize that exercise may: i) activate the heat shock response as a means to enhance macrophage surveillance against potential danger, which in this case are the transformed epithelial cells or ii) induce a DAMP response in stressed tumor cells, potentially by increasing HSP70, which can recruit and activate M1 macrophages for phagocytosis. The anti-cancer response involving DAMP could involve toll-like receptor (TLR) signaling. TLR-4 is a transmembrane protein expressed on monocytes, macrophages and dendritic cells, that functions as a pattern recognition receptor in response to recognition of DAMPs, such as those expressed by bacterial lipoproteins, or other "danger signals" (18). One such "danger signal" would include cancer cells. Indeed, the innate immune system is capable of recognizing

cancer cells *via* TLR activation and the subsequent production of anti-cancer molecules, such as IFN-γ (9). The activation of TLRs *via* ligand binding then results in their binding to intracellular adaptor proteins such as MyD88, and recruits other proteins involved in the inflammatory process, such as IL-1R-associated kinase (IRAK)-1, as well as inducing the production of inflammatory cytokines such as IL-1β and TNF-α, both of which are transcriptional upregulators of iNOS and also products of M1 macrophages (47). However, cancer cells can induce immune tolerance in monocytes by down-regulating the expression of IL-1β and TNF-α by activation of IRAK-M, a negative regulator of the inflammatory response (9). This activation of IRAK-M appeared to be dependent on TLR4 signaling as well, since pre-incubation of human monocytes with TLR4-specific antibodies reduced IRAK-M induction in a dose-dependent manner (9).

In addition to its role in cancer cytotoxicity, TLR4 acts as a functional receptor for serum amyloid A3 (SAA3) on lung endothelial cells and macrophages during the pre-metastatic phase, suggesting that TLR4 expression is up-regulated in TAMs such that they are then recruited to pre-metastatic sites (23). These studies illustrate a mechanistic role for TLR4 in mediating anti-cancer response in innate immune cells, as well as in the chemoattractant response for TAMs to condition the pre-metastatic site for eventual metastasis. These two roles appear to be juxtaposed to each other, such that TLR4 signaling may be detrimental in terms of priming the pre-metastatic site for eventual metastatic colonization, and yet, TLR4 signaling is involved in the activation of the M1 phenotype. To address this dichotomy, it may be required to consider whether TLR4 signaling in a pro-inflammatory cancer microenvironment is associated with a better or poorer clinical prognosis. From a clinical perspective, it was found that physically active and exercise-trained individuals have lower monocyte expression of TLR4 (18), suggesting that physical activity may exert an anti-inflammatory response *via* TLR4 downregulation in monocytes. Curiously, physically active individuals also have lower blood concentrations of inflammatory cytokines such as IL-1β and TNF-α (34). In addition, Timmerman and colleagues (55) also reported that combined resistance and endurance training resulted in a reduction in percentage of $CD14^{+}CD16^{+}$ inflammatory monocytes in circulation as well as reduced LPS-stimulated TNF-α production in whole blood cultures of elderly men and women.

These reports of exercise-induced down-regulation of TLR4 expression and inflammatory cytokine production are not incompatible with the prevailing view that exercise or physical activity improves innate immunity and reduces inflammation (40). In the context of breast cancer, it may mean that in the pre-initiation phase of carcinogenesis, macrophages present in the breast microenvironment should be of the M2 phenotype. This assertion is supported by findings that macrophages are involved in the remodeling of the mammary tissue during development, lactation and involution (6). A healthy mammary microenvironment likely has an influx of both M1 and M2 macrophages to clear the apoptotic epithelial cell and assist in the branching of the terminal milk ducts (41). The balance between M1 and M2 in the mammary microenvironment may favor the development of pre-cancer. Adipocytes in the mammary microenvironment may secrete pro-inflammatory cytokines to recruit M1 macrophages and increase the susceptibility of mammary cancer risk. To illustrate this point, a chronic inflammatory state associated with increased expression of M1 macrophages in adipose tissues

has been reported in diet-induced obesity in mice (33). Conversely, exercise training reduced gene expression of the M1 macrophage marker, CD11c, in adipose tissue as well as inhibited adipose tissue TLR4 expression in C57BL/6 mice fed a high fat diet (28). These studies were not reported in the context of cancer cytotoxicity. Since inflammation is observed in the tumor microenvironment, it may

