

AUTHOR'S VIEW



## Chromatin regulation at the intersection of estrogen receptor and PI3K pathways in breast cancer

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### ABSTRACT

Estrogen Receptor (ER) and the phosphoinositide 3-kinase (PI3K) pathways participate in regulatory crosstalk in breast cancer. We identified that chromatin regulation is at the intersection of oncogenic PI3K and ER. The PI3K effectors AKT, also known as protein kinase B (PKB), and SGK (serum/glucocorticoid-regulated kinase) play a redundant role by phosphorylating the chromatin regulator KMT2D and modulating ER activity and therapy resistance.

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The transcription factor (TF) estrogen receptor (ER), which is present in over 70% of breast cancers, is known to act as a primary driver in breast cancer. The classical mechanism of ER activation includes estrogen binding to the ligand-binding domain of the receptor, which leads to dimerization of ER and its recruitment to the chromatin at sequence-specific response elements known as estrogen response elements (EREs). ER activation in breast cancer leads to the regulation of gene transcription involved in cell growth, proliferation, and cancer progression.<sup>1</sup> As such, ER is targeted clinically at different levels using the direct ER antagonist tamoxifen, the ER degrader fulvestrant, or the aromatase inhibitors letrozole and exemestane.<sup>1</sup> Endocrine therapies have dramatically improved patient survival; however, a significant number of patients develop resistance to these agents. A thorough understanding of the molecular mechanisms of ER activity and the development of new therapeutic strategies is of high clinical importance.

Our knowledge of ER regulation has evolved significantly in recent years and we now appreciate the numerous cofactors that regulate ER activity, the thousands of cis-regulatory elements that ER binds, and the multitude of cross-talks between ER and other signaling pathways. This understanding has been, in part, the result of an emerging comprehensive understanding of chromatin regulation in cancer. Mechanistically, ER associates at the chromatin with a variety of coregulators including FOXA1 (Forkhead Box A1), PBX1 (PBX1 Homeobox 1), and GATA3 (GATA binding protein 3), which have been termed pioneering TFs given their ability to actively open up the local chromatin to initiate ER binding to ERE.<sup>1,2</sup>

The phosphoinositide 3-kinase (PI3K) pathway is essential for cellular growth and survival and is frequently altered in human tumors. Activating mutations in the *PIK3CA*, the gene

encoding the p110 $\alpha$  catalytic subunit of PI3K, occur in 40% of ER+ breast cancer, representing the most common genomic alteration in such tumors.<sup>3</sup> There is important bidirectional regulatory crosstalk between PI3K and ER signaling and both pathways can coordinately support survival. In this regard, we have previously observed that PI3K $\alpha$  inhibition results in enhanced ER function, which confers resistance to PI3K $\alpha$  inhibitors and can be reversed with the addition of anti-endocrine therapies such as fulvestrant.<sup>4</sup> These preclinical efforts have been recently confirmed by a phase III study which has shown that the addition of PI3K $\alpha$  inhibition to anti-endocrine therapy improved progression free survival in ER+/PIK3CA mutant metastatic breast cancers, leading to the recent FDA approval of the first PI3K $\alpha$  inhibitor alpelisib.<sup>5</sup>

Given the importance of these two pathways in breast cancer progression and therapy resistance, and how the chromatin state is a key determinant of ER function, we have been interested in investigating the mechanisms by which oncogenic PI3K-dependent downstream kinases control the cancer epigenome and transcription. To this end, we determined that PI3K inhibition remodels the chromatin landscape towards a more permissive ER state in breast cancer cells.<sup>6</sup> This increased dependency on ER requires the TFs FOXA1 and PBX1, whose knockdown sensitizes cells to anti-PI3K $\alpha$  inhibition by suppressing ER function. Moreover, our data supported a major role for the lysine methyltransferase, KMT2D (also known as MLL2, MLL4) in modulating the chromatin state necessary for the recruitment of the ER-FOXA1-PBX1 transcriptional regulatory network. KMT2D is a member of COMPASS (Complex Of Proteins Associated with Set1) family and is a major histone methyltransferase that regulates transcription by implementing H3 lysing 4 monomethylation and di-methylation.<sup>7</sup> By establishing a direct link between PI3K/AKT signaling and chromatin regulators, we

