Commentary

PTEN deficiency: a role in mammary carcinogenesis

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Abstract

The *PTEN* gene is often mutated in primary human tumors and cell lines, but the low rate of somatic *PTEN* mutation in human breast cancer has led to debate over the role of this tumor suppressor in this disease. The involvement of PTEN in human mammary oncogenesis has been implicated from studies showing that germline *PTEN* mutation in Cowden disease predisposes to breast cancer, the frequent loss of heterozygosity at the *PTEN* locus, and reduced PTEN protein levels in sporadic breast cancers. To assay the potential contribution of *PTEN* loss in breast tumor promotion, Li *et al.* [1] crossed *Pten* heterozygous mice with mouse mammary tumor virus-*Wnt-1* transgenic (*Wnt-1* TG, *Pten*+/–) mice. Mammary ductal carcinoma developed earlier in *Wnt-1* TG, *Pten*+/– mice than in mice bearing either genetic change alone, and showed frequent loss of the remaining wild-type *PTEN* allele. These data indicate a role for PTEN in breast tumorigenesis in an *in vivo* model.

Keywords: mammary carcinogenesis, PKB/Akt, PTEN, Wnt-1

Introduction

Cancer is a multi-step process involving the mutation of genes regulating cell proliferation, differentiation, and survival, leading to escape from normal tissue boundaries and sustained angiogenesis [2]. Given the plethora of genetic alterations observed in primary breast cancers, it has been difficult to establish which are involved in initiation, progression and metastasis. Despite often significant difficulties in the interpretation of their relevance to human disease, mouse models have provided experimental tools to investigate genetic pathways altered in breast cancer. Furthermore, the interbreeding of different TG or gene-deficient mouse models can reveal the potential for co-operation between different signaling pathways.

MMTV-*Wnt-1* TG mice develop mammary tumors

Mammary tumors induced following mouse mammary tumor virus (MMTV) infection have revealed oncogenes involved in murine mammary tumorigenesis. Random insertion of proviral MMTV DNA into mouse mammary epithelial cells results in insertional mutagenesis and oncogenic activation of various genes, including those of the *Wnt*, *Fgf*, and *notch* families, and *eIF-3p48*. The first proto-oncogene to be cloned from MMTV-induced mammary cancers was *Wnt-1* [3], a member of a family of secreted cysteine-rich glycoproteins, which controls cell fate/patterning through stabilization of β-catenin and activation of the downstream transcription factor T cell factor

GSK-3β = glycogen synthase kinase-3β; ILK = integrin-linked kinase; LOH = loss of heterozygosity; MMTV = mouse mammary tumor virus; mTOR = mammalian target of rapamycin; PI3K = phosphatidylinositol 3′-kinase; PKB = protein kinase B; TCF/LEF = T cell transcription factor; TG = transgenic.

(TCF/LEF). MMTV-*Wnt-1* TG mice develop mammary tumors histopathologically similar to human breast cancers. These mice develop extensive mammary hyperplasia, and tumors progress to adenocarcinomas in a temporally predictable manner [4]. Although overexpression of Wnt-1 has not been observed in human breast cancers, several downstream components of the Wnt signaling pathway are deregulated in human cancers, including adenomatous polyposis coli, β-catenin, c-Myc, and cyclin D_1 [5]. Moreover, overexpression of a stable transcriptionally active β-catenin in a mouse mammary model induced multiple aggressive mammary adenocarcinomas [6]. By breeding MMTV-*Wnt-1* TG mice with mice carrying alterations in genes implicated in breast cancer, potential synergies between pathways involved in breast oncogenesis can be defined. Li *et al*. employed this strategy, crossing mice heterozygous for *PTEN* with a MMTV-*Wnt-1* TG model, to assay the relevance of *PTEN* deficiency to murine mammary tumorigenesis in an *in vivo* model [1].

PTEN and cancer

Early cytogenic analysis revealed frequent abnormalities within chromosome 10 involving the 10q23 region in many cancers (reviewed in [7]). The *PTEN* gene, located at chromosome 10q23, was identified as a candidate tumor suppressor gene frequently deleted at chromosome 10q23 in primary cancers, including brain, prostate and breast cancer [8,9]. The tumor suppressor role for PTEN was further supported by the discovery that the autosomal dominant multineoplasia syndrome, Cowden disease, is associated with germ-line *PTEN* mutations [10]. Family members with Cowden disease, both male and female, have an increased incidence of breast cancer [11–13]. Moreover, somatic *PTEN* mutations are found frequently in endometrial carcinomas, malignant gliomas, and melanomas [14–16].

