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

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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.

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.

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. In pre-clinical studies RSK2 (p90 ribosomal S6 kinase 2) was found to associate with ERα and regulate estrogen-mediated gene expression. 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. Interestingly, the RSK2-ERα complex was necessary for tumor growth and was disrupted in response to anti-estrogens, 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. Yet, the requirement for this connection is not understood. We found that estrogen-induced degradation of ERα was regulated by Ser118, 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, 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
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. 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. 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. 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 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. 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 reinivate 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.

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