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Integrin-linked kinase 1: role in hormonal cancer progression

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1. ABSTRACT

Integrin-linked kinase 1 (ILK1) is a serine/threonine kinase that plays important roles in a variety of cellular functions including cell survival, migration and angiogenesis. ILK1 is normally expressed in numerous tissues and activated by growth factors, cytokines and hormones. Dysregulation of ILK1 expression or function is found in several hormonal tumors including breast, ovary and prostate. Emerging evidence suggests that ILK overexpression promotes cellular transformation, cell survival, epithelial mesenchymal transition (EMT), and metastasis of hormonal cancer cells while inhibition of ILK1 reduces tumor growth and progression. The recent development of ILK1 inhibitors has provided novel mechanisms for blocking ILK1 signaling to curb metastasis and therapy resistance of hormonal tumors. This review will focus on recent advances made towards understanding the role of ILK signaling axis in progression of hormonal cancer.

2. INTRODUCTION

ILK1 is a 59 kDa cytoplasmic protein that contains three distinct domains: (a) a phosphoinositide phospholipid-binding domain that mediates phosphoinositide binding, (b) an N-terminal ankyrin repeat domain that facilitates protein interactions and (c) a C-terminal serine/threonine protein kinase domain (1). ILK1 is a serine/threonine kinase that was first discovered as an integrin-binding protein in a yeast two-hybrid screen (2). It is able to directly activate several signaling pathways downstream of integrins and to participate in integrin signaling crosstalk with growth factors and hormones (3, 4). Substrates of the ILK1 include integrin β1 (2), myosin light chain (MLC) (5), protein kinase B /Akt (AKT) and Glycogen synthase kinase 3 (GSK-3) (6). ILK1 is a unique kinase because it also functions as an intracellular adaptor protein, coupling a wide variety of signaling proteins to integrin and growth factor signaling. ILK interacting proteins include Pinch (7), Paxillin (8), Parvins (9), Affixin

(10), ILK BP (11), p21 activated kinase 1 (Pak1) (12) and estrogen receptor (ER) (13). Physiological signals including growth factors (6), cytokines (14, 15) estrogen (16), and the Wnt pathway (17) can activate ILK. Direct regulators of ILK include phosphoinositide 3-kinase (PI3K) (6), phosphatase and tensin homolog (PTEN) (18), protein phosphatase 2C (19), ILKAP (20) and secreted protein acidic and rich in cysteine (SPARC) (21). Accumulating evidence implicates ILK1 as a potential oncogene modulating several signaling pathways for cancer cell survival and tumor progression (22). Here, we will summarize key evidence for ILK signaling in hormonal tumor progression and discuss the possibility of the ILK1 axis as a possible therapeutic target for hormonal cancers.

3. DISCUSSION

3.1. ILK1 signaling and tumorigenesis

ILK1 signaling axis is implicated in many key signaling pathways that are activated in tumor cells promoting anchorage independence, motility, apoptosis, angiogenesis, EMT and tumor progression (22). Overexpression of ILK in epithelial cells enables anchorage-independent growth and survival of tumor cells (22-24) and tumorigenicity in nude mice (25). ILK1 overexpression in prostate cancer cells can suppress anoikis, promote anchorage-independence, and induce tumorigenesis (22). Accordingly, inhibition of ILK1 in prostatic adenocarcinoma (CaP) cells elicits cell cycle arrest and induces apoptosis (26).

Compared to normal cells, breast cancer cells appear to be preferentially dependent on the ILK1 signaling for survival (27). ILK1 function is also required for cytokine osteopontin (OPN)-induced AKT activation and for prostate cancer cell survival (28). Rictor, a known regulator of cytoskeletal dynamics, interacts with ILK1 to promote AKT phosphorylation leading to cell survival in cancer cells (29, 30). Transgenic mice expressing ILK1 in the mammary epithelium (MMTV-ILK1) develop a hyperplastic mammary phenotype and focal mammary tumors (31). These results provide strong evidence for an *in vivo* oncogenic role for ILK1 (31). Given the focal nature and long latency of the tumors, additional genetic events are likely required for tumor induction in MMTV-ILK1 mice.

