BIOLOGIJA. 2005. Nr. 1. P. 61–63

Expression and activity of platelet-derived growth factor receptor-β in breast carcinoma cells

M. Stoškus,
M. Ger,
V. Tunaitis,
M. Valius

Institute of Biochemistry, Department of Developmental Biology, Vilnius, Lithuania

E-mail: Mindaugas@bchi.lt

The platelet-derived growth factor (PDGF) receptor-β is a member of the type III receptor tyrosine kinase subfamily. PDGF-BB and PDGF-DD, ligands for the PDGF receptor-β, activate the receptor by inducing its dimerization and subsequent autophosphorylation at specific tyrosine residues. Phosphorylation causes upregulation of kinase activity of the PDGF receptor and provides binding sites for various downstream signaling molecules. Here we show that breast carcinoma cells obtained from different patients express the PDGF receptor. The PDGF receptor also coimmunoprecipitates with downstream signaling molecules, including phosphatidylinositol 3'-kinase and Ras GTPase activating protein. Our data show that the PDGF receptor-β is activated in breast carcinoma cells and indicate a possible role of the receptor in breast cancerogenesis.

Key words: PDGF receptor-β, breast carcinoma

INTRODUCTION

Receptors are transmembrane proteins that receive and transduce external signals to intracellular signaling molecules, thus causing changes in cell growth, proliferation, differentiation, migration and apoptosis [1]. The PDGF receptor belongs to the type III receptor tyrosine kinase subfamily [1, 2]. Members of this subfamily share a structurally similar kinase domain with an insert and five extracellular Ig-like domains [1, 3]. PDGF-BB and PDGF-DD are the ligands for the PDGF receptor-β [2]. When receptor monomers bind a dimeric ligand (PDGF-BB or PDGF-DD), the latter induces receptor dimerization and subsequent autophosphorylation at tyrosine residues. Kinase activity of the receptor is upregulated by phosphorylation at the specific tyrosine residue in the activation loop of the receptor tyrosine kinase domain. Other autophosphorylated tyrosine residues outside the kinase domain provide binding sites for various downstream signaling molecules. It is known that phosphatidylinositol 3'-kinase (PI3K), Ras GTPase activating protein (RasGAP) and a number of other proteins bind to the activated PDGF receptor-β [1]. The effects of PDGF receptor-β signal transduction depend on the cell type in which the receptor is expressed [4].

In this study, we show that breast carcinoma cells obtained from three different patients express the PDGF receptor-β. Furthermore, the receptor also coimmunoprecipitates with downstream signaling molecules, including PI3K and RasGAP, indicating that the PDGF receptor-β is activated in breast carcinoma cells.

MATERIALS AND METHODS

Cell culture. HepG2 cells were cultured on Dulbecco's modified Eagle's medium (DMEM), supplemented with 100 U/ml penicilin, 100 μg/ml streptomycin and 10% fetal bovine serum (Biochrom, Germany). The HepG2 cell line, which does not express endogenous PDGF receptor-β, was used to create a cell line to express wild-type PDGF receptor-β by using the retroviral infection system described previously [5]. Cell cultures of 70–80% confluence were made quiescent by culturing them in serum-free DMEM overnight and then stimulated or unstimulated with 30 ng/ml PDGF-BB (Amgene, USA) for 30 min at 37 °C.

Preparation extracts from cell culture and breast carcinoma tissue samples. Cultured cells or 100 mg of breast carcinoma tissue minced on ice were washed three times with ice-cold PBS and lysed in EB^{++} buffer (10 mM Tris-HCl, pH 7.4, 50 mM NaCl, 5 mM NaF, 1% Triton X-100, 20 mg/ml aprotinin, 1 mM PMSF, 2 mM $NaVO_4$). The extracts were cleared by centrifugation at 20000 × g for 15 min at 0 °C.

Immunoprecipitation and Western blotting. Cell and tissue extracts were incubated for 3 h at 0 °C with mouse monoclonal antibody against the extracellular domain of PDGF receptor-β. Then, immunoprecipi-