The *PTEN* gene product is a lipid phosphatase [8] that preferentially dephosphorylates phosphoinositides at the D3 position of the inositol ring [17]. It opposes the effects of phosphatidylinositol 3-kinase (PI3K) by dephosphorylating its lipid products. The products of PI3K activity are required for activation of protein kinase B (PKB), also known as Akt, a serine/threonine kinase involved in cell growth and survival (review in [18]), whose constitutive activation has transforming potential [19]. PTEN may thus inhibit carcinogenesis through its inhibitory effect on PKB/Akt. One of the known substrates of PKB is glycogen synthase kinase-3β (GSK-3β). GSK-3β is phosphorylated and inactivated by PKB/Akt [18].

Increasing data have implicated PTEN loss in breast carcinogenesis. While loss of heterozygosity (LOH) of the *PTEN* locus is frequent in sporadic breast carcinoma [20], particularly in late stage disease [21], the very low rate of somatic intragenic *PTEN* mutations led some investigators to suggest that PTEN is not involved in breast tumor progression [22–26]. More recently, however, immunohistochemical analysis revealed frequent loss or reduction of PTEN protein in primary breast cancers [27]. Furthermore, while three different groups showed an embryonic lethal phenotype in *PTEN* knockout mice, *Pten*+/– mice develop breast cancers with relatively long latencies and different frequencies depending on the genetic strain used [28–30]. In one study, one-half of the *Pten*+/– mice developed breast tumors [31]. Despite the differing incidence of mammary cancers in the different genetic backgrounds of these *PTEN*-deficient strains, the evidence from the knockouts, coupled with the data of Li *et al.* [1], support a role for this tumor suppressor in mammary oncogenesis.

Lessons from *Wnt-1* TG, *Pten*+/– mice

By crossing *PTEN* heterozygotes with MMTV-*Wnt-1* TG mice, Li *et al.* [1] demonstrated a synergy between these two pathways and identified a role for PTEN in murine breast tumor promotion or progression. Infiltrating ductal cancers developed earlier in *Wnt-1* TG, *Pten*+/– mice compared with *Pten*+/– mice or MMTV-*Wnt-1* TG animals. The majority of tumors tested from *Wnt-1* TG, *Pten*+/– animals showed loss of the remaining wild-type allele. Moreover, nontumorous mammary glands in *Wnt-1* TG, *Pten*+/– mice showed frequent multifocal intraductal carcinoma both adjacent to and distant from the invasive tumors, while MMTV-*Wnt-1* TG mice showed only ductal hyperplasia in grossly unaffected glands. In addition, the invasive cancers in *Wnt-1* TG, *Pten*+/– mice had a less differentiated histopathology suggestive of a more aggressive phenotype.

PKB activation, as demonstrated by immunostaining of tumors for phospho-PKB, was seen only in a patchy distribution in tumors from *Wnt-1* TG, *Pten*+/– mice. Moreover, PKB activation as assayed by this method was not observed in tumors from *Wnt-1* TG animals. The authors reported reductions in PTEN protein by immunohistochemical analysis in tumors from *Wnt-1* TG, *Pten*+/– mice whether or not they showed *PTEN* LOH. It is thus a little surprising that focal areas of PKB/Akt activation were seen only in tumors from *Wnt-1* TG, *Pten*+/– animals showing *PTEN* LOH. The accuracy of assaying PKB activation by immunohistochemical analysis using antibody against the phosphorylated active form of PKB has not been definitively established.

The relevance of *Wnt-1* TG, *Pten*+/– murine tumorigenesis to spontaneous breast cancer formation in humans is not entirely clear. It is possible that the enhanced tumor formation in the *Wnt-1* TG, *Pten*+/– animals merely reflects synthetic synergy between two susceptible strains of mice. While there may be a synergy between the Wnt-1 and PTEN pathways in the *Wnt-1* TG, *Pten*+/– tumors,

there was no clear evidence for involvement of the PTEN/PKB pathway in MMTV-*Wnt-1*-mediated tumorigenesis. Li *et al*. found no increase in PKB activation in *Wnt-1* TG tumors, and none of these tumors showed reduction to hemizygosity at the PTEN locus.

