乳腺疾病病理学及基础研究专题
· 综述 ·

P21-activated kinase 1 and breast cancer

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The p21-activated kinase 1 (PAK1) belongs to PAKs family, a group of highly evolutionarily conserved protein family of serine/threonine kinases, which acts as a downstream effector of the small GTPases Cdc42 and Rac1, firstly reported in 1994[1]. As a serine/threonine kinase, PAK1 plays an important role in many cellular functions including cell morphogenesis, motility, survival, mitosis, angiogenesis, and tumorigenesis. More than 40 proteins have been reported to be phosphorylated by PAK1[2]. Accumulating experimental data in multiple experimental systems provide compelling evidence that PAK1 plays an important role in breast cancer promotion and progression. PAK1 is overexpressed and/or hyperactivated in more than 50% of breast cancers[3]. On the other hand, PAK1 overexpression in estrogen receptor-alpha (ER-α) positive breast cancer is also closely associated with a reduced responsiveness to tamoxifen therapy[4]. Since PAK1 plays such a vital role in breast cancer, PAK1-targeted therapeutic approaches are likely to be useful in breast cancer treatment as well as in other human cancers with PAK1 upregulation and/or hyperactivation[5].

1 PAK1 signaling in normal mammary development

PAK1 plays an important role in the normal mammary gland development. During pregnancy and lactation, both PAK1 protein and its kinase activity are up-regulated in the murine mammary glands[6]. Interestingly, PAK1 directly phosphorylates Stat5a transcriptional factor at Ser779, leading to translocation of the phosphorylated Stat5a to the nucleus

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and consequently, stimulation of β-casein-promoter activity and increased milk protein expression; mutagenesis of Ser779 of Stat5a to Ala (Stat5a-S779A) completely inhibits the ability of PAK1 to stimulate β-casein promoter activity. These findings support the idea of an essential role of PAK1 and/or its targets in alveolar morphogenesis and differentiation of the mammary gland.

2 PAK1 phosphorylation substrates and pathogenesis of breast cancer

Over the years, PAK1 kinase has been shown to phosphorylate more than 40 substrates, including ER-α[2]. For example, PAK1 phosphorylate ER at Ser305 and promotes its transactivation activity, leading to ER-target gene expression in a ligand-independent manner. These phenotypic effects of PAK1 in-turn translate into hyperplasia in the mammary epithelium[7]. Furthermore, overexpression of catalytically active PAK1 in mammary epithelium also leads to the development of mammary tumors as well as other breast lesions such as adenoma. Interestingly, PAK1 expression and nuclear accumulation progressively increase during the transition from ductal hyperplasia to *in situ* ductal carcinoma and to adenocarcinoma[7]. Moreover, ER-α and progesterone receptor (PR) expressed at the early stage of lesion, but dismissed during the cells' transition to malignant invasive tumors[8]. Estrogen (E2) regulation of PAK1 and forkhead transcription factor (FKHR) signaling pathways are likely to be a potential contributor to the noted phenotypic effects of PAK1 in breast cancer cells. Estrogen induces PAK1 activity which in turn, promotes its ability to interact and phosphorylate FKHR, leading to its perinuclear localization in the cytoplasm in a PAK1-dependent manner[9]. There are also mounting evidence to demonstrate that PAK1 expression correlates well with breast tumor grades in human. For example, PAK1 expression is elevated in less-differentiated breast ductal carcinoma (grade III) than that in grade II and grade I tumors[10].

There is a growing realization of the fact that breast cancer promoting functions of PAK1 may not be necessarily driven by PAK1-overexpression

but are likely to be mediated by the activity of its substrates. For example, dynein light chain 1 (DLC1) protein, a physiological PAK1 substrate, is itself overexpressed in more than 90% of human breast tumors, and overexpression of DLC1 promotes cancerous properties of breast cancer cells[11]. PAK1 interacts with DLC1 by phosphorylating it on Ser88, which plays an essential role in cell survival. Moreover, PAK1 also upregulates Cyclin D1 pathway by stimulating Cyclin D1 transcription through NF-κB-dependent pathway and such events could be also documented in the hyperplastic regions of the mammary glands in a murine model expressing catalytically active PAK1 transgene[3]. In addition to a direct stimulation of the Cyclin D1 transcription independent of ER status, PAK1 can also upregulate Cyclin D1 expression via ER-signaling involving PAK1 phosphorylation of ER-α at Ser305 which in-turn is sufficient to transactivate Cyclin D1 transcription[12]. Interestingly, Cyclin D1 overexpression can be noticed at the earliest stage of breast cancer progression and maintained throughout various stages of progression[13]. ESE-1 is an epithelium-specific ETS (E-twenty six) transcription factor which transforms human breast epithelial cells through a serine- and aspartic acid-rich domain (SAR). Again, PAK1 can selectively phosphorylate ESE-1 at Ser207 which in-turn, might be another contributing component of the mammary gland tumorigenesis[14].

