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Anaplastic lymphoma kinase is expressed in different subtypes of human breast cancer ☆

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Abstract

Pleiotrophin (PTN, *Ptn*) is an 18 kDa cytokine expressed in human breast cancers. Since inappropriate expression of *Ptn* stimulates progression of breast cancer in transgenic mice and a dominant negative PTN reverses the transformed phenotype of human breast cancer cells that inappropriately express *Ptn*, it is suggested that constitutive PTN signaling in breast cancer cells that inappropriately express *Ptn* activates pathways that promote a more aggressive breast cancer phenotype. Pleiotrophin signals by inactivating its receptor, the receptor protein tyrosine phosphatase (RPTP)β/ζ, and, recently, PTN was found to activate anaplastic lymphoma kinase (ALK) through the PTN/RPTPβ/ζ signaling pathway in PTN-stimulated cells, not through a direct interaction of PTN with ALK and thus not through the PTN-enforced dimerization of ALK. Since full-length ALK is activated in different malignant cancers and activated ALK is a potent oncogenic protein, we examined human breast cancers to test the possibility that ALK may be expressed in breast cancers and potentially activated through the PTN/RPTPβ/ζ signaling pathway; we now demonstrate that ALK is strongly expressed in different histological subtypes of human breast cancer; furthermore, ALK is expressed in both nuclei and cytoplasm and, in the "dotted" pattern characteristic of ALK fusion proteins in anaplastic large cell lymphoma. This study thus supports the possibility that activated ALK may be important in human breast cancers and potentially activated either through the PTN/RPTPβ/ζ signaling pathway, or, alternatively, as an activated fusion protein to stimulate progression of breast cancer in humans.

Keywords: PTN; RPTPβ/ζ; ALK; Breast cancer; Tumor progression

Breast cancers progress through genetic and epigenetic mutations that deregulate oncogenic pathways that initiate a more aggressive breast cancer phenotype [1,2]. The identification of these mutations is of major importance, since, once identified, it is likely their identification will lead to new targets for therapeutic development and potentially target directed therapy.

Anaplastic lymphoma kinase (ALK) is a receptor-type transmembrane tyrosine kinase (RTK) that is a member of the insulin receptor superfamily. ALK is functionally important in embryonic development [3] and in determination of cell survival and cell fate [4]. However, ALK was originally discovered when it was found that the chimeric N-terminal nucleophosmin (NPM) domain/cytoplasmic (catalytic) ALK domain fusion protein (NPM–ALK) is the oncoprotein underlying the pathogenesis of anaplastic large cell lymphomas (ALCL) [5,6]. NPM–ALK results from the (2;5) (p23;q35) chromosomal translocation, however, other translocations also lead to constitutive activation of ALK and other malignancies and furthermore, wild-type ALK has been postulated to be involved in the pathogenesis of rhabdomyosarcomas [7], neuroblastoma,

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and neuroectodermal tumors [8,9], glioblastomas [10], and melanomas [11].

ALK is activated through autophosphorylation [12] that has been presumed to result from ligand-enforced dimerization of ALK, a mechanism in common with other receptor tyrosine kinases (RTKs) [13]. Recently, ALK was proposed to be the physiological receptor of the 136 amino acid cytokine pleiotrophin (PTN, *Ptn*) [14]. However, these data were not reconciled with earlier studies that demonstrated that the receptor protein tyrosine phosphatase (RPTP)β/ζ is the functional receptor of PTN [15]; in those earlier studies, PTN was shown to inactivate RPTPβ/ζ and to increase tyrosine phosphorylation of the substrates of RPTPβ/ζ, which results from phosphorylation of these substrates by kinases that phosphorylate the same sites dephosphorylated by RPTPβ/ζ when cells are not stimulated by PTN. The levels of tyrosine phosphorylation of β-catenin [15] and of other substrates of RPTPβ/ζ, including β-adducin [16,17], Fyn [18], GIT1/Cat-1 [19], and P190RhoGAP [20], all are increased in PTN-stimulated cells.

More recently, we demonstrated that ALK is phosphorylated independently of a direct interaction of ALK with PTN (Perez-Pinera et al., submitted for publication[1]); it was demonstrated that ALK phosphorylated in tyrosine in PTN-stimulated cells is a substrate of RPTPβ/ζ and its levels of tyrosine phosphorylation increase in PTN-stimulated cells as also was found with each of the substrates of ALK identified above. It was demonstrated that phosphorylation of ALK in PTN-stimulated cells requires RPTPβ/ζ, and that chemically enforced dimerization of RPTPβ/ζ stimulates phosphorylation of both full-length ALK and ALK that lacks an extracellular domain. Our data furthermore demonstrated RPTPβ/ζ dephosphorylates the same site in ALK that is autophosphorylated in ALK. Thus, the PTN-dependent inactivation of RPTPβ/ζ in PTN-stimulated cells permits unchecked autophosphorylation of ALK and its activation.

