Commentary

Syk: a new player in the field of breast cancer

Zoe A Stewart and Jennifer A Pietenpol

Department of Biochemistry, Center in Molecular Toxicology, and the Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

Correspondence: Jennifer A Pietenpol, Department of Biochemistry, Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

Received: 17 October 2000
Accepted: 20 October 2000
Published: 2 November 2000

Breast Cancer Res 2001, **3**:5–7

© BioMed Central Ltd on behalf of the copyright holder
(Print ISSN 1465-5411; Online ISSN 1465-542X)

Abstract

Breast tumor development and progression are thought to occur through a complex, multistep process, including oncogene activation (eg HER2/neu) and mutation or loss of tumor suppressor genes (eg p53). Determining the function of genetic alterations in breast carcinoma tumorigenesis and metastasis has been the focus of intensive research efforts for several decades. One group of proteins that play a critical role in breast cancer cell signaling pathways are tyrosine kinases. Overexpression of the tyrosine kinase HER2/neu is observed in many human breast cancers and is positively correlated with enhanced tumorigenesis [1]. Recently, another tyrosine kinase, Syk, has been implicated as an important inhibitor of breast cancer cell growth and metastasis [2]. This recent finding was unexpected, since Syk function has been predominantly linked to hematopoietic cell signaling, and is discussed further in this commentary.

Keywords: metastasis, murine xenograft tumors, nonreceptor tyrosine kinase

Introduction

Syk is ubiquitously expressed in hematopoietic cells and has been extensively studied as an effector of B cell receptor (BCR) signaling [3]. BCR engagement induces signaling cascades mediated by three families of nonreceptor tyrosine kinases [3]: the ZAP-70 family (ZAP-70, Syk), the Src family (Lyn, Fyn, Blk), and the Tec family (Btk, Itk, Etk). After BCR activation, Syk-dependent signaling pathways regulate the clonal expansion, differentiation, or apoptosis of B cells. Phospholipase C (PLC)-γ_2 and phosphatidylinositol 3-kinase (PI3-K) are key targets of Syk tyrosine phosphorylation after BCR cross-linking [3]. In B cells, Syk phosphorylation of PLC-γ_2 results in downstream activation of the ERK and JNK kinases [4], whereas PI3-K phosphorylation by Syk mediates Akt activity [5]. Syk also preferentially phosphorylates the α-tubulin subunits of microtubules, which has been proposed to regulate the ability of the microtubule cytoskeleton to function as a scaffold for the assembly of signaling complexes [6].

In addition to breast epithelial cells, Syk expression has also been observed in other nonhematopoietic cells. For example, Syk is expressed in human hepatocytes, and use of the Syk-selective inhibitor, piceatannol, indicated that Syk is necessary for mitogen-activated protein kinase activation by G-protein coupled receptors in this cell type [7]. In human colon carcinoma cells, Syk gene expression is repressed in a *p53*-dependent manner, suggesting that loss of p53 function during tumorigenesis can lead to deregulated Syk activity [8].

Characterization of murine Syk revealed that Syk is expressed at high levels in normal mammary glands [9]. In agreement with this latter study, recent findings of Coopman *et al* [2] showed that Syk is a potent modulator of breast epithelial cell growth. Coopman *et al* initially examined Syk mRNA and protein expression in a panel of well-characterized breast cancer cell lines, normal mammary gland tissue, and a normal breast epithelial cell

BCR = B-cell receptor; PI3-K = phosphatidylinositol 3-kinase; PLC = phospholipase C.

line [2]. Syk expression was readily detectable in normal mammary tissue and epithelium, and in the noninvasive breast carcinoma cell lines; however, Syk expression was reduced or absent in the invasive breast tumor cell lines. Of note, the Syk protein expressed in the noninvasive breast tumor cells was catalytically active, because Syk isolated from these cells was autophosphorylated in *in vitro* kinase assays. *In situ* hybridization of normal and pathologic human breast tissue samples verified Syk expression in normal breast epithelium, whereas Syk expression was reduced in *in situ* carcinomas and was absent in invasive breast carcinomas.

Role of Syk in tumorigenesis

In order to elucidate the role of Syk in tumorigenicity, Coopman *et al* [2] also analyzed Syk in mouse xenograft tumor models. In one set of experiments, a Syk-negative breast cancer cell line was stably transfected with wild-type Syk or vector control, and pooled clones of each were injected into the mammary fat pads of athymic nude mice. Tumors that formed from the Syk-negative vector control cells had a nearly fivefold greater mean tumor volume than did tumors generated by cells that expressed Syk. Furthermore, these engineered cell lines were also tested for their ability to form lung metastases after injection into the tail veins of mice. Injection of Syk-transfected cells resulted in the formation of a single metastatic colony in one of five animals. In contrast, all animals that received vector control-transfected cells had multiple metastatic lung colonies. Additionally, the Syk-expressing cells had a significantly impaired ability to grow in anchorage-independent conditions and to form invasive colonies in matrigel as compared with vector control-transfected cells. In a parallel set of experiments, a Syk-positive breast cell line was stably transfected with a dominant-negative kinase-deficient Syk or empty vector, and pooled clones of each were injected into the mammary fat pads of athymic nude mice. The kinase-deficient Syk-transfected cells had significantly enhanced tumor potency and tumor growth as compared with the vector control-transfected cells. However, expression of the kinase-deficient Syk was not sufficient to inhibit invasive outgrowth in matrigel. Taken together, the data from these tumor studies suggest that Syk can suppress tumor growth and metastasis in breast cells *in vivo*.