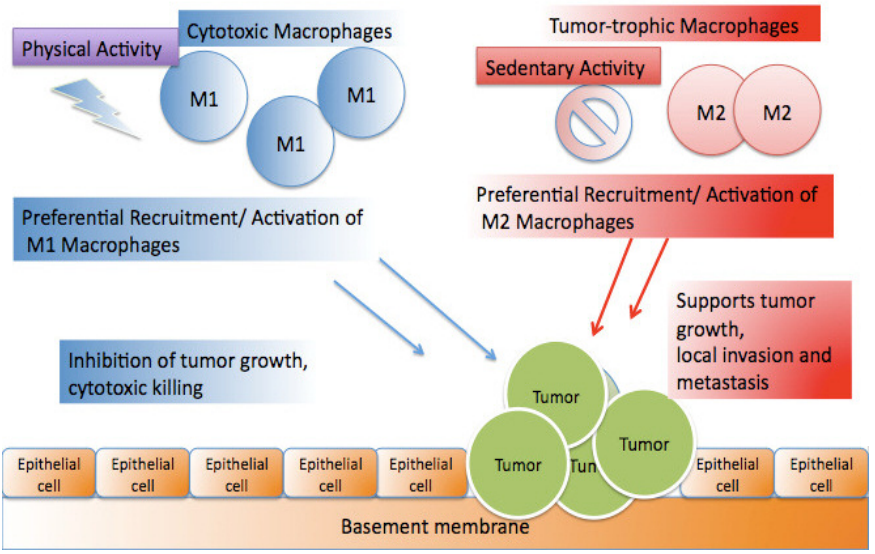

Figure 3: Proposed role of physical activity and exercise on the polarization of macrophages in the tumor microenvironment. Physical activity preferentially polarizes tumor-associated macrophages (TAMs) to an M1 phenotype with anti-tumor affects. Lack of physical activity results in the preferential polarization of TAMs to the M2 phenotype, which supports tumor growth, local invasion and metastasis.

be possible that each specific tissue microenvironment affects the plasticity of TAMs differently. Whereas an M1 cytotoxic macrophage polarization is desirable for the host in the context of cancer cytotoxicity, excessive inflammation, such as in the case of chronic inflammation, may lead to tissue destruction, DNA damage, and oxidative stress, which can paradoxically accelerate carcinogenesis and metastasis (56). Thus, the balance of M1 to M2 macrophages in a normal mammary microenvironment is tightly regulated by their interactions with the epithelial cells and other stromal cells.

Whether an exercise- or physical activity-induced polarization is seen in TAMs within the breast tumor microenvironment is unclear, but indirect evidence from the studies described earlier (29, 32,58) suggest that this may be probable,

as illustrated in Figure 3. In this scenario, physical activity would preferentially polarize tumor-associated macrophages (TAMs) to an M1 phenotype with anti-tumor affects, while lack of physical activity would result in the preferential polarization of TAMs to the M2 phenotype resulting in tumor growth, local invasion and metastasis. It is unclear whether physical activity/ exercise training reduces macrophage infiltration of the tumor microenvironment. According to Czepluch *et al.* (7), young, healthy human subjects undergoing interval training comprising bouts of running and cycling were shown to have attenuated MCP-1 induced migration of monocytes *in vitro*. When the subjects were allowed to recover for 4 weeks after the exercise training period, their serum concentrations of MCP-1 protein remained depressed. Whether this suggests a global attenuation of reduced monocyte trafficking is unclear, and the question then, is whether this outcome is desirable in terms of overall immune function. Certainly, the case for having reduction in macrophage infiltration of the tumor microenvironment is desirable, but only when these macrophage become polarized to that of the M2 phenotype. It is possible that individuals that are endurance-trained or physically active may have reduced monocyte trafficking, which may be concomittant with lower pro-inflammatory cytokines in circulation. In the event that such individuals are diagnosed with breast cancer, their long-term training status may result in a reduction of macrophage infiltration of the tumor microenvironment, and therefore, may also result in a reduction in the quantity of M2 macrophages being polarized. Alternatively, trained individuals may simply have a better ability to resolve M1-type inflammation during the different stages of mammary development, and this ability to down-regulate inflammation could be a protective factor in itself.

