Original Article

J Babol Univ Med Sci
22; 2020. P: 24-30

Effect of Aerobic Interval Training on Expression of Twist and Vimentin and the Rate of Tumor Volume in Mice with Breast Cancer

S. Gholamian (PhD)[1], S.R. Attarzadeh hosseini (PhD)*[1], A. Rashidlamir(PhD)[1], H. Aghaalinejad (PhD)[2]

1.Department of Exercise Physiology, Faculty of Sports Sciences, Ferdowsi University of Mashhad, Mashhad, I.R.Iran

2.Department of Sports Sciences, Faculty of Humanities, Tarbiat Modares University, Tehran, I.R.Iran

J Babol Univ Med Sci; 22; 2020; PP: 24-30

Received: Jan 20th 2019, Revised: May 8th 2019, Accepted: May 22nd 2019.

ABSTRACT

BACKGROUND AND OBJECTIVE: Many deaths from cancer are due to metastases, a process which involves the epithelial-mesenchymal transition (EMT). On the other hand, regular exercise plays an important role in inhibiting the progression of breast cancer. Therefore, the purpose of this study was to investigate the influence aerobic interval training on expression of mesenchymal biomarkers, and tumor volume in mice with breast cancer.

METHODS: In this experimental study, Thirty-two female BALB/c mice, aged 3-5 weeks (17±1g) were used. The mice were allocated to four groups: Exercise Tumor, Exercise (aerobic Interval training was performed six weeks (40 minutes) before and four weeks (30 minutes) after the induction of carcinoma with active recovery), Rest Tumor, Rest (Control-without exercise), Rest, Tumor, Exercise, (four weeks after the induction of carcinoma) and Exercise, Tumor, Rest (six weeks before the induction of carcinoma). The real-time PCR method was used to evaluate the expression of Vimentin and Twist.

FINDINGS: The results of present study demonstrated that tumor tissue Vimentin expression in the Exercise Tumor Exercise (223.0±0.073) group decreased significantly (p= 0.0001), Also, the expression of Twist gene was significantly reduced in Exercise Tumor Exercise group (0.24±0.227) compared to control group (p=0.008). A significant decrease in tumor volume was observed in both RTE and ETE groups compared to the control group (RTR) (p = 0.0001).

CONCLUSION: Based on the results of this study, a period of interval aerobic training can decrease the expression of Vimentin، Twist and decrease the tumor volume ratio.

KEY WORDS: *Breast cancer, Mesenchymal biomarkers, Interval training, Tumor.*

Please cite this article as follows:

Gholamian S, Attarzadeh hosseini S.R, Rashidlamir A, Aghaalinejad H. Effect of Aerobic Interval Training on Expression of Twist and Vimentin and the Rate of Tumor Volume in Mice with Breast Cancer. J Babol Univ Med Sci. 2020; 22: 24-30.

***Corresonding Author: S.R. Attarzadeh hosseini (PhD)**
Address: Faculty of Sports Sciences, Ferdowsi University of Mashhad, Mashhad, I.R.Iran
Tel: +98 51 38805412
E-mail: Attarzadeh@um.ac.ir

[DOR: 20.1001.1.15614107.1399.22.1.4.6]
[DOI: 10.22088/jbums.22.1.24]

Introduction

Breast cancer is the most common type of cancer in women and metastasis is the leading cause of death from this cancer (1,2). Metastasis is one event in which the success of this process requires that cancer cells be able to separate from adjacent cells. Eventually they enter the mesenchymal tissues where they form a secondary tumor. One of the crucial steps in the metastatic cascade is the process of transition from epithelial to mesenchymal state, or Epithelial to Mesenchymal Transition (EMT). Studies have shown that EMT is associated with metastasis and cancer progression (3,4).

EMT is a process in which cells are transformed from epithelial to mesenchymal phenotypes with greater invasion and motility. Epithelial cells are organized into a single unit and by the loss of cellular binding proteins, the cytoskeletal elements become mesenchymal cells by gaining movement (5). EMT stages are regulated by a number of transcription factors, including Vimentin and Twist (6). Vimentin protein is located on chromosome 13p10 and weighs 57 kDa (7).Vimentin is one of the major proteins on the membrane that maintains cell structure, shape and resistance to stress (8).

