How estrogen fuels breast cancer

Ajay Rana¹, Velusamy Rangasamy¹ & Rajakishore Mishra¹

¹Department of Molecular Pharmacology & Therapeutics, Loyola University Medical Center, Building 102, 2160 South First Avenue, Maywood, IL 60153, USA

*Author for correspondence: Tel.: +1 708 216 5761 ■ Fax: +1 708 216 6596 ■ arana@lumc.edu

“...sustained exposure of ER with endogenous or exogenous estrogen ... is well established to cause breast cancer...”

Despite significant progress made to diagnose and treat breast cancer, it still remains the second largest killer in women, just after lung cancer [101]. It is estimated that approximately 190,000 new cases of breast cancer will be diagnosed in USA alone in 2009 [1] and the majority (~70%) of them will be estrogen receptor (ER) positive cases. The ER regulates growth, differentiation and homeostasis of the normal mammary gland [2]; however, sustained exposure of ER with endogenous or exogenous estrogen (E_2) is well established to cause breast cancer [3,4]. Sustained activation of ER in breast epithelial cells causes hyperactivation of ER downstream survival pathways, leading to dysregulated growth and thus breast cancer. Therefore, the agents that block either E_2 synthesis (i.e., aromatase inhibitors) or antagonize E_2 's action (e.g., tamoxifen and other SERMs) are in clinical use to control the growth of ER⁺ breast cancer cells. Tamoxifen is certainly the first line of drug therapy for ER⁺ breast cancer [5], whereas aromatase inhibitors have proven highly effective in postmenopausal women with ER⁺ breast cancer [6]. However, these drugs are not effective for long-term use and are known to cause collateral damage to the heart and other organs. In addition, some women are not responsive to hormonal therapy; and will have to undergo chemotherapy or radiotherapy. Therefore, two of the most challenging tasks for ER⁺ breast cancer treatment are the identification of new therapeutic targets for endocrine resistance and the development of predictive biomarkers of endocrine therapy.

The prevailing hypothesis in breast cancer is that estrogen promotes the survival of ER⁺ breast cancer cells by inhibiting the proapoptotic cellular machinery [7]. It has been demonstrated that ER mediates the downregulation of proapoptotic molecules. However, understanding the detailed network is certainly warranted in finding the primary targets by which ER

promotes dysregulated cell growth and could prove beneficial for ER⁺ breast cancer treatment. Recently, we have identified a novel molecule that could serve as a viable target for ER⁺ breast cancer treatment. The role of this molecule, termed Mixed Lineage Kinase 3 (MLK3) in breast cancer pathogenesis, has never been demonstrated before.

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Mixed Lineage Kinase 3 belongs to the family of MAPKKK, termed Mixed Lineage Kinases (MLKs). The MLK family members are characterized by the presence of signature sequences of serine/threonine and tyrosine kinases within their catalytic domain [8,9]. Previous work from our laboratory has shown that MLK3 activates the c-Jun NH2-terminal kinase (JNK) via activation of its upstream kinase SAPK/ERK kinase 1 (SEK1/MKK4) [10]. Furthermore, we also demonstrated that MLK3 was inhibited directly by protein kinase B (PKB/AKT) [11], a survival protein kinase, which is universally overexpressed in most breast cancer cells and mediates the hormonal or cytotoxic agents' resistance [12]. Since activation of MLK3 causes cell death in neuronal and non-neuronal cells [11,13] and activation of AKT promotes survival, including in breast and other cells, it was tempting to conceive that MLK3 or other MLKs could play a significant role in breast cancer pathogenesis.

We measured MLK3 kinase activities in primary human breast tumors and matching normal breast tissues. Interestingly, the

Keywords

■ breast cancer ■ cytotoxic drug ■ estrogen ■ estrogen receptor

MLK3 kinase activity was significantly higher in some tumors compared with others. Since ER and progesterone receptor (PR) both play a central role in breast cancer pathogenesis, the ER and PR status were also determined in these tumors. Surprisingly, these results revealed that MLK3 kinase activity was approximately five-fold higher, exclusively in the ER⁻ tumors compared with ER⁺ [14]. These results certainly suggested to us that MLK3 is most probably negatively regulated by E₂. Indeed in ER⁺ human breast cancer cell line, MCF7, E₂ inhibited MLK3 kinase activities but not in ER⁻ SkBr3 cells. Furthermore, E₂ action was completely blocked by E₂ antagonist, ICI 182,780. As AKT is overexpressed in most breast cancer cells and promotes cell survival, it was interesting to observe that E₂-activated AKT, blocked MLK3 kinase activity and AKT or PI3K-specific inhibitors, or AKT-specific siRNA, blocked E₂-induced inhibition of MLK3.

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Taxanes, a class of drug that targets the microtubules are often used to treat breast cancer. Taxol, a taxane, has been demonstrated to cause cell death in MCF7 cells and therefore our result that MLK family pan-inhibitor, CEP-11004 inhibited taxol-induced cell death suggests that MLK3 or other MLKs play a central role in cytotoxic drug-induced cell death in ER⁺ breast cancer cells [14]. The interesting part of our observation was that MLK3 kinase activity was significantly elevated in ER⁻ breast tumors compared to ER⁺ ones. Furthermore, the fact that ER⁻ tumors

are more aggressive compared to ER⁺ tumors raises an important question, how MLK3 activation promotes cell death in ER⁺ tumors? This is certainly a most challenging question and is the primary focus of our laboratory to dissect the role of MLK3 and other MLKs in ER⁻ breast tumors.

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It is interesting to note that previously, we have demonstrated that the bioactive lipids, ceramides functions as an agonist of MLK3 [15]. It is already reported that ceramides inhibits AKT; therefore, it appears likely that agents such as ceramides or any small molecule, which could inhibit AKT and at the same time activate MLK3 might prove beneficial to promote cell death in ER⁺ breast cancer cells. Our results certainly open up a new area of research for any future therapeutic intervention by targeting MLK3, and perhaps other MLK family member(s) for ER⁺ breast cancer treatment.

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