SUMMARY

This review has discussed the effects of physical activity and aerobic exercise on the biology of breast cancer and the possible modulatory effects on TAMs. Not much is known about other forms of physical activity and exercise training, such as the impact of occupational and household physical activity, swimming, weight lifting etc. For individuals with limited access to recreational physical activity, it may be more applicable to determine whether being physically active at work or doing household chores could provide improved immuno-modulation of TAMs.

Some crucial questions remain in order to elucidate the role of physical activity/or exercise on TAMs: i) does exercise training and/ or physical activity reduce the number of monocytes recruited to the cancer microenvironment, ii) does exercise training and/ or physical activity alter the phenotype of macrophages within the cancer microenvironment, but not the trafficking/ recruitment of monocytes to the specific cancer microenvironment? iii) What is the optimal "dose" of physical activity or exercise training in eliciting a beneficial macrophage polarization response? iv) Are there differences in macrophage polarization in the pre-cancer and cancer microenvironment? Addressing these questions would allow investigators to enhance the knowledge of clinically relevant markers of prognosis, and determine whether physical activity and exercise training can be used routinely as primary or adjunctive prevention methods to modulate these markers.

LITERATURE CITED

1. Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett Jr DR, Tudor-Locke C et al. Compendium of physical activities: a second update of codes and MET values. Med Sci Sports Exerc 43: 1575-1581, 2011.
2. Andersson H, Bohn SK, Raastad T, Paulsen G, Blomhoff R and Kadi F. Differences in the inflammatory plasma cytokine response following two elite female soccer games separated by a 72-h recovery. Scan J Med Sci Sports 20: 740-747, 2010.
3. Asea A, Kraeft SK, Kurt-Jones EA, Stevensonn MA, Chen LB, Finberg RW, Koo GC, Calderwood SK. HSP70 stimulates cytokine production through a CD14-dependent pathway, demonstrating its dual role as a chaperone and cytokine. Nature Med 6;435-442, 2000.
4. Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise and physical fitness: definitions and distinctions for health related research. Pub Health Rep 100: 126-131,1985.
5. Ceddia MA and Woods JA. Exercise suppresses macrophage antigen presentation. J Appl Physiol 87:2253-2258, 1999.
6. Chua ACL, Hodson LJ, Moldenhaver LM, Roberson SA, Ingman WV. Dual roles for macrophages in ovarian cycle-associated development and remodeling of the mammary gland epithelium. Development 137: 4229-4238, 2010.
7. Czepluch FS, Barres R, Caidahl K, Olieslagers S, Krook A, Rickenlund A, Zierath JR and Waltenberger J. Strenuous physical exercise adversely affects monocyte chemotaxis. Thrombosis Haemostasis 105: 638-647, 2011.
8. De Boo S, Kopecka J, Brusa D, Gazzano E, Matera L, Ghigo D, Bosia A and Riganti C. iNOS activity is necessary for the cytotoxic and immunogenic effects of doxorubicin in human colon cancer cells. Mol Cancer 108. DOI:10.1186/1476-4598-8-108, 2009.
9. Del Fresno C, Otero K, Gomez-Garcia L, Gonzalez-Leon MC, Soler-Ranger L, Fuentes-Prior P, Escoll P, Baos R, Caveda L, Garcia F, Arnalich F and Lopez-Collazo E. Tumor cells deactivate human monocytes by upregulating IL-1 receptor associated kinase-M expression *via* CD44 and TLR4. J Immunol 174: 3032-3040, 2005.
10. Emtage PC, Clarke D, Gonzalo-Daganzo R and Junghans RP. Generating potent Th1/Tc1 T cell adoptive immunotherapy doses using human IL-12: harnessing the ummunomodulatory potential of IL-12 without the in vivo associated toxicity. Immunother 26: 97-106, 2003.
11. Fairey AS, Courneya KS, Field CJ, Bell GJ, Jones LW, Mackey JR. Randomized controlled trial of exercise and blood immune function in postmenopausal breast cancer survivors. J Appl Physiol 98: 1534-1540, 2005.
12. Fehr HG, Lotzerich H and Michna H. The influence of physical exercise on peritoneal macrophage functions: histochemical and phagocytic studies. Int J Sports Med 9: 77-81, 1988.
13. Forner MA, Collazos ME, Barriga C, De La Fuente M, Rodriguez AB and Ortega E. Effect of age on adherence and chemotaxis capacities of peritoneal macrophages. Influence of physical activity stress. Mech Ageing Dev 75: 179-189,1994.
14. Forner MA, Barriga C and Ortega E. Exercise-induced stimulation of murine macrophage phagocytosis may be mediated by thyroxine. J Appl Physiol 80: 899-903, 1996.