Twist belongs to the protein family (helix -loop-helixld). As a transcription factor 21 kDa, it appears in human solid tumors, including a variety of cancers (9). Also, overexpression of Twist and Vimentin by inhibiting E-cadherin and inducing EMT enhances the ability of invasion and metastasis of cancer cells (9). The results of many studies confirm the importance of Vimentin and Twist in different cancer types, especially the EMT process, as metastatic biomarkers (10). On this basis, they have recently been considered as a target for cancer treatment (7).

Moderate-intensity physical exercise, by enhancing the immune system and releasing myokines, play an important role in preventing inflammatory diseases such as breast cancer. Exercise reduces body fat percentage, reduces obesity, and reduces systemic inflammation. Each of these factors is involved in the pathogenesis of cancer; according to research reports, physical activity can prevent 50% of cancers (11). Despite studies of the effect of exercise training on the EMT process, very little research has been done on the role of exercise training in the expression of mesenchymal biomarkers in cancer. In this way, Zhang et al observed a decrease in the expression of mesenchymal marker genes in mice with liver cancer after 9 weeks of moderate-intensity swimming training (11). They emphasized the nervous system that moderate-intensity swimming exercise suppresses the EMT process and, consequently, suppresses tumor growth (12,13). Intermittent exercise is effective in increasing aerobic capacity, endurance, weight loss and cardiac metabolic function for people with breast cancer (14), but the effect of this type of aerobic exercise as an exercise in cellular and molecular mechanisms and their effects on effective signal pathways in tumor growth and low metastasis have been investigated. Since exercise training can be an appropriate method for cancer prevention and an important part of the treatment process, therefore, the purpose of the present study was to investigate the effect of a period of aerobic exercise training on mRNA expression of mesenchymal markers and tumor volume changes in tumor tissue of mice with breast cancer.

Methods

This experimental study, in vitro, was approved by the Ethics Committee of Ferdowsi University (IR.MUM.FUM.REC.1397.038) was done on 32 Balb/c mice (3 to 5 weeks, 17 1 1 g average weight) that were purchased from Pasteur Institute. The mice were kept in the laboratory under conditions of light control (12 h light and 12 h dark) temperature (22±3°C) and humidity (about 45%). Animal feed consisted of mice' usual water and food and free access to water and food. Because mice were homogenous in terms of diet, weight, age, etc., they were randomly divided into four groups: Exercise-Tumor-Exercise (ETE), Rest-Tumor-Rest (RTR), and Rest-Tumor-Exercise (RTE) and Exercise-Tumor-Rest (ETR) groups. The RTR team continued their normal life in the cage. The ETR group performed the exercise protocol 5 days a week for 6 weeks before tumor formation, the RTE group performed the exercise protocol 5 days a week for 4 weeks after tumor formation, and the ETE group performed the exercise protocol 5 days a week for 6 weeks before tumor formation and 4 weeks after tumor formation (Table 1).

The training protocol is based on the protocol of Ranjbar et al. (15). Cell culture: The tumor was generated from 4T-1 cell line by subcutaneous injection of the cell. Each mouse was injected about one million cells subcutaneously and centrally to the upper thigh. Tumor volume was measured in two longitudinal and transverse axes. The largest dimension of the tumor was considered as the length (L) of the tumor and the other dimension (at 90°) as the width (W) of the tumor, using

[DOR: 20.1001.1.15614107.1399.22.1.4.6]
[DOI: 10.22088/jbums.22.1.24]

the tumor volume formula of Jones et al. (16) as V= ½ (L2×W). To determine the ratio of tumor volume changes in each group, the tumor volume of the fourth week was divided into the tumor volume of the first week. 24 hours after the last training session, mice with tumor of all groups were anesthetized by intra-peritoneal injection of the combination of ketamine (90 mg/kg) and xylazine (10 mg/kg) and surgically, the tumor tissue was removed. In the laboratory, 50-100 mg of tumor tissue along with 1 cc of trisol was poured into a homogeneous tube and homogenized.

