Nuclear p21-Activated Kinase 1 in Breast Cancer Packs Off Tamoxifen Sensitivity

Suresh K. Rayala,[1] Poonam R. Molli,[1] and Rakesh Kumar[1,2]

[1]Department of Molecular and Cellular Oncology, The University of Texas M.D. Anderson Cancer Center and [2]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas

Abstract

There is significant clinical interest in the factors that influence the development of tamoxifen resistance in estrogen receptor-α (ER-α)–positive breast cancers. Recent studies suggest that in ER-positive breast tumor cells, elevated protein levels, and in particular, nuclear localization of p21-activated kinase 1 (PAK1), is associated with the progressive limitation of tamoxifen sensitivity. These phenotypic effects of PAK1 in model systems are mechanistically linked with the ability of PAK1 to phosphorylate ER-α on serine 305 and subsequent secondary activation of serine 118. These findings prompt further investigation of how nuclear signaling by PAK1 may affect estrogen's action and whether tamoxifen resistance might be prevented or reversed by PAK1 inhibition. (Cancer Res 2006; 66(12): 5985-8)

Introduction

Tamoxifen, a nonsteroidal antiestrogen, has been the preferred antiestrogen hormonal treatment for breast cancer for the past three decades. It is an established therapy which is relevant to the treatment of all stages of breast cancer. However, resistance to tamoxifen frequently develops with extended treatment. This resistance is a serious obstacle in the management of breast cancer. Scientists do not yet have a clear understanding of the molecular mechanisms that underlie the failure of the antiestrogenic action of tamoxifen (tamoxifen resistance) or of the breast cancer progression to more invasive phenotypes or the development of estrogen receptor (ER)–negative breast cancer. Emerging data suggests that elevated p21-activated kinase 1 (PAK1) expression in premenopausal breast cancer patients correlates well with a lack of tamoxifen response despite the presence of ER-α expression (1). This relationship was even distinctly stronger in breast tumors with nuclear PAK1. In this review, we have summarized the current knowledge on recent advances in the field of PAK research with a special emphasis on PAK1 nuclear localization and its role in tamoxifen resistance.

PAK1, one of the major downstream signaling nodules of extracellular stimuli, is the prototypic member of the evolutionarily conserved PAK family of serine-threonine protein kinases with roles in cell survival, migration, and invasion (2–4). Binding of the small GTPases to PAK1 results in autophosphorylation and serine-threonine kinase activity, leading to reorganization of the actin cytoskeleton as well as phosphorylation of a large number of cellular proteins with roles in survival, energy metabolism, proliferation, invasion, and motility and gene expression (3). A number of recent studies have now shown PAK1 in the nucleus and its nuclear functions that are just beginning to be appreciated. These findings offer an exciting opportunity for the discovery of new nuclear PAK1 functions, including, possible roles in cell cycle regulation, mitosis, and cancer.

PAK1 and the Cell Cycle

Although a large body of work implicates PAK1 in the regulatory processes in the cytoplasm, accumulating evidence now suggests a role for PAK in nuclear and mitotic events. The first conclusive evidence that PAK1 has a role in cell cycle regulation was the finding that overexpression of activated PAK1 in human breast cancer cells leads to the abnormal accumulation of centrosomes and aberrant mitoses (5). Thereafter, Li et al. (6) showed that PAK1 becomes activated and translocates to the nucleus before the onset of mitosis. From this point on, PAK1 behaves like a chromosomal passenger protein. The authors observed a punctated pattern of PAK1 localization in the nucleus which suggested that activated PAK1 might be associated with the kinetochore complex/centromeric region of mitotic chromosomes during prophase. Subsequently, PAK1 was shown to localize to centrosomes in mitotic cells and to become phosphorylated by the mitotic kinase Cdc2. More recently, PAK1 was shown to induce cytoskeletal reorganization and microtubule-organizing centers, in part, by regulating microtubule dynamics by phosphorylating tubulin cofactor B and colocalizing with tubulin cofactor B on newly polymerized microtubules and on centrosomes (7). Earlier studies have shown that PAK1 also phosphorylates histone H3.3a (6) and stathmin (8), undergoes phosphorylation during mitosis (9), is enriched in microtubule-organizing centers (10), and induces mitotic spindle abnormalities in breast cancer cells (5). Cumulatively, these reports suggest that in addition to its established involvement in actin cytoskeletal signaling, PAK1 plays an important role in mitosis and microtubule dynamics. Recently, it was also shown that PAK1 can bind to and phosphorylate Aurora-A during the mitotic phase of the cell cycle (11), and that PAK1 phosphorylates Aurora-A on Thr^{288} and Ser^{342}, two sites that are important for Aurora-A activation, whereas the active form of PAK1 binds Aurora-A, inactive PAK1 binds the PIX/GIT1 complex. Thus, the centrosomal PIX/GIT1 complex activates PAK1, which then dissociates from the complex and phosphorylates Aurora-A. Collectively, these observations highlight the significance of PAK1 activity in cell cycle regulatory events in mammalian cells.