15. Friedenreich CM and Cust AE. Physical activity and breast cancer risk: impact of timing, type and dose of activity and population subgroup effects. Br J Sports Med 42: 636-647, 2008.
16. Garg AD, Nowis D, Golab, Vandenabeele P, Krysko DV, Agostinis P. Immunogenic cell death, DAMPs and anticancer therapeutics: An emerging amalgamation. Biochim Biophys Acta 1805: 53-71, 2010.
17. Giraldo E, Garcia JJ, Hinchado MD and Ortega E. Exercise intensity-dependent changes in the inflammatory response in sedentary women: role of neuroendocrine parameters in the neutrophil phagocytic process and the pro-/anti-inflammatory cytokine balance. Neuroimmunomodulation 16: 237-244, 2009.
18. Gleeson M, McFarlin B and Flynn M. Exercise and Toll-like receptors. Exerc Immunol Rev 12: 34-53, 2006.
19. Goede V, Brogelli L, Ziche M and Augustin HG. Induction of inflammatory angiogenesis by monocyte chemoattractant protein-1. Int J Cancer 82: 765-70, 1999.
20. Goswani S, Sahai E, Wyckoff JB, Cammer M, Cox D, Pixley FJ, Stanley ER, Segall JE and Condeelis JS. Macrophages promote the invasion of breast carcinoma cells via a colony-stimulating factor-1/ Epidermal growth factor paracrine loop. Cancer Res 65: 5278-5283, 2005.
21. Hagemann T, Lawrence T, McNeish I, Charles KA, Kulbe H, Thompson RG, Robinson SC and Balkwill FR. "Re-educating" tumor-associated macrophages by targeting NF-κB. J Exp Med 205: 1261-1268, 2008.
22. Hicks AM, Riedlinger G, Willingham MC, Alexander-Miller MA, Von Kap-Herr C, Pettenati MJ et al. Transferable anticancer innate immunity in spontaneous regression/ complete resistance mice. Proc Natl Acad Sci USA 103: 7753-7758, 2006.
23. Hiratsuka S, Watanabe A, Aburatani H and Maru Y. Tumor-mediated upregulation of chemoattractants and recruitment of myeloid cells predetermines lung metastasis. Nature Cell Biol 8: 1369-1375, 2006.
24. Holick CN, Newcomb PA, Trentham-Dietz, Titus-Ernstoff L, Bersch AJ, Stampfer MJ, Baron JA, Egan KM and Willett WC. Physical activity and survival after diagnosis of invasive breast cancer. Cancer Epidemiol Biomark Prev 17: 379-386, 2008.
25. Holmes M, Chen WDF, Kroenke C and Colditz G. Physical activity and survival after breast cancer diagnosis. JAMA 293: 2479-2486, 2005.
26. Hutnick NA, Williams NI, Kraemer WJ, Orsega-Smith E, Dixon RH, Bleznak AD, Mastro AM. Exercise and lymphocyte activation following chemotherapy for breast cancer. Med Sci Sports Exerc 37: 1827-1835, 2005.
27. Irwin ML, Smith AW, McTiernan A, Ballard-Bardash R, Cronin K, Gilliland FD, Baumgartner RN, Baumgartner KB and Bernstein L. Influence of pre- and postdiagnosis physical activity on mortality in breast cancer survivors: the health, eating activity, and lifestyle study. J Clin Oncol 26: 3958-3964, 2008.
28. Kawanishi N, Yano H, Yokogawa Y and Suzuki K. Exercise training inhibits inflammation in adipose tissue *via* both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet-induced obese mice. Exerc Immunol Rev 16: 105-118, 2010.
29. Kizaki T, Takemasa T, Sakurai T, Izawa T, Hanawa T, Kamiya S, Haga S, Imaisumi K and Ohno H. Adaptation of macrophages to exercise training improves innate immunity. Biochem Biophys Res Commun 372: 152-156, 2008.