The supernatant was then poured into a new tube to extract RNA. RNA extraction was performed using Qiazol solution according to the manufacturer's instructions. PrimerScript RT Regent Kit was used to transcribe RNA to cDNA.The obtained cDNA was stored at -20°C. Real time PCR was performed using Stepone plus, according to SYBER-Green and Realtime according to the kit instructions. The ACTB gene was used as a reference gene to formalize the gene expression results. Formula 2-ΔΔ ct was used to quantify gene expression. Statistical methods: Kolmogorov-Smirnov test was used to check the natural distribution of variables. One-way ANOVA and Tukey's post hoc tests were also used for data analysis. All statistical operations were performed using SPSS software (version 22) and P <0.05 was considered as significant.

Results

The results showed a significant difference in Vimentin gene expression in the RTR and ETE groups, with a significant decrease in the ETE group (0.222±0.073) compared to the RTR group (F= 270.85, p<0.0001). But there was no significant difference between RTE group (0.825±0.34) (p= 0.156) and ETR group (0.912±0.5) (p= 0.810). The results showed a significant difference in the expression of Twist gene in RTR and ETE groups, with a significant decrease in ETE group (0.27±0.24) compared to RTR group (F= 14.35, p= 0.008) But there was no significant difference between RTE group with mean of 1.06±0.9 (p= 0.156) and ETR group with mean of 1.59±0.981 (p= 0.810).

The results show that there was a significant difference between two groups that did the aerobic exercise after the cancer and the two groups that did the rest. In fact, there was a significant difference in tumor volume ratio between RTR group with ETE group (p= 0.0001) and RTE group (p= 0.0001) and ETR group with ETE (p= 0.042) and RTE groups (p= 0.0001). As in the ETE group, tumor volume in the fourth week had a 4.79-fold growth compared to the first week. This ratio was 5.51-fold higher in the RTE group, 7.5-fold higher in the ETR group, and in the RTR-control group, tumor volume growth was 8.70-fold higher than in the first week (Figure 1).

Table 1. Practice Protocol

Training period				Steps
	Third two weeks Speed 25-30 20 intervals of 2-minute	Second two weeks Speed: 20-25 20 intervals of 2-minute	The first two weeks Speed: 15-20 20 intervals of 2-minute	Before tumor induction
Forth week Speed: 10-15 15 intervals of 2-minute	Third week Speed: 15-20 15 intervals of 2-minute	second week Speed: 20-25 15 intervals of 2-minute	The first week Speed: 25-30 15intervals of 2-minute	After tumor induction

Table 2. Sequences of primers

Genes	Forward sequence	Reverse sequence
Vimentin	ACATCATACGGCTGCGAGAG	GACTTGCTGTTCCTGAATCTGG
Twist	AGCAAAGCCTTCTCCGTCTG	CCTCCTCTGGAAACAATGACATC
ACTB	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT

[DOR: 20.1001.1.15614107.1399.22.1.4.6]
[DOI: 10.22088/jbums.22.1.24]

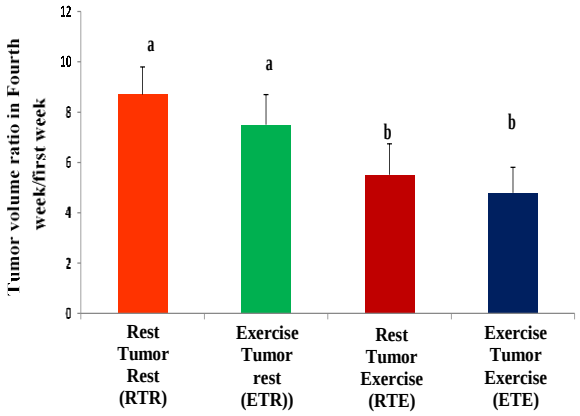

Figure 1. Fourth to first week tumor volume ratio. Data are presented as mean±SD. The dissimilar letters indicate a significant difference. P≥0.05 is considered significant.