Preliminary studies by Coopman *et al* [2], exploring Syk mechanism of action in breast cells, failed to reveal significant differences in the rates of apoptosis in Syk-expressing cells and Syk-deficient cells. Cells from Syk-containing tumors were enlarged and had multilobed or multiple nuclei. Furthermore, abnormal mitotic spindles were detected in Syk-positive cells, suggesting that Syk may inhibit tumor growth in breast cells by regulation of microtubules. Interestingly, both Syk kinase activity and intact Syk SH2 sites were necessary to suppress the invasive outgrowth of cells in matrigel. However, mutation of the autophosphorylation site (Y348F) in the Syk linker region did not impair the ability of Syk to suppress invasive growth. Because autophosphorylation of the Syk linker region is required for the interaction of Syk with Vav1 and PLC-γ_1 [10], this latter finding suggests that other Syk effectors mediate Syk-dependent inhibition of invasive growth in breast epithelial cells.

The findings by Coopman *et al* support a role for Syk function in breast tumor development and metastasis, and raise many interesting questions as to potential Syk-effector pathways in breast epithelial cells, as well as the role of other nonreceptor tyrosine kinases in breast cell signaling cascades. In B cells, Syk activity is a key regulator of Akt kinase activity after BCR engagement, because Syk induces PI3-K-dependent Akt activation and thus inhibition of apoptosis [5]. Because numerous studies have demonstrated deregulated Akt activity in breast cancer cells [11], it is tempting to speculate that Syk may also regulate Akt activity in breast cells. However, only signaling pathways that generate elevated Akt activity have been identified in breast tumor cells. For example, *PTEN* is a negative regulator of Akt activity and mutation in *PTEN* leads to increased Akt activity and increased mammary tumors in mice [12], whereas overexpression of HER2 results in increased Akt activity in human breast tumor cells [13]. However, if Syk expression is reduced during human breast cancer progression, then it is unlikely that Syk-dependent signaling contributes to the increased Akt activity observed in breast tumor cells.

Expression of the Syk-related ZAP-70 kinase was not detected in any of the breast cancer cell lines examined by Coopman *et al*. However, the role of other nonreceptor tyrosine kinases in breast epithelial cell signaling remains to be determined. Lyn, a member of the Src family of nonreceptor tyrosine kinases, opposes Syk function for Akt activation in B lymphocytes, because Lyn-dependent signaling reduces Akt activity [14]. Although Lyn expression has been observed in a noninvasive breast cancer cell line, a rigorous examination of Lyn expression during breast cancer progression has not been performed [15]. If Lyn expression is reduced concurrent with Syk expression during breast cancer development, the loss of Lyn-dependent inhibition may be one contributor to the elevated Akt activity observed during tumorigenesis.

Several other lines of evidence support a role for Syk-dependent signaling in breast tumorigenesis. First, allelic loss of the human Syk locus on chromosome 9q22 has been linked to lymph node metastasis of primary breast cancer [16]. Furthermore, knockout of the negative Syk regulator c-Cbl in mice leads to development of mammary epithelial hyperplasia [17]. Given the results of Coopman *et al*, it will be interesting to evaluate Syk activity and microtubule structures in c-Cbl-deficient mammary epithelial cells.

In contrast to what was observed by Coopman *et al* in human breast samples, examination of Syk expression in murine breast biopsies demonstrated that aggressive, metastasizing mammary gland tumors had upregulated Syk expression as compared with well differentiated, non-metastasizing tumors [9]. However, tumor epithelial cells were not separated from coassociated lymph node tissues by laser capture microdissection in this latter study. Additionally, Syk activity was not directly examined in the mammary gland tumors, and thus it is unclear whether the Syk protein retained wild-type function. Alternatively, it is possible that the murine tumors contained mutations in the downstream effector(s) responsible for Syk-dependent growth inhibition and metastasis in human breast epithelial cells. Clearly, further *in vivo* experimentation using isogenic murine mammary cell models is necessary before definitive conclusions can be made regarding the role of Syk in murine mammary epithelial cell tumorigenesis.