ILK1-mediated AKT Ser473 phosphorylation may be celltype and context dependent. For example, genetic studies in *Drosophila Melanogaster* and *Caenorhabditis elegans* show AKT phosphorylation on Ser473 was not affected in ILK 1mutants (32, 33) and levels of Ser473 phosphorylation on AKT were equivalent in ILK1-null and wild-type mouse fibroblasts (34). The majority of analyses using tumor cells indicate that AKT Ser473 phosphorylation is dependent on a functional ILK1 axis; hence, the ILK1 pathway may be important during epithelial tumor progression presumably by promoting cell survival. In breast cancer cells, inhibition of ILK1 activity results in a decrease in AKT Ser473 phosphorylation and induction of apoptosis; whereas inhibition of ILK1 in normal cells has no such effects. These findings suggest that ILK1 promotes survival function uniquely in breast cancer cells. ILK1 targeted treatment using specific ILK1 inhibitors may therefore have potential to reduce side effects in cancer patients (27).

Evidence also implicates ILK1 in regulating tumor angiogenesis; ILK1 increases vascular endothelial growth factor (VEGF), modulate levels of hypoxia inducible factor (HIFα ____ blood-vessel formation and tumor growth of VEGF-treated endothelial cells (35, 36). In ovarian cancer cells, ILK1 serves as a key mediator in transforming growth factor (TGF) β1 regulation of uPA/PAI-1 system, which is critical for the invasiveness of human ovarian cancer cells (37). ILK1 promotes epithelial to mesochymal transformation (EMT) of cancer cells by modulating β-catenin/TCF, Snail and TGFβ pathways (38-40). Collectively, these evolving findings indicate ILK1 signaling has the potential to activate multiple signaling pathways that contribute to the growth advantage of cancer cells.

3.2. Expression of ILK in hormonal cancers

While ILK1 is normally expressed in many hormonal tissues, emerging evidence implicates dysregulation of ILK1 expression and/or activity in many cancers including those of the breast, prostate and ovary (22). ILK1 expression increases as ovarian tumor grade and its expression can be sustained by peritoneal tumor fluid (PTF). PTF-induced over-expression of ILK correlates with the activation of the AKT pathway (41). Thus, ILK1 has potential to serve as a biological marker for early detection and a therapeutic target for ovarian cancer (41).

One study found that serum from ovarian cancer patients contains cell-free immunoreactive ILK1 at statistically elevated levels compared to controls without ovarian cancer (42); ILK1 was present at elevated levels in both the serum and PTF of ovarian cancer patients. The correlation between ILK1 expression with CA125 concentration in these biological fluids suggests a potential role of ILK1 as a serological ovarian tumor marker for early detection and treatment monitoring (43). Integrin alphavbeta3 upregulates ILK1 expression in human ovarian cancer cells via enhancement of ILK1 gene transcription. Mechanistic studies show that transcription factor Ets contributes to alphavbeta3-mediated ILK1 upregulation. By increasing ILK1 as an important integrin-proximal kinase, alphavbeta3 may promote its intracellular signaling and tumor biological processes (42).

ILK1 mRNA is upregulated in prostate adenocarcinoma cells compared to normal epithelial cells and therefore, can be a useful internal reference gene marker (44). ILK1 expression also increases with prostate tumor grade and is specifically associated with the increased proliferative index that typifies CaP progression. Further, enhanced ILK1 expression is inversely related to 5-year patient survival linking increased ILK1 expression in prostate tumor progression (26). b-parvin (ParvB) is an adaptor protein that binds to the ILK1. Expression studies indicated ParvB expression was down regulated in breast tumors compared to ParvB expression in patient-matched

normal mammary gland tissue. These results suggest that loss of ParvB expression could be a mechanism for upregulating ILK1 activity in tumors (9).

3.3. Role of ILK1 in cancer cell metastasis

Metastasis is a frequent and fatal culmination to hormone-sensitive cancers, particularly of ovarian origin. Acquisition of invasive and migratory characteristics in cancer cells results primarily from adopting an EMT phenotype. This phenotype is supported by various pro-metastatic factors. Emerging studies have unraveled the role of ILK1 in governing metastatic features in various cancers, predominantly for acquiring the mesenchymal phenotype and promoting cell invasion through increased expression of various matrix-degrading proteases.