Discussion

In the present study, expression of Twist and Vimentin gene demonstrated a significant decrease after 10 weeks of periodic training in tumor training group that performed periodic training for 6 weeks before tumor induction and 4 weeks after tumor induction, compared to resting tumor group with no exercise. The results also showed that tumor growth in the two training groups (RTE and ETE) was significantly lower than the ETR and RTR control groups. Concerning the effects of exercise on the expression of mesenchymal markers in tumor tissue, there are few conclusions, and in agreement with the findings of the present study, in the study of Zhang et al., examined the effect of moderate-intensity swimming exercise on mesenchymal markers expression for 9 weeks, the results showed a decrease in Twist and Vimentin gene expression and tumor volume in mice with liver cancer (12).

The contribution of specific cytokines as inflammatory mediators of EMT has been widely reported in studies, most notably TGF-β, which interacts with several pro-inflammatory cytokines during the progression of the EMT process (17). There is clear evidence that TGF-β gene expression enhances Twist and Vimentin expression. On the other hand, TGF-β1 / Smad3 signaling is a common pathway for EMT induction. Phosphorylated Smad3 upregulates the expression of Twist, Vimentin and N-cadherin and negatively regulates E-cadherin (17,18). TGF-β is increased by signal phosphorylation (ERK), which has been seen in the invasion of many tumors (19,20).

Zhang et al., emphasizing on the nervous system stated the mechanism of the effect of exercise on the process of epithelial to mesenchymal transition (EMT), so that moderate-intensity swimming exercise increases dopamine (DA) followed by dopamine 2 receptor (DR2) (Which has anti-tumor activity) is activated, and since ERK signaling pathway increases invasion of various tumors, it increases TGF-β expression (19,20), with activation of DR2 signaling through exercise, it reduces cAMP and inhibits ERK1 / ERK2 activity, resulting in negative regulation of TGF-β, Smad3 in animal models. Thus, inhibition of Smad3 nuclear translocation can decrease EMT-related TGF-β (21), which results in reduced Vimentin and Twist gene expression in tumor tissue, thereby inhibiting EMT, tumor growth, and metastases in cancer (22).

The results also showed a decrease in both Vimentin and Twist mesenchymal markers in the two groups (RTE and ETE) that performed aerobic exercise. Moreover, it has been well established that IL-6 promotes osteosarcoma proliferation, metastasis and angiogenesis and is capable of inducing EMT in a variety of cancer cells through JAK signaling. Downstream IL-6 signals include STAT3, Akt, and ERK1 / 2 MAPK. Among them, STAT3 has been shown to play an important role in IL-6 EMT modulation (23).

In a study of patients with estrogen receptor-positive breast cancer, IL-6 disrupted E-cadherin expression and increased Vimentin and Twist (24). On the other hand, many studies have shown that some exercise activities decrease IL-6 pro-inflammatory cytokines. It seems that exercise through a number of factors, such as its effect on the JAK / STAT3 pathway, can decrease tumor IL-6 and STAT3 expression, thereby reducing cell proliferation, cell deformation, metastasis and volume. On the other hand, the tumor factor as an extracellular stress enhances the expression of these proteins, but aerobic exercise due to its different nature, despite constant exercise, prevents the tumor from continually and doubly increasing heat and blood flow. This type of activity, unlike continuous activity, causes less stress in the tumor area, which may be one of the reasons for the decrease in protein and tumor volume.

The results also showed that tumor growth in the two training groups (RTE and ETE) was significantly lower than the ETR and RTR control groups, so that in the ETE group, tumor volume in the fourth week, growth was 79 / 4 was equal to the first week. This ratio was 5.51-fold higher in the RTE group, 7.5-fold in the ETR

[DOR: 20.1001.1.15614107.1399.22.1.4.6]
[DOI: 10.22088/jbums.22.1.24]

group, and 8.70-fold higher in the RTR control group than in the first week. Therefore, the tumor growth rate in the group that had never exercised was higher than other groups. The results also showed that in the two groups ETR and ETE that performed 6 weeks of periodic exercise before tumor induction compared to the RTR group that did not have any periodic training, the tumor volume ratio was lower. Exercise can play a preventive role in cancer in addition to its role in cancer treatment. Consistent with the present study, other studies have shown a decrease in tumor volume as a result of exercise (25,26).

