PKC\(\lambda/\iota\) signaling—a common node for normal cellular development and breast oncogenesis

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Abbreviations: DCIS, ductal carcinoma \textit{in situ}; ESC, embryonic stem cell; EMT, epithelial to mesenchymal transition; IDC, invasive ductal carcinoma; IL1\(\beta\), interleukin-1 \(\beta\); iPSC, induced pluripotent stem cell; PSC, pluripotent stem cell; MET, mesenchymal to epithelial transition; PKC, protein kinase C; PKC\(\lambda/\iota\), atypical protein kinase C lambda/iota; PKC\(\zeta\), atypical protein kinase C zeta; TGF\(\beta\), transforming growth factor \(\beta\); NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; TNBC, triple-negative breast cancer.

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We recently demonstrated that PKC\(\lambda/\iota\) signaling is an important contributor to breast cancer development. Strikingly, PKC\(\lambda/\iota\) signaling is also important to balance self-renewal \textit{versus} differentiation in pluripotent stem cells and is essential for embryonic development. This commentary highlights some key functions of PKC\(\lambda/\iota\) signaling that are integral to both normal development and cancer progression.

Breast cancer is a heterogeneous disease and the coexistence of intratumor and intertumor heterogeneity imposes extreme challenges in the development of successful therapy. A single tumor contains multiple cell types and tumors can differ from each other significantly. Considering the similarities between tumor development and embryonic development, intratumor cellular heterogeneity in cancer models is often explained by the existence of cancer stem cell-like or tumor-initiating cell populations. These stem-like tumor initiating cells are implicated in recurrence of the disease after successful initial therapy. In fact, signaling pathways that govern normal cellular differentiation processes are often linked to cancer development, and recent studies suggest that embryonic stem cells (ESCs) share overlapping gene expression signatures, which predict the clinical outcome of cancer patients.\(^1,3\) These discoveries indicate that detailed research is required to define and understand the signaling pathways that regulate stem cell populations during normal development as well as tumor progression. Understanding these pathways may lead to the development of more robust therapeutic options for the management of heterogeneous diseases like breast cancer.

Recently, we discovered that protein kinase C (PKC) signaling, especially the functions of the atypical PKC isozymes PKC zeta (PKC\(\zeta\)) and PKC\(\lambda/\iota\), is crucial for balancing self-renewal \textit{versus} differentiation of pluripotent stem cells (PSCs).\(^4-6\) We found that inhibition of PKC isozymes maintains self-renewal in ESCs and facilitates reprogramming of differentiated cells toward induced pluripotent stem cells (iPSCs). We also observed that PKC inhibition-mediated maintenance of ESC self-renewal and cellular iPSC generation is associated with a molecular signature characteristic of the mesenchymal to epithelial transition (MET) process. Since the reverse process of MET, epithelial to mesenchymal transition (EMT), has been implicated in self-renewal of cancer stem/progenitor cells and tumor invasion,\(^7\) we investigated the activity of one of the PKC isozymes, PKC\(\lambda/\iota\), during breast cancer progression\(^8\) and found that expression and activation of PKC\(\lambda/\iota\) were induced in human invasive ductal carcinoma (IDC) with triple-negative status compared to non-invasive ductal carcinoma \textit{in situ} (DCIS) and normal breast tissue. In addition, metastatic breast cancer, an advanced stage of disease, also showed high levels of PKC\(\lambda/\iota\) expression and activation. RNA interference-mediated inhibition of PKC\(\lambda/\iota\) signaling dramatically prevented growth, invasion, and metastasis of triple-negative breast cancer (TNBC) in experimental animal models. These observations highlighted PKC\(\lambda/\iota\) signaling as a rational clinical target for breast cancer.
Interestingly, PKC\(\lambda/\iota\) signaling is therapeutically targetable. Thus, our discovery is highly encouraging since the paucity of targeted therapy for TNBC remains a critical problem in breast oncology. Another intriguing question in the field of breast cancer is how to predict the progression of invasive disease. Importantly, we described a PKC\(\lambda/\iota\)-regulated gene signature that is differentially expressed in normal breast, DCIS, and IDC tissue, and demonstrated that differential expression of this gene signature significantly predicted poor clinical outcome (relapse or death) of breast cancer patients. These observations indicate that PKC\(\lambda/\iota\)-regulated genes have the promise to predict invasive progression of breast cancer as an independent variable and that specific patterns of gene expression are significantly associated with poor clinical outcome in breast cancer patients. Thus, our discoveries shed light on a very challenging and clinically impactful area of breast cancer and could possibly serve as the starting point for further preclinical evaluation of PKC\(\lambda/\iota\)-targeted therapies during invasive progression of breast cancer.

Breast cancer stem cells or tumor initiating cells are resistant to chemotherapy and major research efforts are currently focusing on identifying treatments that shift them toward a more differentiated phenotype, thus making them more susceptible to chemotherapy. Interestingly, our study on PKC\(\lambda/\iota\) signaling in PSCs indicated that inhibition of PKC\(\lambda/\iota\) signaling in these cells allows them to self-renew and inhibits their multilineage differentiation. Using breast cancer models we have demonstrated the putative benefits of targeting PKC\(\lambda/\iota\) signaling in cancer treatment, showing that inhibition of PKC\(\lambda/\iota\) signaling is effective for inhibition of tumor progression, at least in animal models. One might imagine that if tumor cells could be restricted at their primary site of origin by inhibiting differentiation processes, the chances of metastasis will be reduced significantly and surgical removal of the primary tumor will be more effective with less fear of recurrence.

We found that functionally active phospho-PKC\(\lambda/\iota\) molecules are predominantly localized in the nucleus of breast cancer cells. Cytokines like TGF\(\beta\) and IL1\(\beta\) could promote phosphorylation of PKC\(\lambda/\iota\) and facilitate nuclear translocation of phospho-PKC\(\lambda/\iota\). Furthermore, our global gene expression analysis indicated that PKC\(\lambda/\iota\) signaling regulates a number of transcription factors, including NFkB p65, in breast cancer cells. Interestingly, many of these PKC\(\lambda/\iota\) regulated transcription factors are indicated to be important for breast cancer progression. Thus, our study identified a TGF\(\beta\)/IL1\(\beta\)–PKC\(\lambda/\iota\)–NFkB p65 signaling axis that appears to be important for acquisition of metastatic potential in breast cancer (Fig. 1). The detailed mechanism of this signaling axis and its regulation is yet to be defined. Nevertheless, the nuclear localization event of active PKC\(\lambda/\iota\) molecules opens up several possibilities. It is tempting to speculate that functionally active phospho-PKC\(\lambda/\iota\) might be directly influencing the cellular transcription program in cancer cells. Future investigations will define how phospho-PKC\(\lambda/\iota\) nuclear translocation affects the gene expression program to promote breast cancer progression.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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**Figure 1.** Involvement of PKC\(\lambda/\iota\) signaling in invasive progression of breast cancer. Cytokines such as transforming growth factor \(\beta\) (TGF\(\beta\)) or interleukin-1 \(\beta\) (IL1\(\beta\)) can induce phosphorylation (indicated by attached “\(p\)”) and nuclear translocation of atypical protein kinase C lambda/iota (PKC\(\lambda/\iota\)) in invasive breast cancer cells. Active PKC\(\lambda/\iota\) signaling regulates the nuclear factor kappa-\(\beta\) p65 (NFkB p65)-mediated transcription program to express genes associated with invasive progression/metastasis of breast cancer.
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