Increased expression of ILK1 correlates significantly with higher grade of ovarian tumors (41, 43). ILK1 played a predominant role in endothelin-1 (ET-1/ETAR)–induced EMT and in the development of the invasive phenotype in ovarian cancer by increasing levels of Snail, stabilizing beta-catenin and suppressing E-cadherin expression through a PI3K-dependent signaling pathway. Also, enhanced expression and activity of matrix metalloproteinases (MMP-2 and MMP-9) mediated by ET-1 correlated with increased ILK expression (45). In addition, PI3K-ILK1 axis played a critical role in TGF β1-mediated invasive phenotype in ovarian epithelial cancer cells via up-regulation of urokinase-type plasminogen activator (uPA) and PA inhibitor 1 (PAI-1). It is worthwhile to note that expression of uPA and PAI-1 were reported to correlate with advanced stages of ovarian cancer (46). Unlike ET-1, the TGF β1-mediated increase in MMP-2 expression was found to be independent of ILK1 signaling (37). Similarly, Y-box-binding protein 1 (YB-1), a known, poor prognostic marker of ovarian cancer, localized in the nucleus and enhanced CXCR4 expression for acquiring the malignant phenotype (47). Interestingly, siRNA depletion of ILK1 and AKT affects both nuclear translocation of YB-1 and expression of CXCR4 in ovarian cancer cells, suggesting that disrupting ILK1-AKT pathway can be used to block YB-1–mediated metastasis (48).

One of the earliest insights into the potential of ILK1 to govern the metastatic phenotype came from the Dedhar lab. They demonstrated that stable overexpression of ILK1 in scp2 murine mammary gland epithelial cell lines induced the classic EMT phenotype including reduction in E-cadherin along with translocation of β-catenin and formation of β-catenin/LEF complex inside the nucleus and thus upregulating expression of various mesenchymal genes (49). Similarly, Somasiri *et al.*, found that forced exogenous expression of wild type ILK1 but not the dominant negative kinase-dead version of ILK in scp2 murine mammary epithelial cell lines induced the EMT phenotype via reduction in E-Cadherins and acquisition of vimentin filaments (39). Suppression of anoikis, a unique process of apoptosis resulting from insufficient cell-matrix adhesion, appears to be an important event in the development of metastasis (50). Studies using both scp2 murine mammary cell lines and human breast cancer cell lines implicated ILK1 as a suppressor of anoikis (24). Inhibition of cell death/anoikis by ILK overexpression supports the idea that ILK is a predominant player in regulating the emergence of the metastatic phenotype. Subsequent to these studies, ILK-mediated induction of the invasive phenotype in mammary epithelial cells was found to be associated with increased MMP-9 expression. This increase in MMP-9 proteins was attributed to ILK-mediated activation of GSK-3beta and AP-1 transcription factor (51). The ILK1-AP1 axis was further shown to contribute to the invasive phenotype in mammary gland epithelial cells mediated by osteopontin (OPN), a metastasis-associated glyco-phosphoprotein. Using the murine metastatic mammary epithelial cells 4T1, Mi *et al.*, demonstrated that an OPN-mediated increase in expression of MMP2 and uPA can be attributed to ILK1-dependent AP-1 activation (14). Transgenic mice specifically expressing ILK in mammary glands had increased mesenchymal-like cell populations within their tumors, suggesting that stand-alone ILK1 overexpression can initiate the EMT phenotype (31). Another piece of evidence connecting ILK1 to anoikis is from a study that demonstrated the role of the tumor suppressor DOC-2/hDab-2 in the induction of anoikis in breast cancer cells. DOC-2 was shown to induce anoikis by down regulating ILK activity but this activity was found to be independent of the PI3K/AKT and MAPK pathways, suggesting that ILK1 may utilize alternate pathways to suppress anoikis and promote anchorage independence (52). Estrogen-mediated extranuclear functions are also shown to activate ILK1. Since PELP1 expression is upregulated in metastatic breast cancer (53); modulation of the ILK1 pathway by PELP1 may represent a potential mechanism by which estrogen signaling promotes metastasis in breast cancer cells (54).

Relatively fewer studies have been done to elucidate the role of ILK1 in metastatic prostate, cervical and endometrial cancers. A recent study using prostate cancer cell lines implicated ILK1 as a downstream effector for talin1-mediated resistance to anoikis (55). It is worthy to note that talin1 was found to be overexpressed in metastatic prostate cancer and tumors with a high Gleason score when compared to its expression in normal Gleason scores and benign tumors (55). PI3K/AKT-dependent anoikis has also been found in endometrial cancer cell lines but the role of ILK1 in governing this anoikis has not yet been demonstrated (56). Similarly, Notch1-Rhoc axis and anoikis are reported to regulate the metastatic potential in cervical cancer progression but the role of ILK1 in these cancers remains elusive (57).