3 The role of PAK1 in invasiveness of breast cancer

Accumulating evidence suggests that PAK1 is involved in breast cancer invasiveness. PAK1 activation in breast cancer is accompanied by the disassembly of stress fibers and focal adhesion complexes, a fundamental requirement for cancer cell invasion. Heregulin-beta1 (HRG), a ligand for Her3 and Her4, stimulation of non-invasive breast cancer cells has been shown to promote conversion of globular to filamentous actin and formation of membrane ruffles, stress fibers, filopodia, and lamellipodia, and collectively, all these events promote cell migration and invasiveness. Since HRG stimulates PAK1 kinase activity via phosphatidylinositol-3 kinase

(PI3-K), inhibition of the PI3-K activity blocks the activation of PAK1 activity as well as invasiveness of HRG-stimulated breast cancer cells[15]. To further support the role of PAK1 in breast cancer, Vadlamudi *et al*. have demonstrated that regulatable expression of PAK1 stimulated anchorage-independent growth of human breast cancer cells in soft agar in a preferential mitogen-activated protein kinase (MAPK)-sensitive manner[16]. There is also evidence of a close correlation between the levels of PAK1 activity and baseline invasiveness of human breast cancer cells as well as breast tumor grade[16].

Furthermore, PAK1 signaling also participates in the breast cancer progression by supporting the process of epithelial-mesenchymal transition (EMT) which plays a key role in the conversion of early stage tumor into invasive malignancy[17]. For example, PAK1 phosphorylates Snail, a master regulator of EMT, on Ser246 which in-turn, leads to activation of the corepressing activity of Snail for the E-cadherin promoters, allowing transcriptional repression of E-cadherin and associated promotion of EMT. In support of this notion, a Ser (246) Ala substitution in Snail or PAK1 knockdown by short interference RNA blocked PAK1-mediated Snail phosphorylation, leading to increased cytoplasmic accumulation of Snail and attenuation of Snail repressor activity in breast cancer cells[17]. In another study involving 3D-culture model, the levels of PAK1 expression and activation were increased as a function of malignant progression, while selective PAK1-knockdown reduced pericelluar proteolysis, cell proliferation, and migration/invasion. Thus, it appears that PAK1 overexpression and activation act as a coordinator for aberrant cell survival and proteolysis in breast cancer progression[18]. A recent study further demonstrated that SCRIB-complex associates with PAK1 or PAK2 and plays a crucial role in the modulation of the cell migration. In this study, PAK1 behaved as a binding partner for the SCRIB-complex at the leading edge of migratory epithelial cells, and thus, promoting actin polymerization and contributing to cytoskeleton remodeling[19].

4 PAK1 and angiogenesis in growth and metastasis of breast cancer

The process of tumor growth and metastasis is also profoundly influenced by angiogenesis, a biologic process responsible for the formation of new blood vessels from existing blood vessels, which provides nutrient flow to solid tumor. The onset of tumor angiogenesis relies on the production of angiogenic factors by tumor cells and/or by tumor microenvironment. One of the best characterized angiogenic proteins is vascular endothelial growth factor (VEGF), which is regulated by a number of cytokines, growth factors, and oncogenes. PAK1 signaling is also essential for VEGF expression and consequently, its function[20]. For example, kinase-active T423E PAK1 promotes the expression and secretion of VEGF in human breast cancer cells, while kinase-dead K299R PAK1 suppresses VEGF promoter activity, as well as VEGF mRNA expression and VEGF protein secretion. Furthermore, Heregulin-β1 regulates angiogenesis through up-regulation of VEGF, while PAK1 is essential in controlling VEGF expression, secretion, and function. Treatment of cells with a dominant-negative PKA1 mutant or an N-terminal proline-rich PAK1 peptide specifically inhibits endothelial cell migration and contractility, hence specifically blocks angiogenesis[21]. In addition, the Rac/PAK1 pathway also contributes to ephrinA1-mediated inhibition of cell spreading, which participates in blood vessel destabilization, a prerequisite for angiogenesis[22]. There are also examples wherein integrin $\alpha_v\beta_3$ regulates PAK1 activity during bFGF-induced angiogenesis via stimulation of extracellular signal-related kinase (ERK) activity[23]. Prostate-specific membrane antigen (PSMA), a transmembrane peptidase overexpressed in the vasculature of solid tumors, might be another contributing player to the noted effects of PAK1 upon angiogenesis. In PSMA-null animals, angiogenesis is severely impaired because endothelial cells can't invade through the extracellular matrix barrier. It has been reported that PSMA is a component of the regulatory loop which modulates laminin-specific integrin signaling and PAK1 activity. PSMA is an important molecular