Zielinski et al. attributed the decrease and delay of tumor growth in the experimental group to the control group after 4 weeks of endurance training to a decrease in the amount of immune cells in the tumor (27). According to Murphy et al.'s research, aerobic activity decreased tumor growth in the training group compared to the control group (28); however, some studies have shown no effect of exercise on tumor volume changes (29,30). The training protocol of the present study is defined on the treadmill; therefore, differences in training style, training duration, and type of induced tumor may be the reason for the results inconsistent with the results of the present study. The decrease in tumor volume growth with periodic aerobic exercise in addition to the decreased expression of the Twist and Vimentin genes may imply a positive effect of this type of exercise on epithelial to mesenchymal transition in mice with breast cancer. It is worth noting that very little research has been done to investigate the gene expression changes of mesenchymal biomarkers as well as the tumor volume in tumor specimens and patients with breast cancer in pathologic conditions compared to exercise training, especially aerobic interval training. This makes the background difficult. Positive results indicate that processes such as decreased blood supply to tumor cells may be involved in the slowdown of tumor growth, given that there are many factors contributing to the EMT process and other factors may be involved, it is not possible to quantify the tumor volume differences in the groups solely due to the variables in the present study, so it is advisable to evaluate other factors and mechanisms involved in the EMT process so that the results of the present study can be interpreted more clearly.B ased on the results of this study, it can be concluded that a period of aerobic exercise can decrease the expression of Vimentin, Twist genes and decrease the tumor volume ratio.

Acknowledgment

Hereby, we would like to thank the Vice Chancellor for Research and Technology of Ferdowsi University of Mashhad for supporting this research.

[DOR: 20.1001.1.15614107.1399.22.1.4.6]
[DOI: 10.22088/jbums.22.1.24]

References

1.Enayatrad M, Amoori N, Salehiniya H. Epidemiology and trends in breast cancer mortality in iran. Iran J Public Health. 2015;44(3):430-31.

2.Motamedi M, Hashemzadeh Chaleshtori M, Ghasemi S, Kheiri S, Haji Gholami A. The association of mir-451 and mir-21 in plasma with lymph node metastases in breast cancer. J Babol Univ Med Sci. 2018;20(4):12-16. [In Persian]

3.Chai JY, Modak C, Mouazzen W, Narvaez R, Pham J. Epithelial or mesenchymal: Where to draw the line? Biosci trends. 2010;4(3):130-42.

4.Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009;119(6):1420-8.

5.Kalcheim C. Epithelial-mesenchymal transitions during neural crest and somite development. J Clin Med. 2016;5(1):1.

6.Wu Y, Sarkissyan M, Vadgama JV. Epithelial-mesenchymal transition and breast cancer. J Clin Med. 2016;5(2):13.

7.Satelli A, Li S. Vimentin in cancer and its potential as a molecular target for cancer therapy. Cell Mol Life Sci. 2011;68(18):3033-46.

8.Wu Y, Zhang X, Salmon M, Lin X, Zehner ZE. TGFbeta1 regulation of vimentin gene expression during differentiation of the C2C12 skeletal myogenic cell line requires Smads, AP-1 and Sp1 family members. Biochim Biophys Acta. 2007;1773(3):427-39.

9.Hwangbo C, Tae N, Lee S, Kim O, Park OK, Kim J, et al. Syntenin regulates TGF-β1-induced Smad activation and the epithelial-to-mesenchymal transition by inhibiting caveolin-mediated TGF-β type I receptor internalization. Oncogene. 2016;35(3):389-401.

10.Li X, Yang J, Wang X, Li X, Liang J, Xing H. Role of TWIST2, E-cadherin and Vimentin in epithelial ovarian carcinogenesis and prognosis and their interaction in cancer progression. Eur J Gynaecol Oncol. 2016;37(1):100-8.

11.Lee IM. Physical activity and cancer prevention-data from epidemiologic studies. Med Sci Sports Exerc. 2003;35(11):1823-7.

12. Zhang LJ, Liu W, Gao YM, Qin YJ, Wu RD. The expression of IL-6 and STAT3 might predict progression and unfavorable prognosis in Wilms' tumor. Biochem Biophys Res Commun. 2013;435(3):408-13.

