Physiology & Behavior 169 (2017) 52–58

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Physical activity induced protection against breast cancer risk associated with delayed parity

Kathleen M. Sturgeon [a,*], Aaron Schweitzer [b], John J. Leonard [b], Deirdre K. Tobias [c,d], Ying Liu [e], Elizabeth Cespedes Feliciano [f], Vasanti S. Malik [c], Amit Joshi [c], Bernard Rosner [c], Bart C. De Jonghe [g]

[a] Pennsylvania State University, School of Medicine, Hershey, PA, USA
[b] University of Pennsylvania, School of Arts and Sciences, Philadelphia, PA, USA
[c] Harvard T.H. Chan School of Public Health, Boston, MA, USA
[d] Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, MA, USA
[e] Washington University, School of Medicine, St. Louis, MO, USA
[f] Kaiser Permanente Northern California, Division of Research, Oakland, CA, USA
[g] University of Pennsylvania, School of Nursing, Philadelphia, PA, USA

HIGHLIGHTS

- Patterns of delayed parity induced changes to the breast tissue which are both dependent and independent of exercise training completed between menarche and first pregnancy.
- While exercise training was beneficial for tumor latency and size, it did not mitigate enhanced collagen levels found in mammary tissue of delayed parity animals.
- Similarly, exercise training did not mitigate enhanced expression levels of several genes associated with breast cancer.
- However, there were exercise-dependent changes in the mammary gland.
- Exercise training prevented the development of inflammation and ductal hyperplasia.
- Exercise training also led to improved directional regulation of gene expression levels for *Cdkn1c* and *Plau*.
- Differential gene expression levels of mammary tissue *Cdkn1c* and *Plau* in animals physically active between menarche and first pregnancy suggest that these genes may play a role in exercise-induced protection against breast cancer later in life.

ARTICLE INFO

Article history:
Received 15 August 2016
Received in revised form 12 November 2016
Accepted 18 November 2016
Available online 21 November 2016

Keywords:
Neoplasm
Exercise
Delayed parity
Gene expression

ABSTRACT

Epidemiological evidence indicates that physical activity between menarche and first pregnancy is associated with a lower risk of breast cancer among women with at least 20 years between these reproductive events. The mechanism by which physical activity during this interval confers protection is unknown. This study used a novel animal model to assess potentially protective effects of physical activity on tumor development in delayed parity. Thirty-six female Sprague Dawley rats received an i.p. injection of 50 mg/kg *N*-methyl-*N*-nitrosourea (MNU) at 5 weeks of age. Estrogen and progesterone pellets were implanted subcutaneously 1 week (early parity, EP, $n = 8$) or 4 weeks (delayed parity, DP, $n = 11$) following MNU injection. An additional group of DP rats were progressively exercise trained (Ex + DP, $n = 9$) on a treadmill following MNU injection for 7 weeks (up to 20 m/min at 15% incline for 30 min). We observed the greatest tumor latency and smallest tumor burden in Ex + DP animals. Ductal hyperplasia and inflammation of non-tumor bearing mammary glands were only found in DP, and we detected a significant increase in collagen for DP and Ex + DP compared to EP. Exercise induced differential gene expression of cyclin-dependent kinase-inhibitor 1C (*Cdkn1c*) and urokinase-plasminogen activator (*Plau*) in mammary tissue of Ex + DP animals compared to DP alone. While there are delayed parity-induced changes in mammary gland collagen and gene expression levels, Ex + DP animals had longer tumor latency, smaller tumor burden, and glandular tissue resistant to ductal hyperplasia. Exercise may induce protection through beneficial regulation of gene expression profiles.

1. Introduction

Breast cancer is the most common malignancy among women in the United States [1]. Reproductive factors such as earlier age at menarche,

* Corresponding author at: Pennsylvania State University, Cancer Institute, 500 University Drive, Hershey, PA, 17033, USA.
E-mail address: sturgeon.katie@gmail.com (K.M. Sturgeon).

http://dx.doi.org/10.1016/j.physbeh.2016.11.026
0031-9384/

later age at first pregnancy, and a longer interval between these two reproductive events are consistently associated with higher risk of breast cancer [2–4]. Available global evidence indicates the menarche to first pregnancy interval has lengthened in recent years [5], supporting a need to identify lifestyle interventions to mitigate this accumulating excess risk. There is convincing epidemiological evidence that moderate-to-vigorous intensity physical activity is associated with a 10–25% lower risk of breast cancer, compared to inactivity [6]. Pregnancy- and exercise-induced protections against breast cancer are also well-established in female rats using carcinogen-induced breast cancer models [7–10]. Also consistent with human data, delayed parity in rats increases breast cancer risk compared to rats with pregnancy induced earlier in life [11].

We have recently shown in epidemiological data that the influence of leisure-time physical activity between menarche and first pregnancy on future breast cancer risk varies by the length of the interval between menarche and first pregnancy [12]. Physical activity was significantly associated with a 27% lower breast cancer risk for high risk women with the longest time between menarche and first pregnancy (≥20 years). This was not observed among women with a shorter time between menarche and first pregnancy. Here, a parallel pre-clinical rat model was devised to examine potential mechanisms concerning the role of physical activity in mammary gland tumorigenesis associated with delayed parity. We investigated the role of exercise not only on breast cancer incidence, but latency, mammary gland morphology, and pertinent gene expression levels.