3.4. ILK and ER signaling crosstalk

Several lines of evidence implicate ILK1 axis crosstalk with ER signaling. One of the lacunae in our understanding of the mechanistic details of the metastatic evolution of these hormonal cancers and how hormones like estrogen and their respective steroid receptors regulate ILK1 pathway. Dr. Kumar's group provided first evidence of estrogen receptor (ER)-ILK crosstalk. They showed a direct interaction of ER with ILK1 and that the interaction occurred through the nuclear receptor box (i.e., LXXLL) located between the ILK1 pleckstrin homology–like domain and the ankyrin repeats (58). In addition, we

recently identified ILK1 as a novel interacting protein of PELP1, an ER-coregulator protein (54). Our study demonstrated that ILK functions as a downstream effector of ER extranuclear signaling, leading to cytoskeleton reorganization. These extranuclear actions of estrogen facilitated activation of the ILK enzyme via the PI3K pathway and inhibition of ILK functions significantly affected the estrogen-mediated cell migratory potential. The proposed signaling pathway is E2>PELP1>PI3K>ILK>CDC42 and it may contribute to estrogen mediated cytoskeleton changes (54). Earlier evidence suggests that the ILK1 axis is a major signaling node that links integrins and growth factor signaling to a variety of cellular responses. The ability of ILK1 to interact with ER and growth factor/integrin signaling components suggests that deregulation of ILK has the potential to promote ER growth factor crosstalk and thus a potential to contribute to therapy resistance.

3.5. ILK1 and hormonal therapy resistance

Deregulation of human epidermal growth factor receptor 2 (ErbB2) expression and /or signaling has emerged as the most significant factor in the development of hormonal resistance (59). ErbB2 is an oncogene that has been shown to be over expressed, amplified, or both, in several human malignancies including breast tumors. ER expression occurs in ~50% ErbB2 positive breast cancers and crosstalk between the ER and ErbB2 pathways promotes endocrine therapy resistance (58, 59). Disruption of ILK expression by siRNA or inhibition of ILK1 function in ErbB2-expressing cells with a small molecule inhibitor resulted in a profound block in invasive properties resulting from the induction of apoptotic cell death. These observations support the concept of ILK1 having a critical role in the initiation phase of ErbB2 tumor induction (61).

AKT signaling plays an important role in the development of hormonal therapy resistance (62). Many hormonal tumors exhibit an increase in constitutively active AKT; however, mutations in AKT are rare in breast tumors (62). Therefore, proteins contributing to AKT activation may play a role in the development of therapy resistance. In this context, several lines of evidence indicate that ILK is a receptor-proximal effector for the PI3K-dependent, extracellular matrix- and growth factor-mediated activation of PKB/AKT and inhibition of GSK-3 (6). Since ILK1 expression is deregulated in hormonal cancer, increases in ILK1 signaling has the potential to contribute to therapy resistance.

Nuclear localization of PAK1, a proto-oncogene (63), is associated with the progressive limitation of tamoxifen sensitivity and implicated in development hormonal therapy resistance (64). ILK1 is a PAK1 substrate, and undergoes phosphorylation-dependent shuttling between the cell nucleus and cytoplasm, and interacts with gene-regulatory chromatin, thus ILK1-PAK1 interactions may have a role in therapy resistance (12).

Cyclin D1 overexpression commonly occurs in breast cancer. The level of cyclin D1 expression and activated STAT3 are important markers to predict response to tamoxifen treatment (65). ILK1 signaling increases cyclin D1 protein levels (65). Mechanistic studies showed that ILK-induced CREB transactivation and CREB binding to the cyclin D1 promoter CRE led to cyclin D activation. Wnt-1, an oncogene implicated in mammary tumorigenesis also induced cyclin D1 mRNA via ILK pathway (65).

ER-coregulators play an essential role in hormonal therapy responsiveness and cancer progression (67). Recent findings suggest that ILK1 interacts with ER-coregulator PELP1 (54) and that such interactions enhance ILK1-kinase activity. Since PELP1 expression is commonly deregulated in many hormone-responsive tissues (16), the PELP1-ILK1 interaction is likely to have significant implications in tumor cell survival and therapy resistance.