Conclusion

The recent identification of Syk as a potent modulator of breast epithelial cell growth has generated a need for further exploration of the role of nonreceptor tyrosine kinases in breast cancer progression and metastasis. These initial results offer the promise that novel molecular targets may be identified both for the prevention of breast cancer development and for inhibition of metastatic breast cancer spread. The continual elucidation of novel targets such as Syk is a critical part of the endeavor to eradicate breast cancer.

References

1. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL: **Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene.** *Science* 1987, **235**:177–182.
2. Coopman PJP, Do MTH, Barth M, Bowden ET, Hayes AJ, Basyuk E, Blancato JK, Vezza PR, McLeskey SW, Mangeat PH, Mueller SC: **The Syk tyrosine kinase suppresses malignant growth of human breast cancer cells.** *Nature* 2000, **406**:742–747.
3. Turner M, Schweighoffer E, Colucci F, Di Santo JP, Tybulewicz VL: **Tyrosine kinase SYK: essential functions for immunoreceptor signalling.** *Immunol Today* 2000, **21**:148–154.
4. Jiang A, Craxton A, Kurosaki T, Clark EA: **Different protein tyrosine kinases are required for B cell antigen receptor-mediated activation of extracellular signal-regulated kinase, c-Jun NH2-terminal kinase 1, and p38 mitogen-activated protein kinase.** *J Exp Med* 1998, **188**:1297–1306.
5. Craxton A, Jiang A, Kurosaki T, Clark EA: **Syk and Bruton's tyrosine kinase are required for B cell antigen receptor-mediated activation of the kinase Akt.** *J Biol Chem* 1999, **274**:30644–30650.
6. Faruki S, Geahlen RL, Asai DJ: **Syk-dependent phosphorylation of microtubules in activated B-lymphocytes.** *J Cell Sci* 2000, **113**:2557–2565.
7. Tsuchida S, Yanagi S, Inatome R, Ding J, Hermann P, Tsujimura T, Matsui N, Yamamura H: **Purification of a 72-kDa protein-tyrosine kinase from rat liver and its identification as Syk: involvement of Syk in signaling events of hepatocytes.** *J Biochem (Tokyo)* 2000, **127**:321–327.
8. Okamura S, Ng CC, Koyama K, Takei Y, Arakawa H, Monden M, Nakamura Y: **Identification of seven genes regulated by wild-type p53 in a colon cancer cell line carrying a well-controlled wild-type p53 expression system.** *Oncol Res* 1999, **11**:281–285.
9. Fluck M, Zurcher G, Andres AC, Ziemiecki A: **Molecular characterization of the murine syk protein tyrosine kinase cDNA, transcripts and protein.** *Biochem Biophys Res Commun* 1995, **213**:273–281.
10. Deckert M, Tartare-Deckert S, Couture C, Mustelin T, Altman A: **Functional and physical interactions of Syk family kinases with the Vav proto-oncogene product.** *Immunity* 1996, **5**: 591–604.
11. Page C, Huang M, Jin X, Cho K, Lilja J, Reynolds RK, Lin J: **Elevated phosphorylation of AKT and Stat3 in prostate, breast, and cervical cancer cells.** *Int J Oncol* 2000, **17**:23–28.
12. Stambolic V, Tsao MS, Macpherson D, Suzuki A, Chapman WB, Mak TW: **High incidence of breast and endometrial neoplasia resembling human Cowden syndrome in pten+/- mice.** *Cancer Res* 2000, **60**:3605–3611.
13. Liu W, Li J, Roth RA: **Heregulin regulation of Akt/protein kinase B in breast cancer cells.** *Biochem Biophys Res Commun* 1999, **261**:897–903.
14. Li HL, Davis WW, Whiteman EL, Birnbaum MJ, Pure E: **The tyrosine kinases Syk and Lyn exert opposing effects on the activation of protein kinase Akt/PKB in B lymphocytes.** *Proc Natl Acad Sci USA* 1999, **96**:6890–6895.
15. Liu X, Gupta AK, Corry PM, Lee YJ: **Hypoglycemia-induced c-Jun phosphorylation is mediated by c-Jun N-terminal kinase 1 and Lyn kinase in drug-resistant human breast carcinoma MCF-7/ADR cells.** *J Biol Chem* 1997, **272**:11690–11693.
16. Minobe K, Onda M, Iida A, Kasumi F, Sakamoto G, Nakamura Y, Emi M: **Allelic loss on chromosome 9q is associated with lymph node metastasis of primary breast cancer.** *Jpn J Cancer Res* 1998, **89**:916–922.
17. Murphy MA, Schnall RG, Venter DJ, Barnett L, Bertoncello I, Thien CB, Langdon WY, Bowtell DD: **Tissue hyperplasia and enhanced T-cell signalling via ZAP-70 in c-Cbl-deficient mice.** *Mol Cell Biol* 1998, **18**:4872–4882.