Pleiotrophin is expressed in breast cancers and in cell lines derived from human breast cancers [21]; since targeting constitutive PTN signaling by a dominant negative PTN reversed the malignant phenotype of human breast cancer cells *in vivo* [22], it is suggested that constitutive PTN signaling contributes to the pathogenesis of advanced breast cancer. Recently, different models to determine the role of inappropriate expression of *Ptn* in breast cancer were tested (Chang et al., submitted for publication[2]); it was found that inappropriate expression of *Ptn* that was targeted to breast epithelial cells by the mouse mammary tumor virus (MMTV) promoter does not induce breast cancer in a MMTV-*Ptn* transgenic mice breast cancer model; however, MMTV-driven PTN signaling cooperated with the oncoprotein polyoma middle T-antigen (PyMT) to promote progression of breast cancer in MMTV-*PyMT*-*Ptn* bitransgenic mice. It was furthermore found that secretion of PTN alone through activation of stromal cells and induction of marked remodeling of the microenvironment was sufficient to account for significant features of breast cancer progression, thus, the data supports potentially a very important role of PTN signaling in promoting a more aggressive breast cancer phenotype.

Based on our recent findings that inappropriate expression of *Ptn* in transgenic mice stimulates breast cancer progression (Chang et al., submitted for publication[2]), that PTN signaling may be important in breast cancer [22], and that PTN activates the potent oncoprotein ALK through the PTN/RPTPβ/ζ signaling pathway in PTN-stimulated cells (Perez-Pinera et al., submitted for publication[1]), we explored different databases to learn whether ALK is expressed in human breast cancers; only a single manuscript described ALK expression in breast cancer cell lines using RT-PCR [11]. The significance of the transcripts of ALK detected by PCR is unknown and, in the database search, we failed to uncover that ALK is expressed in human breast cancer tissues.

We have now analyzed expression of ALK in tissues derived from human breast cancers using immunohistochemistry. We demonstrate that ALK is highly expressed in each of the different subtypes of human breast cancer studied; we furthermore observed that the cellular location and patterns of ALK expression in the breast cancer cells differs significantly from its pattern of expression in normal breast tissues; this study thus is consistent with the possibility that ALK may be activated through a constitutively activated PTN/RPTPβ/ζ signaling pathway in breast cancers that inappropriately express *Ptn*; the data also raise the alternative possibility that constitutively activated ALK fusion oncoproteins may contribute to the pathogenesis of breast cancers.

Methods

Breast cancer tissue arrays (Catalog No. CC08-01-005) were obtained from Cybrdi (Frederick, Maryland). Tissue slides were deparaffinized (2 × 10 min) in xylene, and hydrated (2 × 10 min) with 100%, 95% (2 × 10 min), (1 × 10 min) 90%, (1 × 10 min) 70% ethanol, and distilled water (10 min). The slides were then incubated in antigen retrieval solution (Trypsin 0.05%, $CaCl_2$ 0.1%, pH 7.8) for 20 min at 37 °C and then for 10 min at room temperature in a humidified chamber as previously described [23]. Endogenous peroxidase was quenched by incubating the sections with 3% hydrogen peroxide for 5 min and the tissues were permeabilized by incubating the samples in Tris-buffered saline (TBS, 10 mM Tris, pH 7.6, 150 mM NaCl) with 1% Triton X-100 for 30 min.

Non-specific binding of the antibodies was reduced by incubating the sections for 30 min in a blocking solution containing 2% bovine calf serum, 2% goat serum, 1% BSA, 0.1% gelatin, 0.1% Triton X-100, 0.05%

[1] P. Perez-Pinera, W. Zhang, Y. Chang, J.A. Vega, T.F. Deuel, Anaplastic lymphoma kinase (ALK) is activated through the pleiotrophin (PTN)/receptor protein tyrosine phosphatase (RPTP)β/ζ signaling pathway: an "alternative mechanism of receptor tyrosine kinase (RTK) activation", submitted for publication.

[2] Y. Chang, M. Zuka, P. Perez-Pinera, A. Astudillo, J. Mortimer, J.R. Berenson, Z. Wang, T.F. Deuel, Inappropriate expression of pleiotrophin (PTN) stimulates breast cancer progression through secretion of PTN and PTN-dependent remodeling of the tumor microenvironment, submitted for publication.