3.6. Therapeutic potential of targeting ILK in hormonal cancers

Current strategies to block the ILK1-mediated phenotype using *in vitro* systems include the usage of small molecular inhibitors (like KP-392/KP-SD-1 and QLT-0267) (27, 57, 68) and ILK-targeted siRNAs and antisense oligonucleotides (69). Also, the use of a dominant negative ILK (ILK-E359K) has been proposed. Initial work towards generating potent small molecule inhibitors against ILK1 was accomplished by Dedhar and colleagues in collaboration with Kinetek Pharmaceuticals (now a part of QLT Inc). As a result the KP-392/KP-SD-1 and KP-SD-2 compounds were identified and shown to inhibit ILK-mediated AKT activation and the EMT phenotype when tested using *in vitro* model cells. The use of KP-SD-1 was highly encouraging when tested on human colon carcinoma cells using an *in vivo* xenograft transplantation assay (70). QLT-0267 is a second-generation ILK1 inhibitor that is more potent with increased sensitivity over the parental KP-392 compound (27). Interestingly, QLT-2067 induces apoptosis in the breast cancer cell lines MDA-MB-231, MDA-MB-435, BT-549, and MDA-MB-468, but not in normal human breast epithelial cells at a concentration of 10 μmol/L or less.

Some evidence indicates blockage of ILK1 signaling along with conventional chemotherapy may be beneficial. QLT-0267 in combination with docetaxel exhibited synergistic effects on reducing the viability of various breast cancer cells (68). A remarkable observation made in this study was that the ErbB2 status of cells has a definitive effect on this combinatorial treatment. Low ErbB2-expressing cells were more sensitive to this combination when reduction of phospho-AKT was used as endpoint for assessing the efficacy. Further, this combination was found to be more effective than the single treatment in reducing the tumor burden and prolonging survival in an orthotopic breast cancer model using transplanted LCC6 cells, which have reduced ErbB2 expression. Similarly, the ILK1 inhibitor KP-307-2, an analog of KP-392, was found to suppress tumorigenesis in xenograft tumor models using the prostate cancer cell line PC3. Surprisingly, a novel feedback mechanism between ILK and VEGF expression was also observed in this study and therefore treatment with ILK1 inhibitor causes a