30. Kumar S, Deepak P, Acharya A. Autologous Hsp70 immunization induces anti-tumor immunity and increases longevity and survival of tumor-bearing mice. Neoplasma 56: 259-268, 2009.
31. Lin EY, Nguyen AV, Russell RG and Pollard JW. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. J Exp Med 193; 727-739, 2001.
32. Lu Q, Ceddia MA, Price EA, YE SM and Woods JA. Chronic exercise increases macrophage-mediated tumor cytolysis in young and old mice. Am J Physiol Regul Integr Comp Physiol 276: R482-R489, 1999.
33. Lumeng CN, Bodzin JL and Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 2007;117: 175-184.
34. McFarlin BK, Flynn MG, Campbell WW, Craig BA, Robinson JP, Stewart LK, Timmerman KL and Coen PM. Physical activity status, but not age, influences inflammatory biomarkers and toll-like receptor 4. J Gerontol A Biol Sci Med 61: 388-393, 2006.
35. McKinnell RG, Parchment RE, Perantoni AO, Damjanov I, Pierce GB. The biological basis of cancer, 2nd edition. Cambridge University Press, 2006.
36. Mendoza M and Khanna C. Revisiting the seed and soil in cancer metastasis. Int J Biochem Cell Biol 41: 1452-1462, 2009.
37. Ostrand-Rosenberg S. Myeloid-derived suppressor cells: more mechanisms for inhibiting antitumor immunity. Cancer Immunol Immunother 59: 1593-1600, 2010.
38. Patel AV, Callel EE, Bernstein L, Wu AH and Thun MJ. Recreational physical activity and risk of post-menopausal breast cancer in a larger cohort of US women. Cancer Causes Control 14: 519-529, 2003.
39. Patel AV, Press MF, Meeske K, Callel EE and Bernstein L. Lifetime recreational exercise activity and risk of breast carcinoma in situ. Cancer 98: 2161-2169, 2003.
40. Petersen AMW and Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol 98: 1154-1162, 2005.
41. Pollard JW. Trophic macrophages in development and disease. Nat Rev Immunol 9: 259-270, 2009.
42. Pollard JW. Macrophages define the invasive microenvironment in breast cancer. J Leukoc Biol 84: 623-630, 2008.
43. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer 4: 71-78, 2004.
44. Polyak K, Kalluri R. The role of the mammary microenvironment in mammary gland development and cancer. Cold Spring Harb Perspect Biol doi:10.1101/cshperspect.a003244, 2010.
45. Radons J, Multhoff G. Immunostimulatory functions of membrane-bound and exported heat shock protein 70. Exerc Immunol Rev 11: 17-33, 2005.
46. Ripperger T, Gadzicki D, Meindl A, Schlegelberger B. Breast cancer susceptibility: current knowledge and implications for genetic counseling. Eur J Hum Genet 17: 722-731, 2009.
47. Schmid MC and Varner JA. Myeloid cells in the tumor microenvironment: Modulation of tumor angiogenesis and tumor inflammation. J Oncol doi: 10.1155/2010/201026, 2010.
48. Schmidt KH. Exercise for secondary prevention of breast cancer: moving from evidence to changing clinical practice. Cancer Prev Res (Phila) 4: 476-480, 2011.