found KMT2D to be directly phosphorylated at S1331 by the PI3K effector, the serine/threonine kinase AKT, also known as protein kinase B (PKB). This phosphorylation event suppresses its function, attenuating the recruitment of ER-FOXA1-PBX1 regulatory network, and subsequently the ER activity (Figure 1A).<sup>6</sup> In contrast, upon PI3K inhibition and full suppression of AKT1, KMT2D activity is augmented, facilitating the recruitment of ER, and promoting ER-dependent transcription and tumor growth. Knockdown of KMT2D, in turn, enhanced the anti-tumor effect of PI3K $\alpha$  inhibitors in ER+ xenografts models. This AKT-dependent mechanism of KMT2D regulation, suggested a path towards novel combinatory treatments involving inhibitors of oncogenic signaling pathways and epigenetic regulators for ER+/PIK3CA mutant breast cancers.<sup>6</sup>

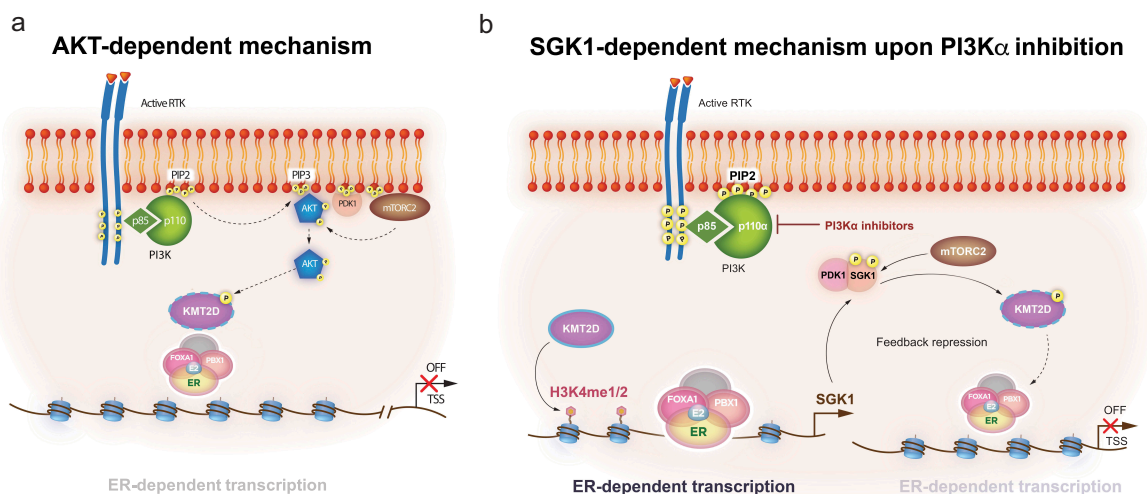
Further examination revealed that the SGK (serum/glucocorticoid-regulated kinase) family of kinases is a major gene target of ER. SGK's are 55% homologous to AKT within its catalytic domain and have a consensus motif similar to those of AKT (RXXRXXS/T), suggesting that both kinases can compensate for each other by phosphorylating overlapping substrates.<sup>3</sup> We previously showed in a subset of cancer cells that upon PI3K $\alpha$  inhibition, SGK1 can compensate for AKT loss by sustaining mTORC1 (mTOR Complex 1) activity and cell growth through direct phosphorylation and inhibition of TSC2 (TSC complex subunit 2), a *bona-fide* AKT target, mediating resistance to PI3K $\alpha$  inhibitors.<sup>8</sup> This also seems to be the case for SGK3, which is transcriptionally upregulated in response to PI3K/AKT inhibitors.<sup>9</sup> Thus, although AKT has been considered the dominant tumor-promoting mechanism of PI3K signaling, there are AKT-independent mechanisms by which the PI3K pathway propagates its cellular effects especially in scenarios where AKT activity is being challenged. Taking this into consideration, we studied whether ER activation by PI3K $\alpha$  inhibition can transcriptionally upregulate SGKs, which in turn, would

phosphorylate KMT2D to inhibit ER-mediated transcription as a negative regulatory feedback loop.

