MOLECULAR & CELLULAR ONCOLOGY
2020, VOL. 7, NO. 6, e1825916 (3 pages)
https://doi.org/10.1080/23723556.2020.1825916

Taylor & Francis
Taylor & Francis Group

COMMENTARY

RSK2 and ERα comrades-in-arms in homeostasis and transformation

Katarzyna A. Ludwik[a*] and Deborah A. Lannigan[a,b,c]

[a]Pathology, Microbiology & Immunology, Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN, USA; [b]Biomedical Engineering, Vanderbilt University, Nashville, TN, USA; [c]Cell and Developmental Biology, Vanderbilt University, Nashville, TN, USA

ABSTRACT

The physiological response to estrogen differs according to the developmental stage. We show, in the adult, estrogen-responsiveness is driven by ERK1/2 (extracellular signal-regulated kinase 1/2) whereas its downstream effector, RSK2 (p90 ribosomal S6 kinase 2), prevents continuous ERK1/2 activity through regulation of oxidative stress. Bioinformatic analysis revealed RSK2 association with breast cancer risk and oral contraceptives.

ARTICLE HISTORY
Received 10 September 2020
Revised 15 September 2020
Accepted 16 September 2020

KEYWORDS
Estrogen; estrous cycle; ERK1/2; RSK2; p90 ribosomal S6 kinase; p90RSK2; oral contraceptives; exogenous estrogens; breast cancer

The steroid hormone, estrogen, regulates numerous developmental and homeostatic processes. The mammary gland is extremely sensitive to estrogen and therefore, is an excellent model system for understanding the physiological effects of estrogen. Estrogen acts mainly through interaction with its receptors, estrogen receptor alpha (ERα) and beta (ERβ). At puberty, increased estrogen levels are responsible for the dramatic expansion of the mammary ductal network throughout the mammary fat pad. In contrast, in the adult, the estrogen pulse that occurs with each menstrual cycle until menopause generates a very limited response. A central question in the field is to define the regulatory mechanism that must exist to limit estrogen responsiveness after puberty to prevent the inappropriate expansion that occurs in breast cancer. In the U.S. ~ 12.5% of women will be diagnosed with invasive breast carcinoma during their life time and ~ 60% of these cancers will be positive for ERα (ER+). The ability of estrogen to act as a driver in these tumors is demonstrated by the effectiveness of therapies designed to lower or antagonize estrogen. Disease processes frequently co-opt mechanisms that are important for development and homeostasis. Therefore, to identify possible novel targets for therapeutic intervention in ER+ breast cancer we investigated and described a mechanism that limits estrogen responsiveness in the adult mammary gland .[1]

Estrogen induces increased expression of growth factors and their receptors, resulting in activation of their downstream signaling pathways, such as RAF-MEK1/2-ERK1/2 (extracellular signal-regulated kinase 1/2) -RSK (p90 ribosomal S6 kinase). RSK correlates positively with patient response to hormone-based therapies, which suggests that RSK is an indicator of intact estrogen signaling and sensitivity to anti-estrogen therapy .[2] In pre-clinical studies RSK2 (p90 ribosomal S6 kinase 2) was found to associate with ERα and regulate estrogen-mediated gene expression.[3] Targeting RSK2 (*Rps6ka3*) to the nucleus of mammary epithelial cells induced high grade ER+ ductal carcinoma in situ and these transformed cells were highly metastatic.[3] Interestingly, the RSK2-ERα complex was necessary for tumor growth and was disrupted in response to anti-estrogens, [3] which may explain the clinical connection between active RSK, anti-estrogens and overall survival. These data suggest that RSK2 acts as a required participant for ERα function in tumorigenesis, which led us to investigate whether RSK2 was involved in estrogen homeostasis in the mammary gland.

In the mouse, the estrous cycle is divided into four stages based on vaginal cytology: proestrus, estrus, metestrus, and diestrus. In response to the estrogen burst in proestrus, degradation of ERα through the 26S proteasome is necessary for increased ERα-dependent gene expression .[4] Yet, the requirement for this connection is not understood. We found that estrogen-induced degradation of ERα was regulated by Ser-118, a previously identified ERK1/2 phosphorylation site in ERα. We observed that ERK1/2 activity was regulated by the estrous cycle. High ERK1/2 activity occurred in estrus and was dependent on the estrogen pulse in proestrus. In contrast, in diestrus, when estrogen levels are the lowest, ERK1/2 was inactive. Our data support a model in which the cyclic changes in degradation of ERα and estrogen-mediated gene expression that occur during the estrous cycle are driven by ERK1/2 activity (Figure 1). Our observations are the first to demonstrate that ERK1/2 activity responds to the estrous cycle and is required for estrogen responsiveness.