PAK1 in Transformation: Does Localization Play a Role?

There is sufficient experimental evidence to link PAK1 to cellular mechanisms important for cellular transformation and tumor progression. PAK1 activity has been shown to stimulate, and be

Requests for reprints: Rakesh Kumar, The University of Texas M.D. Anderson Cancer Center, Box 108, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: 713-745-3558; Fax: 713-745-3792; E-mail: rkumar@mdanderson.org.

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required for, increased migration and invasiveness in breast cancer cell lines. PAK1 overexpression and hyperactivation have been linked to the invasiveness of human breast cancer cells and breast tumors (2). Expression of PAK1 in breast tumor tissue positively correlates with tumor grade, with higher expression in less differentiated ductal carcinomas of the breast (grade 3 tumors) than in grade 2 and grade 1 tumors. PAK1 functionality is also required for the transformation of fibroblasts by a variety of the small GTPases and by Ras. Combined-array comparative genomic hybridization analysis of ovarian cancer cell lines and tissue microanalysis suggest that PAK1 is a candidate oncogene that can be coamplified with cyclin D1 on chromosome 11q13 (12). In this context, Balasenthil et al. (13) showed that hyperplastic mammary glands from catalytically active PAK1 transgenic mice exhibit a 5- to 7-fold increase in cyclin D1 expression as compared with stage-matched wild-type mice. In addition, PAK1 levels were reported to be elevated in human breast tumors: 15% of the samples showed nuclear staining that was positively correlated with increased cyclin D1 expression. Wang et al. (14) showed that transgenic mice expressing kinase-active PAK1 developed hyperplasia in the mammary epithelium and had increased expression of ER-α target genes. PAK1 was found to interact with and phosphorylate ER-α at Ser^{305} and to promote its transactivation functions, thereby leading to up-regulation of cyclin D1 and promotion of hormone independence (15). This finding provided the first *in vivo* evidence for a role of the PAK1-ER pathway in promoting the process of mammary gland tumorigenesis. Because several PAK1 substrates such as DLC1 and Aurora kinase are overexpressed in breast cancer (4), it is possible that the presumed breast cancer–promoting activity of PAK1 may be driven by specific PAK1 substrates or regulated targets such as cyclin D1. These observations raise the possibility that PAK1 hyperstimulation might contribute to oncogenic cell transformation using mechanisms that are either direct or indirect or both.

All the above findings raise the possibility that hyperactivation of PAK1 may promote breast cancer tumorigenesis. Indeed, transgenic overexpression of a catalytically active PAK1 mutant in the mammary epithelium was found to cause mammary gland tumors and lead to a variety of other breast lesions, including focal solid nodules, ductal hyperplasia, and mini-intraductal neoplasms and adenomas (16). This study provided the first direct evidence that PAK1 deregulation is sufficient for the formation of mammary gland tumors and emphasized the emerging importance of nuclear PAK1 in breast cancer cells. Furthermore, consistent with its role in breast tumor progression, PAK1 expression and its nuclear accumulation were progressively increased during the transition from ductal hyperplasia to ductal carcinoma *in situ* to adenocarcinoma in the established progression model of polyoma-middle T antigen transgenic mice (16).

Nuclear PAK1 and Tamoxifen Response

The fact that PAK1 could be detected in the nucleus in a variety of experimental breast cancer model systems raises the obvious question about the significance, prognostic, and predictive value of PAK1 expression and subcellular localization in the pathogenesis of breast cancer. In this context, Holm et al. examined PAK1 protein expression and localization in 403 primary breast tumors from premenopausal patients randomized to 2 years of adjuvant tamoxifen or no treatment (1). They report that high PAK1 expression correlates well with a lack of tamoxifen response in patients with breast cancer despite the presence of ER-α expression. Interestingly, breast tumors with nuclear PAK1 exhibited a pronounced impaired tamoxifen effect, thus strengthening the notion that elevated PAK1 expression and nuclear localization may be intimately linked to lack of tamoxifen response in premenopausal breast cancer. Overall, the results suggest that PAK1 activation and its nuclear localization might be one of the mechanisms responsible for reduced tamoxifen sensitivity of breast tumor cells. It is nevertheless clear that premenopausal patients with PAK1-overexpressing breast cancer do not respond to tamoxifen, and such patients might be more effectively treated with alternative endocrine treatment.