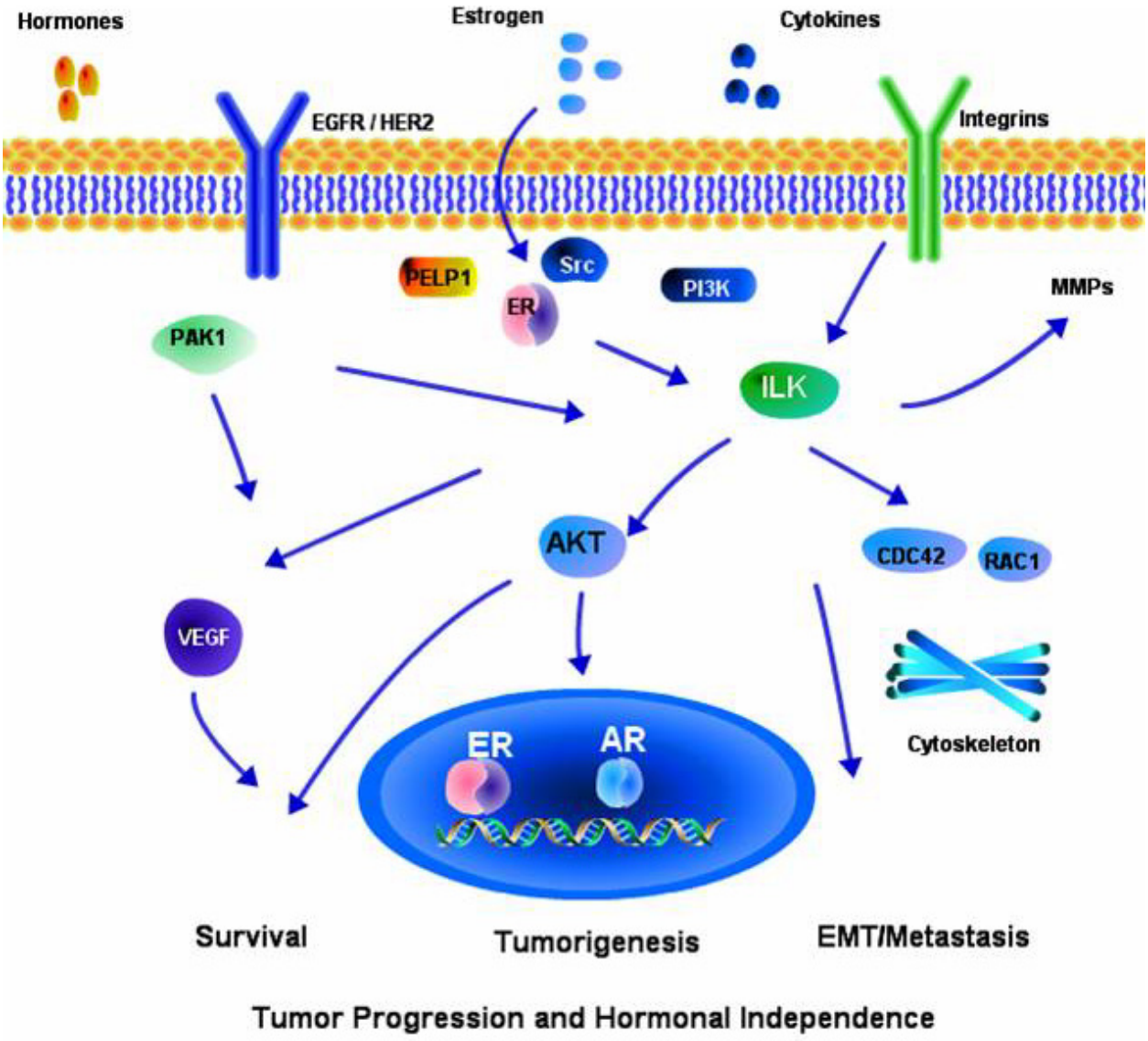

Figure 1. Schematic representation of the current understanding of ILK1 signaling crosstalk with pathways that are commonly deregulated in hormonal cancers. Crosstalk of ILK axis with the estrogen receptor, ERBB2, Pak1 signaling pathways suggest that deregulation of ILK expression and/or function is likely to contribute to the hormonal cancer progression and development of therapy resistance.

'double jeopardy' situation in the cells by causing inhibition of tumorigenesis and suppressing angiogenesis (35).

The second generation ILK1 inhibitor QLT-0267 may be useful in radiosensitizing cancer cells, particularly squamous cell carcinoma cells of head and neck and also, engenders the possibility of similar effect on gynecologic cervical carcinoma that is predominantly squamous cell carcinoma upon histological type (71). Overall, it appears that ILK1 inhibitors, although at various levels of development, have the potential to down regulate ILK1 activity, the ILK1-mediated EMT phenotype and tumorigenesis when tested using *in vitro* and various pre-clinical mouse models.

4. FUTURE PERSPECTIVES

In summary, the data reviewed herein provides support for the following conclusions:(a) deregulation of ILK1 expression and/or functions occurs in human hormonal cancers; (b) inhibition of ILK1 correlates with delayed tumor growth in preclinical models; (c) ILK1 can modulate key signaling pathways including cell survival, tumor growth, angiogenesis, EMT and metastasis; and (d) ILK1 crosstalk with various signaling pathways that are commonly deregulated in hormonal cancers including

ErbB2, PAK1 and ER (Figure 1). Thus, these data strongly support a role of ILK1 in the hormonal cancer progression. The recent availability of drugs that specifically target ILK1 has begun to open up new avenues for targeting hormonal tumors. Most interesting is the difference in sensitivity to the effects of ILK inhibition between normal breast epithelial and breast cancer cells, provide a potential for the use of ILK1 inhibitors in patient therapy. Future studies using combination of ILK inhibitors with drugs that target hormone therapy could possibly be done to achieve significant reduction in various hormonal cancers. Future studies are warranted to identify the signaling pathways that regulate ILK1 expression in hormonal cancers and to examine the prognostic / diagnostic significance of ILK1 using larger number of tumor samples. A better understanding of the ILK1 signaling and its crosstalk with hormonal signaling components is expected to assist in the development of an integrated model for targeting ILKs in the management of hormone-driven tumors.

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Abbreviations: AKT: protein kinase B; EMT:epithelial mesenchymal transition; ErbB2: epidermal growth factor receptor2; ER: estrogen receptor; ILK1: integrin linked kinase 1; Pak1:p21 activated kinase 1; ; PI3K: phosphotidyl inositol 3 kinase. TGF:transforming frowth factor.

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