interface that integrates both extracellular and intracellular signals during angiogenesis[24].

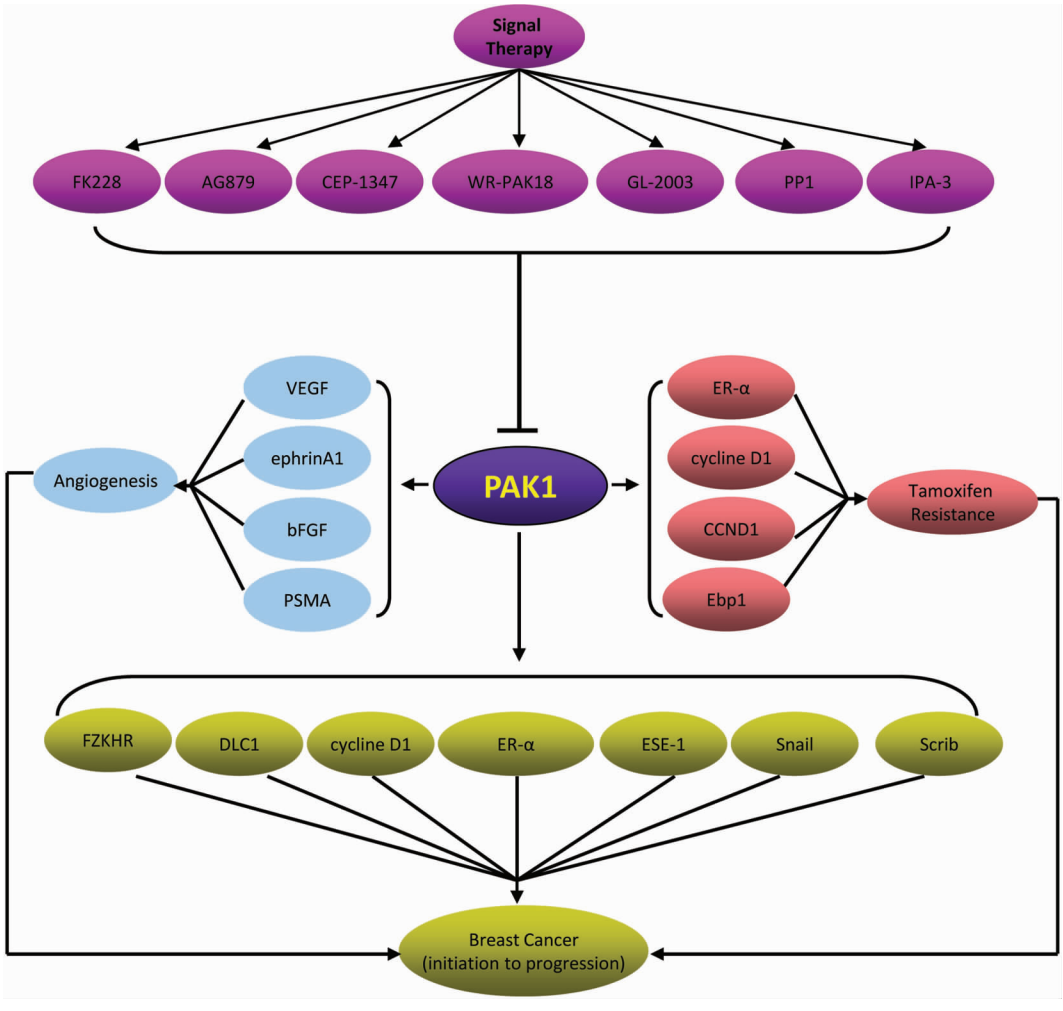
5 The role of PAK1 in tamoxifen resistance