As a potential mechanistic explanation of the results of the above study, very recently, Rayala et al. (17) found that deregulated PAK1 cooperated with tamoxifen in stimulating ER transactivation, leading to blockage of tamoxifen-inhibitory effects in hormone-sensitive breast cancer cells. Interestingly, tamoxifen treatment resulted in the up-regulation of ER target genes in PAK1-overexpressing breast cancer cells and increased PAK1-ER interaction in tamoxifen-resistant but not in tamoxifen-sensitive cells. These findings imply that PAK1 signaling–dependent activation of ER-S305 leads to an improved ER transactivation function of tamoxifen (Fig. 1). The underlying mechanism involves S305-linked regulation of Ser^{118} phosphorylation and the cooperative influence of both Ser^{305} and Ser^{118} in promoting the transactivation activity of ER. A similar model of tamoxifen agonism was also previously proposed by Michalides et al. (18), in which another signaling kinase, protein kinase A, also phosphorylated S305, leading to an active conformational arrest upon tamoxifen binding and, in turn, tamoxifen-induced transactivation. Because growth factor signaling could activate PAK1, and because the consensus phosphorylation motif in the PAK substrates is not restricted and is common with other kinases such as protein kinase A and ribosomal S6 kinase, these above findings suggest a broader role of ER-S305 phosphorylation resulting from growth factor signaling in conferring tamoxifen-resistant phenotypes. Tamoxifen resistance can also be conferred by stimulation of upstream PAK1 activators such as AND-34/BCAR3 (19). Clues about the therapeutic significance of PAK1 regulation of tamoxifen-resistance are derived from a recent study by Hirokowa et al. (20), wherein the authors show that treatment of breast cancer in experimental tumor models with a PAK1 inhibitor, FK228, blocks the Ras-induced activation of PAK1 and abrogates tamoxifen resistance. All of the above studies suggest that activation of either upstream or downstream signals in the PAK1 pathway plays a pivotal role in tamoxifen resistance in human breast cancer.

Bridging the Link between Cytosolic and Nuclear PAK1 Functions

The notion of nuclear PAK1, conceived a few years ago, may elucidate the beginnings of a new model in which PAK1 signaling may have an extensive network in the nucleus. It will be interesting to see the dynamic strength behind PAK1 transition to the nucleus: what drives PAK1 to the nucleus, how nuclear PAK1 regulates critical steps involved in mitotic progression, and how exactly PAK1 gets to the centrosome. Given that the nuclear characteristics of PAK1 are similar to those of the Aurora kinases, and that Aurora-A is a substrate of PAK1, we speculate that both of these kinases are likely to influence the functions of each other and thus, collectively, may control specific regulatory mitotic targets that have yet to be identified. Furthermore, it is expected that many of the PAK1-interfacing molecules may also participate in the control of cell cycle checkpoints. Because a large number of regulatory