49. Schmidt KH, Courneya KS, Matthews C, Demark-Wahnefried W, Galvao DA, Pinto BM, Irwin ML, Wolin KY, Segal RJ, Lucia A, Schneider CM, Von Gruenigen VE and Schwartz AL. American College of Sports Medicine roundtable on exercise guidelines for cancer survivors. Med Sci Sports Exerc 42: 1409-1426, 2010.
50. Sica A and Bronte V. Altered macrophage differentiation and immune dysfunction in tumor development. J Clin Invest 117: 1155-1166, 2007.
51. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 62: 10-29, 2012.
52. Sinha P, Clements VK, Bunt SK, Albelda SM and Ostrand-Rosenberg S. Crosstalk between myeloid derived suppressor cells subverts tumor immunity toward a type 2 response. J Immunol 179: 977-983, 2007.
53. Song M, Lee KM, Kang D. Breast cancer prevention based on gene-environment interaction. Mol Carcinog 50: 280-290, 2011.
54. Steding CE, Wu ST, Zhang Y, Jeng MH, Elzey BD and Kao C. The role of interleukin-12 on modulating myeloid derived suppressor cells, increasing overall survival and reducing metastasis. Immunol 133: 221-238, 2011.
55. Timmerman KL, Flynn MG, Coen PM, Markofski MM and Pence BD. Exercise training-induced lowering of inflammatory (CD14+ CD16+) monocytes: a role in the anti-inflammatory influence of exercise? J Leukoc Biol 84: 1271-1278, 2008.
56. Van Ginderachter JA, Movahedi K, Hassanzadeh Ghassabeh G, Meerschaut S, Beschin A, Raes G and De Baetselier P. Classical and alternative activation of mononuclear phagocytes: picking the best of both worlds for tumor promotion. Immunobiol 211: 487-501, 2006.
57. Vasievich EA and Huang L. The suppressive tumor microenvironment: a challenge in cancer immunotherapy. Mol Pharmceutics dx.doi.org/10.1021/mp1004228, 2011.
58. Verma VK, Singh V, Singh MP and Singh SM. Effect of physical exercise on tumor growth regulating factors of tumor microenvironment: Implications in exercise-dependent tumor growth retardation. Immunopharmacol Immunotoxicol 31: 274-282, 2009.
59. Watkins SK, Li B, Richardson KS, Head K, Eligmez NK, Zeng Q, Suttles J and Stout RD. Rapid release of cytoplasmic IL-15 from tumor associated macrophages is an initial and critical IL-12-initiated tumor regression. Eur J Immunol 39: 2126-2135, 2009.
60. Westerlind KC, McCarty HL, Schultheiss PC, Story R, Reed AH, Baier ML and Strange R. Moderate exercise training slows mammary tumor growth in adolescent rats. Eur J Cancer Prev 12: 281-287, 2003.
61. Woods JA. Exercise and resistance to neoplasia. Can J Physiol Pharmacol 76: 581-588, 1998.
62. Woods JA and Davis JM. Exercise, monocyte/macrophage function, and cancer. Med Sci Sports Exerc 26: 147-157, 1994
63. Woods JA, Davis JM, Kohut ML, Ghaffar A, Mayer EP and Pate RR. Effects of exercise on the immune response to cancer. Med Sci Sports Exerc 26:1109-1115, 1994.
64. Woods JA, Davis JM, Mayer EP, Ghaffar A and Pate RR. Effects of exercise on macrophage activation for antitumor cytotoxicity. J Appl Physiol 76:2177-2185, 1994.
65. Woods JA, Davis JM, Mayer EP, Ghaffar A and Pate RR. Exercise increases inflammatory macrophage antitumor cytotoxicity. J Appl Physiol 75: 879-886, 1993.

66. Woods JA, Lu Q, Ceddia MA and Lowder T. Exercise-induced modulation of macrophage function. Immunol Cell Biol 78: 543-553, 2000.
67. Zielinski MR, Muenchow M, Wallig MA, Horn PL and Woods JA. Exercise delays allogeneic tumor growth and reduces intratumoral inflammation and vascularization. J Appl Physiol 96: 2249-2256, 2004.