One intriguing possibility is that Wnt-1 overexpression and PTEN loss may interact by each contributing to inhibition of GSK-3β [32]. Following insulin or receptor tyrosine kinase stimulation, PKB phosphorylates and inhibits GSK-3β. Wnt signaling causes a conformational inhibition of GSK-3β within a complex with axin, the adenomatous polyposis coli protein and β-catenin. Whereas in normal cells cross-talk between Wnt and receptor tyrosine kinase signaling does not usually occur, there is some evidence that the phosphorylation state of GSK-3β can influence Wnt signaling ([32] and references therein). GSK-3β inhibition would lead to increased cyclin D_1 stability [33], and stabilization of β-catenin with increased mitogenic transcriptional activity via the β-catenin–TCF/LEF complex [34]. PKB may not be an obligate target of PTEN loss since only 'patchy' activation of PKB was seen in *Wnt-1* TG, *Pten*+/– mammary tumors, as assessed by immunohistochemical analysis. Recent reports suggest that the integrin-linked kinase (ILK) can mediate PI3K-dependent inactivation of GSK-3β [35–37]. Although ILK can phosphorylate and activate PKB [38], it may also inhibit GSK-3β directly, independent of PKB in some cells. In oncogenesis, ILK-dependent GSK-3β inhibition could potentially contribute to Wnt-1 signaling and β-catenin-dependent transcriptional effects. In *Wnt-1* TG, *Pten*+/– tumors, where PKB activation is 'patchy', activation of ILK via PTEN loss may lead directly to GSK-3β inactivation. PTEN loss may synergise with Wnt-1 through activation of ILK, with or without PKB activation, leading to GSK-3β inhibition. While this mechanistic model for cooperation between PTEN loss and Wnt-1 activation may be provocative, evidence for cross-talk between these pathways in human mammary tumorigenesis has yet to be established.

Since PKB, a key PI3K effector downstream of PTEN, has been shown to inhibit apoptosis and promote cell cycle progression, the authors compared proliferation rates (by Ki67 staining) and assayed apoptosis in tumors from *Wnt-1* TG, *Pten*+/– mice and in tumors from MMTV-*Wnt-1* TG animals. Surprisingly, no differences in either were found. Activated PKB/Akt has been shown to enhance survival signals in breast epithelial cells [39]. Other methods to assay apoptosis would have strengthened the conclusions drawn by Li *et al*. Since the tumors in these two models both have very high proliferation rates, a subtle difference may have been missed by Ki67 staining. Flow cytometric analysis might have been useful, but the high content of stromal cells observed in the *Wnt-1* TG, *Pten*+/– tumors would have precluded accurate analysis without tumor microdissection. Since PTEN inhibits S phase entry by increasing levels of the cdk inhibitor, p27, in certain cell types [40,41], and since activated PKB/Akt can stabilize cyclin D1 through its inhibitory action on GSK-3β [33], an increased rate of cellular proliferation might have been expected in *Wnt-1* TG, *Pten*+/– tumors.

An additional possibility is that PTEN loss may influence tumorigenesis and the rapidity of tumor growth *in vivo* by increasing tumor cell invasiveness. It was recently shown that HER2/ErbB2 activation of PI3K-dependent signaling increases mammary epithelial cell invasive potential *in vitro* [42]. Moreover, PTEN dephosphorylates focal adhesion kinase and inhibits integrin-mediated cell spreading and cell migration [43]; thus, reduced PTEN expression could favor a metastatic phenotype. It is unfortunate that the small number of animals assayed for metastatic tumors precluded a definitive conclusion regarding the role of PTEN in metastasis in Li *et al*.'s study [1].

Given that increased PI3K and PKB/Akt signaling have also been shown to mediate angiogenesis through increased expression of vascular endothelial growth factor [44], loss of PTEN may promote tumor growth *in vivo* by an angiogenic mechanism. While reconstitution of PTEN expression in U87MG glioma cells failed to inhibit proliferation in culture, it caused a dramatic reduction in tumor growth *in vivo* in a murine orthotopic brain tumor model [45]. The PTEN-mediated inhibition of tumor growth seen in this brain tumor model may reflect a reduction in tumor angiogenesis. It would be interesting to compare the extent of angiogenesis in mammary cancers arising in MMTV-*Wnt-1* mice, in *Wnt-1* TG, *Pten*+/– mice, and in *Pten*+/– mice. These models may be useful to assay the potential role of PTEN in regulating breast cancer growth through an effect on tumor vasculature.

Conclusion

Li *et al*. provide compelling evidence for synergy between Wnt activation and loss of the PTEN tumor suppressor in promoting mammary carcinoma development and growth *in vivo* in mice. These data support further investigation of how downstream Wnt targets and PTEN inactivation may cooperate in mammary tumorigenesis. While the understanding of mechanisms of disease is in itself a laudable goal, an added utility of murine models lies in the preclinical testing of novel therapeutic agents. Since breast tumors form with predictable rapid kinetics in this model, *Wnt-1* TG, *Pten*+/– mice may allow the testing of molecular-based therapies of potential utility in human tumors showing *PTEN* loss/mutation. One such candidate, the rapamycin analog CC1779, which inhibits the potential PKB/Akt target mTOR, is under investigation by several groups [46]. Novel drugs that inhibit other cell cycle players may also prove to have therapeutic efficacy.

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