13. Gibala MJ, Little JP, MacDonald MJ, Hawley JA. Physiological adaptations to low-volume, high-intensity interval training in health and disease. J physiol. 2012;590(5): 1077-84.

14. Meyer K, Samek L, Schwaibold M, Westbrook S, Hajric R, Lehmann M, et al. Physical responses to different modes of interval exercise in patients with chronic heart failure- Application to exercise training. Eur Heart J. 1996;17(7):1040-7.

15. Ranjbar K, Agha Alinejad H, Shahbazi Sh, Molanouri Shamsi M, Chekachak S, Chenari J, et al. Interval aerobic exercise and selenium nanoparticle stimulate autophagy in mice with cancer cachexia. Int J Cancer Oncol. 2018;5(1):35-40.

16. Jones LW, Viglianti BL, Tashjian JA, Kothadia SM, Keir ST, Freedland SJ, et al. Effect of aerobic exercise on tumor physiology in an animal model of human breast cancer. J Appl Physiol. 2010;108(2):343-8.

17.Massague J. TGFbeta in Cancer. Cell. 2008;134(2):215-30.

18.Tian F, Byfield DS, Parks WT, Yoo S, Felici A, Tang B, et al. Reduction in Smad2/3 signaling enhances tumorigenesis but suppresses metastasis of breast cancer cell lines. Cancer Res. 2003;63(23):8284-92.

19. Lee J, Roh KB, Kim SC, Lee J, Park D. Soy peptide-induced stem cell proliferation: involvement of ERK and TGF-β1. J Nutr Biochem. 2012;23(10):1341-51.

20. Mulder KM. Role of Ras and Mapks in TGFβ signaling. Cytokine Growth Factor Rev. 2000;11(1-2):23-35.

21. Medeiros A, Oliveira EM, Gianolla R, Casarini DE, Negrão CE, Brum PC. Swimming training increases cardiac vagal activity and induces cardiac hypertrophy in rats. Braz J Med Biol Res. 2004;37(12):1909-17.

22. Terada Sh, Tabata I, Higuchi M. Effect of high-intensity intermittent swimming training on fatty acid oxidation enzyme activity in rat skeletal muscle. Jpn J Physiol. 2004;54(1):47-52

23. Kumari N, Dwarakanath BS, Das A, Bhatt AN. Role of interleukin-6 in cancer progression and therapeutic resistance. Tumour Biol. 2016;37(9):11553-72.

[DOR: 20.1001.1.15614107.1399.22.1.4.6]
[DOI: 10.22088/jbums.22.1.24]

24.Sullivan NJ, Sasser AK, Axel AE, Vesuna F, Raman V, Ramirez N, et al. Interleukin-6 induces an epithelial–mesenchymal transition phenotype in human breast cancer cells. Oncogene. 2009;28(33):2940-7.
25. Friedenreich CM, Orenstein MR. Physical activity and cancer prevention: Etiologic evidence and biological mechanisms. J Nutr. 2002;132(11 Suppl):3456-64.
26. Liu X, Chu KM. E-cadherin and gastric cancer: Cause, consequence, and applications. Biomed Res Int. 2014;2014:637308.
27. Zielinski MR, Muenchow M, Wallig MA, Horn PL, Woods JA. Exercise delays allogeneic tumor growth and reduces intratumoral inflammation and vascularization. J Appl Physiol. 2004;96(6):2249-56.
28.Murphy EA, Davis JM, Barrilleaux TL, McClellan JL, Steiner JL, Carmichael MD, et al. Benefits of exercise training on breast cancer progression and inflammation in C3 (1) SV40Tag mice. Cytokine. 2011;55(2):274-9.
29.Betof AS, Dewhirst MW, Jones LW. Effects and potential mechanisms of exercise training on cancer progression: A translational perspective. Brain Behav Immun. 2013;30 Suppl:S75-87.
30.Woods JA, Vieira VJ, Keylock KT. Exercise, inflammation, and innate immunity. Immunol Allergy Clin North Am. 2009; 29(2):381-93.

[DOR: 20.1001.1.15614107.1399.22.1.4.6]
[DOI: 10.22088/jbums.22.1.24]