A major and surprising finding, given the association of RSK2 with cancer,[3,5] was the observation that RSK2 acts as a brake on ERK1/2 activity. We found that loss of RSK2 resulted in increased ERα degradation and estrogen-mediated gene expression in the ERα+ cells within the mammary gland. In contrast to the wild type, ERK1/2 was continuously active

CONTACT Deborah A. Lannigan deborah.lannigan@vumc.org Pathology, Microbiology & Immunology, Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN 37232, USA

*Present address: K.A.L. is Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine, Hannoversche Str. 28, 10115 Berlin, Germany.

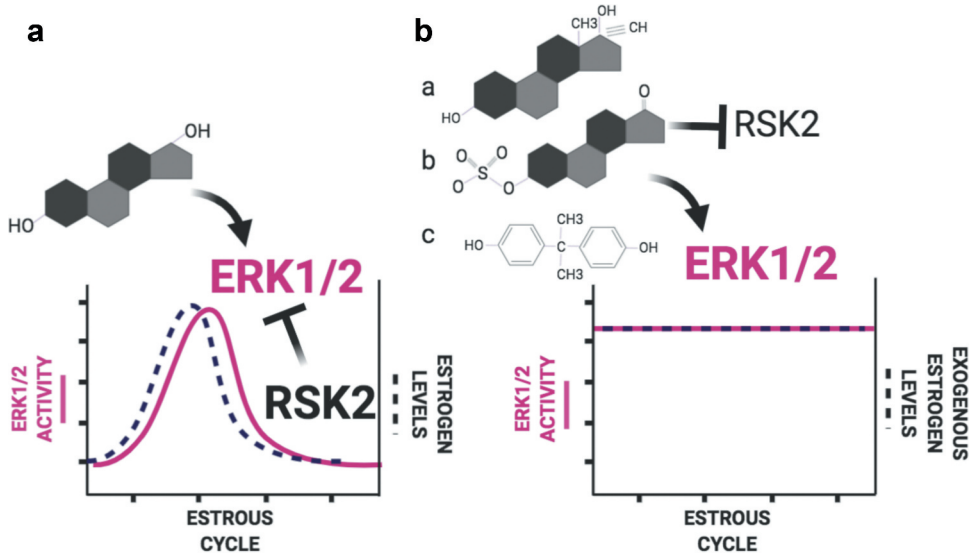

Figure 1. ERK1/2 activity drives estrogen responsiveness. The left panel (a) indicates that ERK1/2 (extracellular signal-regulated kinase 1/2) is activated in response to the estrogen burst (estradiol shown) at proestrus to drive estrogen-mediated gene expression. RSK2 (p90 ribosomal S6 kinase 2) facilitates the inactivation of ERK1/2 to ensure estrogen homeostasis. The right panel (b) shows that in response to continuous exposure to oral contraceptives (e.g. ethinylestradiol shown (a)), hormone replacement therapy (e.g. conjugated estrogens shown (b)) or environmental estrogens (e.g. bisphenol A shown (c)) RSK2 levels are decreased. Reduced RSK2 results in chronic activation of ERK1/2 independent of the estrous cycle. Figure generated with Biorender.

throughout the estrous cycle in the mammary glands of the RSK2 (*Rps6ka3*) knockout mice. We found that ERK1/2 remained active because the loss of RSK2 resulted in elevated reactive oxygen species (ROS). ROS is known to inactivate phosphatases by oxidizing cysteine residues present in their catalytic site .[6] Therefore, it is possible that ERK1/2 remains active through the estrous cycle due to the loss of phosphatase activity, which is necessary to remove the activating phosphorylation on ERK1/2. Our results are the first demonstration that RSK2 negatively regulates ROS production and the mechanism responsible has not yet been identified.

In humans starting at puberty estrogen levels continuously cycle with the highest levels occurring in the follicular phase, akin to the mouse proestrus stage, and the lowest levels occurring in the luteal phase. These cycles continue until menopause. Approximately 140 million women take oral contraceptives containing estrogens, which increases breast cancer risk .[7] In contrast to the menstrual cycle, oral contraceptives result in increased estrogen levels over a sustained period, which is important in the contraception mechanism. To investigate whether our observations were relevant to humans we analyzed gene expression data obtained from normal breast tissue of women who were cycling or taking oral contraceptives .[8] A significant correlation with an estrogen responsive gene signature was observed in response to the estrogen pulse that occurs in the normal cycle and in individuals taking oral contraceptives. Consistent with our in vivo mouse data, this estrogen-responsive gene signature was inversely correlated with RSK2 mRNA levels. Decreased RSK2, which facilitates estrogen signaling, results in DNA damage. We speculate that the increase in breast cancer risk associated with oral contraceptives may be the result of continuously reduced RSK2 by the prolonged increase in estrogen levels (Figure 1). In addition to women taking oral contraceptives, hormone therapy containing estrogens are given for ovarian insufficiency including menopausal symptoms and extremely high doses of estrogens are given in transgender therapy. Environmental pollutants also serve as a source of exogenous estrogenic compounds. We postulate that the increase in breast cancer risk associated with prolonged exposure to exogenous estrogens[9,10] is due to a less effective RSK2 brake on ERK1/2 activity.