In a recent study,<sup>10</sup> we found that in ER+ breast cancer cells treated with PI3K $\alpha$  inhibitors, transcriptionally upregulated SGK1 phosphorylates KMT2D, attenuates its activity and recruitment, and subsequently downregulates ER-dependent transcription. We demonstrated that the SGK's transcription and protein levels are enhanced upon PI3K $\alpha$  inhibition through increased occupancy of ER and phosphorylated (S5) Pol II, a marker of transcriptional activation, at the promoters of SGKs. Elevated SGK1 phosphorylates KMT2D at S1331, leading to a loss of the occupancy of the chromatin marks which KMT2D catalyzes, H3K4me1/2, at ER loci and a loss of binding of ER regulatory network, which in turn downregulated ER target gene expression (Figure 1B). This suggests a role for SGK1, or possibly other SGK's, to program chromatin and ER transcriptional output under scenarios of AKT inactivation.

The precise mechanism that allows an ER+ breast cancer cell to upregulate SGK1 upon PI3K inhibition and affect ER-dependent transcription via KMT2D remains to be determined. We hypothesize that the ER-dependent increase in SGK1 transcription upon PI3K inhibition is rapid and transiently regulated, consistent with the fact that SGK1 expression is short-lived at the mRNA and protein level, while the overall ER function is sustained. This suggests that this mechanism is likely to represent a regulatory feedback. Future work will be required to predict which tumors will exhibit a robust AKT or SGK dependent mechanism and whether the chromatin regulation of ER response by AKT or SGK may differ, perhaps through the phosphorylation of distinct unknown chromatin regulators.

In summary, we and others have highlighted the importance of elucidating the overlooked role of SGK's in cancer and therapy response.<sup>8–10</sup> For instance, previous studies have shown SGK's to be an important mediator of resistance to PI3K inhibitors<sup>8,9</sup> and, in our recent work, we speculate that



**Figure 1.** AKT1 and SGK1-dependent mechanism/s of estrogen receptor (ER) regulation by KMT2D.

elevated SGK's may be a biomarker of resistance to the concomitant inhibition of PI3K and ER.<sup>10</sup> Moreover, our observations provide mechanistic understanding of ER regulation by the PI3K signaling and underscore the relevance of the chromatin state in connecting the oncogenic signaling responses with gene activity.

(A) AKT1 (also known as protein kinase B, PKB), phosphorylates the H3K4 histone lysine methyltransferase KMT2D at S1331 and negatively regulates its activity leading to a repression of ER, FOXA1 (Forkhead Box A1), and PBX1 (PBX Homeobox 1) transcriptional regulatory network recruitment and attenuation of ER-dependent transcription (AKT1-dependent mechanism). Modified from.<sup>6</sup> Reprinted with permission from AAAS.

(B) In the presence of PI3Kα inhibitors, *SGK1* (serum/glucocorticoid-regulated kinase 1) is transcriptionally upregulated by ER. Elevated SGK1 phosphorylates KMT2D at S1331 to repress H3K4me1/2 occupancy at ER loci and ER-dependent transcription (SGK1-dependent mechanism).

PI3K (Phosphoinositide 3-kinase); PDK1 (3-phosphoinositide-dependent Protein Kinase 1) also known as PDK1; PIP2 (Phosphatidylinositol 4, 5-bisphosphate); PIP3 (Phosphatidylinositol 3, 4, 5-trisphosphate); TSS (Transcription Start Site); RTK (Receptor Tyrosine Kinase); mTORC2 (mTOR Complex 2).

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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