Many cellular adaptations occur in response to exercise and there are marked differences between mammary tissue of animals with early parity and with delayed parity. We hypothesized several pathways would be modified by our intervention. Preliminary data indicated the gene *Cdkn1c* was differentially regulated by exercise. *Cdkn1c* encodes for the protein cyclin dependent kinase 1c, which is an inhibitor of several G1 cyclin/CDK complexes and a negative regulator of the cell cycle at the G1 checkpoint [13]. Decreased mRNA levels of *CDKN1C* are associated with multiple cancer types [14–18], and several observations implicate a role for *CDKN1C* in breast tumorigenesis. Decreased *CDKN1C* expression occurs during human mammary epithelial cell immortalization [19]. Additionally, decreased *CDKN1C* gene expression is seen in the large majority of human breast cancers and is associated with poor prognosis [20,21]. No study to-date has investigated potential mechanisms underlying the role of physical activity in preventing increased mammary gland tumorigenesis associated with delayed parity. Therefore, we seek to identify candidate genes associated with breast cancer which may be beneficially modulated by exercise during the menarche-to-first-pregnancy interval.

2. Methods

2.1. Animals and experimental protocol

The paradigm of the study is presented in Fig. 1A. Five-week old female Sprague Dawley rats (Harlan Laboratories) were randomly divided into four cohorts as described below. Of note, thirty-five days of age is the approximate age of reproductive maturity for female Sprague Dawley rats [9]. All rats ($n = 38$) were injected (week 0) intraperitoneally with 50 mg/kg *N*-methyl-*N*-nitrosourea (MNU) in sterile 0.85% NaCl solution at pH 5.0 as an accepted model of mammary tumor induction [11]. Pregnancy was mimicked by estrogen (35 mg) and progesterone (35 mg) 21 day release hormone pellets (Innovative Research of America, FL, USA) implanted subcutaneously (anesthesia: (60 mg/kg) ketamine/(7.5 mg/kg) xylazine/(1.0 mg/kg) on alternate sides of the scruff of the neck [22]. Our early parity (EP, $n = 9$), group was implanted with hormone pellets 1 week following MNU injection as it has been shown that inducing pregnancy 1 week following the administration of MNU confers protection against mammary gland tumor development [8]. In contrast, the delayed parity (DP, $n = 11$) group was implanted with hormone pellets 4 weeks following MNU injection. Others have observed tumor development as early as 5 weeks following MNU administration in female Sprague Dawley rats administered MNU at 5 weeks of age [23]. Therefore, a 4 week time frame was chosen for delayed parity.

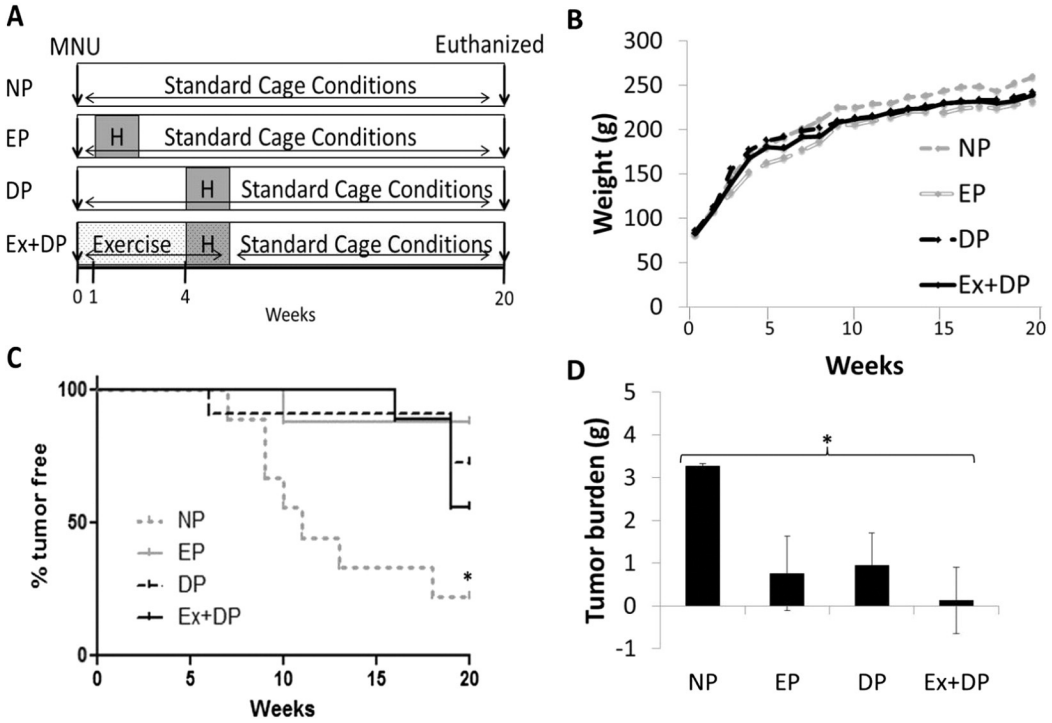

Fig. 1. Experimental paradigm and physical characteristics. Thirty-four to thirty-five day old animals were injected with MNU at time 0 (weeks) and followed for 20 weeks. Animals were progressively exercise trained (Ex), or remained sedentary, prior to and during exposure to hormone (H) pellets (A). Exercise training did not alter body weight (B), but did increase tumor latency (C) leading to smaller tumors (D) in exercise trained animals. *$p < 0.05$. Nulliparous (NP), early parity (EP), delayed parity (DP).