Interestingly, ERK1/2 and RSK2 are not active prior to sexual maturity, which we defined as the occurrence of regular estrous cycles. We hypothesize that the ERK1/2-RSK2 signaling pathway evolved as a necessary braking mechanism to prevent ductal expansion after maturity. However, in breast cancer the ability of RSK2 to restrain ERK1/2 activation of ERα does not occur and both ERK1/2 and RSK2 appear to drive the transformation phenotype .[5] High ROS production frequently occurs in cancer and it is possible that RSK2 regulation of ROS is bypassed in breast cancer, which ultimately results in constitutive activation of the ERK1/2-RSK2 pathway. Identifying the mechanism by which RSK2 regulates ROS would provide insight into homeostatic mechanisms but also provide a way to reinitiate RSK2 control of ROS and prevent inappropriate ERK1/2 activation.

Disclosure of potential conflicts of interest

The author D.A.L. has a patent related to this work.

Funding

This work was supported by NIH grant DK113423 (D.A.L.) and CA213201 (D.A.L.).

ORCID

Deborah A. Lannigan http://orcid.org/0000-0001-8380-9240

References

1. Ludwik KA, Sandusky ZM, Stauffer KM, Li Y, Boyd KL, O'Doherty GA, Stricker TP, Lannigan DA. RSK2 maintains adult estrogen homeostasis by inhibiting ERK1/2-mediated degradation of estrogen receptor alpha. Cell Reports. 2020;32:107931. doi:10.1016/j.celrep.2020.107931.
2. Yamashita H, Nishio M, Kobayashi S, Ando Y, Sugiura H, Zhang Z, Hamaguchi M, Mita K, Fujii Y, Iwase H, *et al*. Phosphorylation of estrogen receptor alpha serine 167 is predictive of response to endocrine therapy and increases postrelapse survival in metastatic breast cancer. Breast Cancer Res. 2005;7:R753–764. doi:10.1186/bcr1285.
3. Ludwik KA, McDonald OG, Brenin DR, Lannigan DA. ERα-mediated nuclear sequestration of RSK2 is required for ER+ breast cancer tumorigenesis. Cancer Res. 2018;78:2014–2025. doi:10.1158/0008-5472.CAN-17-2063.
4. Nawaz Z, Lonard DM, Dennis AP, Smith CL, O'Malley BW. Proteasome-dependent degradation of the human estrogen receptor. Proc Natl Acad Sci U S A. 1999;96:1858–1862. doi:10.1073/pnas.96.5.1858.
5. Ludwik KA, Campbell JP, Li M, Li Y, Sandusky ZM, Pasic L, Sowder ME, Brenin DR, Pietenpol JA, O'Doherty GA, *et al*. Development of a RSK inhibitor as a novel therapy for triple-negative breast cancer. Mol Cancer Ther. 2016;15:2598–2608. doi:10.1158/1535-7163.MCT-16-0106.
6. Meng TC, Fukada T, Tonks NK. Reversible oxidation and inactivation of protein tyrosine phosphatases in vivo. Mol Cell. 2002;9:387–399. doi:10.1016/S1097-2765(02)00445-8.
7. Lovett JL, Chima MA, Wexler JK, Arslanian KJ, Friedman AB, Yousif CB, Strassmann BI. Oral contraceptives cause evolutionarily novel increases in hormone exposure: A risk factor for breast cancer. Evol Med Public Health. 2017;2017:97–108. doi:10.1093/emph/eox009.
8. Pardo I, Lillemoe HA, Blosser RJ, Choi M, Sauder CAM, Doxey DK, Mathieson T, Hancock BA, Baptiste D, Atale R, et al. Next-generation transcriptome sequencing of the premenopausal breast epithelium using specimens from a normal human breast tissue bank. Breast Cancer Res. 2014;16:R26. doi:10.1186/bcr3627.
9. Chlebowski RT, Anderson GL, Aragaki AK, Manson JE, Stefanick ML, Pan K, Barrington W, Kuller LH, Simon MS, Lane D, et al. Association of menopausal hormone therapy with breast cancer incidence and mortality during long-term follow-up of the women's health initiative randomized clinical trials. JAMA. 2020;324:369–380. doi:10.1001/jama.2020.9482.
10. de Blok CJM, Wiepjes CM, Nota NM, van Engelen K, Adank MA, Dreijerink KMA, Barbé E, Konings IRHM, den Heijer M. Breast cancer risk in transgender people receiving hormone treatment: nationwide cohort study in the Netherlands. BMJ. 2019;365:l1652. doi:10.1136/bmj.l1652.