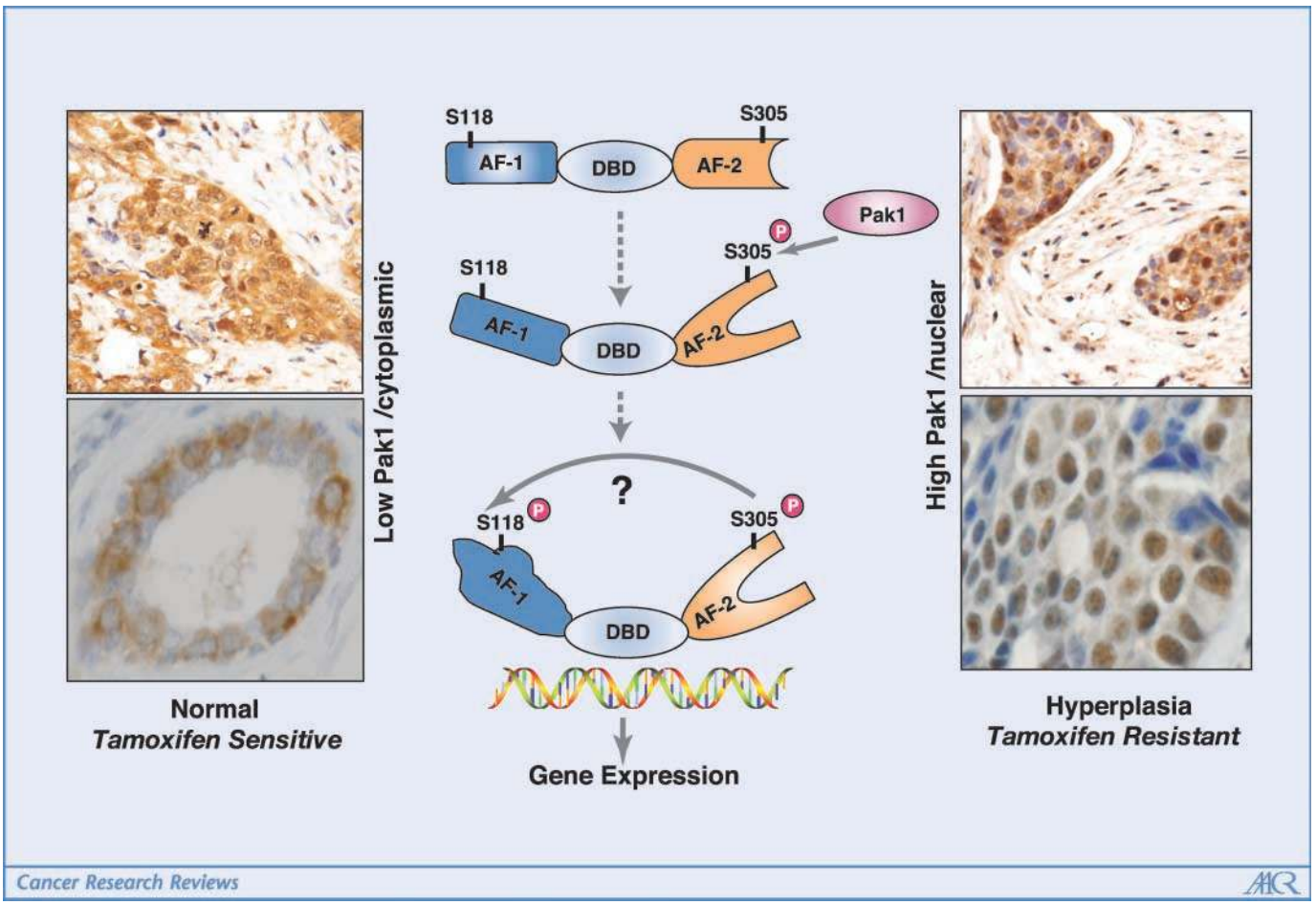

Figure 1. PAK1 enhancement of ER transactivation requires ER Ser^{305}-dependent Ser^{118} stimulation, presumably due to conformational changes. *Top left* and *top right,* Pak1 localization and expression in human breast tumors. *Bottom left* and *bottom right*, Pak1 localization in PyMT-induced mammary gland tumors. *AF1,* activation function 1; *DBD,* DNA binding domain; *AF2,* activation function 2. *Broken arrow,* potential involvement of more than one step.

proteins shuttle between the nucleus and cytoplasm, it is very likely that that PAK1 phosphorylation of its putative nuclear substrates may affect the subcellular distribution and functions of such PAK1 targets. In addition to its substrates, it will be also important to gain a deeper insight into the mechanisms by which PAK1 is recruited to specific chromatin targets (21), which could influence the expression of, and thus, the functions of such chromatin targets. In the context of breast cancer, it is possible that many of the above PAK1 substrates, regulatory targets, or both, might be differentially affected by tamoxifen versus hormone signals depending on the overexpression status of PAK1. Furthermore, despite the clear importance of PAK1 overexpression and its nuclear localization in tumorigenesis, the most crucial question that needs to be addressed is the potential role of nuclear PAK1 per se in regulating the genes involved in tumorigenesis. This could possibly be accomplished via gene profiling analysis of the cells expressing nuclear versus cytoplasmic PAK1. Finally, because PAK1 nuclear localization correlates with tamoxifen resistance, searching for methods to prevent the entry of PAK1 into the nucleus might constitute one experimental approach to interfering with tamoxifen resistance in breast cancer.

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