Endocrine therapies for breast cancer have been in use for more than a century[25]. Tamoxifen, a nonsteroidal antiestrogen, which blocks estrogen-stimulated breast cancer growth via binding to ER, has been the preferred endocrine therapy for breast cancer in the past three decades. However, resistance to tamoxifen is a serious obstacle in breast cancer treatment[26]. It's reported that PAK1 expression and subcellular localization is tightly associated with tamoxifen resistance in breast cancer patients. PAK1 expression, especially PAK1 nuclear localization, is intimately linked to tamoxifen resistance in premenopausal breast cancer patients. Among 403 primary breast cancer patients, those who had ER-α positive tumor with low PAK1 expression had better recurrence-free survival after 2 years of adjuvant tamoxifen treatment than those who received no treatment. In vitro study showed that overexpression of wild type PAK1 compromised tamoxifen response *via* stimulating cyclin D1 expression[27]. Another study demonstrated that increased PAK1 activity in breast tumor cells led to acquired-resistance to tamoxifen[28]. Whereas conditional down-regulation of PAK1 in tamoxifen-resistant cells partially restored antiestrogen action of tamoxifen, while transient up-regulation of PAK1 inhibited antiestrogen effect of tamoxifen[28]. PAK1 gene locates at 11q13, a gene-rich region, which contains oncogenes that more or less contribute to positive selection for cell proliferation and survival. One of the most studied oncogenes in this region is the CCND1 gene which encodes the cell-cycle regulatory protein cyclin D1. Cyclin D1 can ligand-independently activate ER-α *via* direct binding, which results in the induction of anti-estrogen insensitivity[29]. PAK1 and CCND1 amplifications in the postmenopausal breast cancer patients are good predictors for tumor recurrence and tamoxifen resistance[30]. Two recent clinical studies show that phosphorylation of ER-α at Ser305 by PAK1 or PKA as well as overexpression of PAK1 are

associated with sensitivity to tamoxifen in breast cancers[4, 31]. Moreover, PAK1 phosphorylates ErbB3 binding protein Ebp1 at Thr261 and then leads to tamoxifen resistance in breast cancer cells[32].

6 PAK1 targeted signal therapeutics and breast cancer

Since PAK1 overexpression is found in 55% human breast cancers[3], as well as PAK1 plays an essential role in breast cancer progression, PAK1 targeted therapeutic approaches are likely to provide an exciting avenue for human breast cancer treatment. FK228, a histone deacetylase (HDAC) inhibitor, can block PAK1 activation in human breast cancer cells, as well as block estrogen-dependent growth of both tamoxifen-sensitive and resistant breast cancers *in vivo*[33]. There are also several small molecule inhibitors such as AG879, CEP-1347 and WR-PAK18, PP1 and GL-2003, which have also been shown to effectively inhibit PAK1 activity in many experimental systems[34-36]. Moreover, allosteric inhibitor such as IPA-3 is a kind of highly selective, cell-permeable PAK1 inhibitor, which covalently binds to PAK1 regulatory domain, prevents PAK1 binding to its upstream activator Cdc42 and thus inhibits PAK1 activation[37-38]. These studies confirm that PAK1 is a potent biomarker for breast cancer (Figure 1). Most recently, Murray and colleagues reported an ATP-competitive, pyrrolopyrazole inhibitor of PAK4, PF-3758309[39]. In cells, PF-3758309 inhibits phosphorylation of the PAK4 substrate GEF-H1 and anchorage-independent growth of a panel of tumor cell lines. In tumor models, PF-3758309 inhibits PAK4-dependent pathways in proteomic studies and regulates functional activities related to cell proliferation and survival. These results provide support for PAK4 as a therapeutic target and identify a potent and orally available small-molecule PAK inhibitor with significant promise for the treatment of human cancers[39].



PAK1 plays an important role in the multistage development process of breast cancer by phosphorylating and activating its substrates. PAK1 overexpression is also associated with resistance to tamoxifen in estrogen receptor positive breast cancer. Thus, PAK1 targeted therapeutic approaches provide a new avenue for human breast cancer treatment.

Figure 1 Summary of the functional roles of PAK1 in breast cancer

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