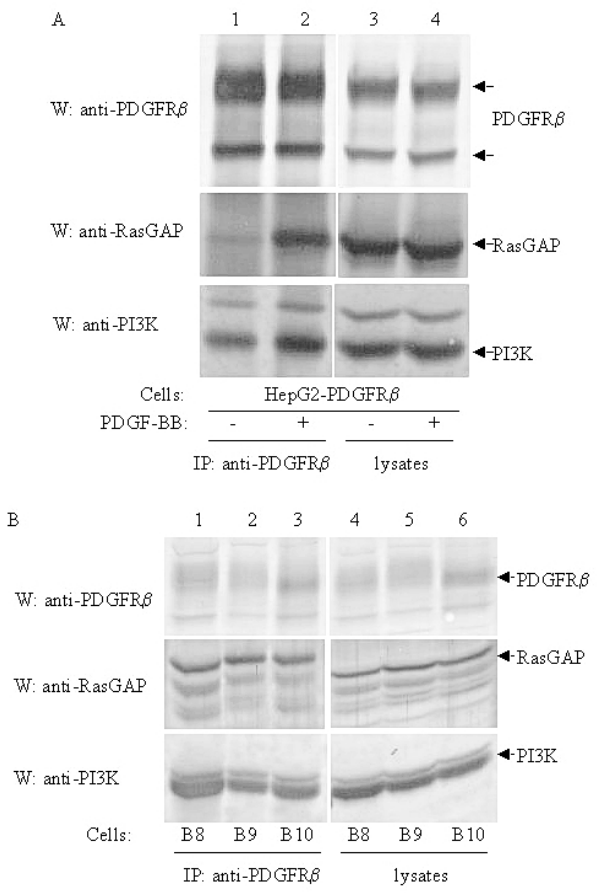

Figure. Expression and activity of PDGF receptor-β in breast carcinoma cells.
Control HepG2 cells that express wild-type PDGF receptor-β (HepG2-PDGFRβ) were either treated or untreated with PDGF-BB (A). Postnuclear lysates of HepG2-PDGFRβ cells (A) and of three different breast carcinoma tissue samples B8, B9 and B10 (B) were immunoprecipitated with specific anti-PDGF receptor-β antibody, proteins were analyzed by SDS-PAGE and subsequent protein immunoblotting using antibodies specific for PDGF receptor-β (PDGFRβ), RasGAP or PI3K. Immunoprecipitated proteins are indicated by arrows on the right

tates were incubated for 1 h at 4 °C with 20 μl of ProtA-Sepharose beads (Amersham Biosciences, UK). The beads were washed four times with EB^{++} buffer and heated for 3 min at 100 °C with SDS-PAGE sample buffer. The supernatants were run in 8% SDS-PAGE, transferred on PVDF membrane and incubated with antibodies against PDGF receptor-β, RasGAP and PI3K. Antibodies used for protein immunoblotting were crude rabbit antisera raised against an appropriate protein. Then, membranes were probed with a goat anti-rabbit alkaline phosphatase-conjugated secondary antibody (Sigma, USA). The blots were visualised in a solution containing nitro-blue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate tolidium salt (Karl Roth, Germany).

RESULTS AND DISCUSSION

To demonstrate immunoprecipitation and the ability of the activated PDGF receptor-β to coimmunoprecipitate RasGAP and PI3K, we have performed an immunoprecipitation assay using lysates from PDGF-treated and PDGF-untreated HepG2 cells expressing a wild-type PDGF receptor-β (HepG2-PDGFRβ). Data show that only a ligand-activated receptor is able to coimmunoprecipitate both RasGAP and PI3K (Figure 1A, lanes 1 and 2). Lanes 3 and 4 in Figure 1A show a total expression level of the respective proteins in the lysate. To show the expression of PDGF receptor-β and to find out whether it is activated in breast carcinoma cells, we have performed an analogous immunoprecipitation assay using lysates of breast carcinoma cells obtained from three different patients. Data showed that the PDGF receptor-β was expressed in all three breast carcinoma samples (Figure 1B, lanes 1–3 and 4–6). Furthermore, coimmunoprecipitation of both RasGAP and PI3K shows that the PDGF receptor-β is activated in these breast carcinoma samples (Figure B, lanes 1–3).

Although expression of the PDGF receptor-β and PDGF-BB proteins in breast carcinoma have bean shown in previous studies [6, 7], evidence describing activity of the PDGF receptor-β itself has been lacking. Taken together, our data show that the PDGF receptor-β is activated in breast carcinomas. Further studies are needed to determine the localization of the activated of PDGF receptor-β and its ligands, as well as the mechanisms of the receptor activation in a complex breast carcinoma tissue.

ACKNOWLEDGEMENTS

This work was supported by the Lithuanian Science and Studies Foundation (Grants: K-005 and G-046).

Received 27 January 2004
Accepted 15 November 2004

References

1. Heldin CH, Ostman A, Ronnstand L. Biochim et Biophys Acta 1998; 1378: 79–113.
2. Li X, Eriksson U. Cytokine and Growth Factor Reviews 2003; 14: 91–8.
3. Blume-Jensen P, Hunter T. Nature 2001; 411: 355–65.
4. Heldin CH. FEBS Letters 1997; 410: 17–21.
5. Lint JV, Ni Y, Valius M et al. J Biol Chem 1998; 273(12): 7038–43.
6. Kommoss F, Colley M, Hart CE, Franklin WA. Moll Cell Probes 1990; 4(1): 11–23.
7. Coltera MD, Wang J, Porter PL, Gown AM. Cancer Res 1995; 55(12): 2703–8.

M. Stoškus, M. Ger, V. Tunaitis, M. Valius

PLOKÐTELIØ KILMËS AUGIMO VEIKSNIO β RECEPTORIAUS EKSPRESIJA IR AKTYVUMAS KRÛTIES KARCINOMOS LÀSTELËSE

Santrauka

Plokðteliø kilmës augimo veiksnio (PDGF) β receptorius priklauso treèiajam tirozino kinaziø poðeimiui. PDGF β receptoriaus ligandai PDGF-BB ir PDGF-DD aktyvuoja receptoriø, indukuodami jo dimerizacijà ir tirozino radikalø autofosforilinimà. Fosforilinimo metu aktyvuojama PDGF receptoriaus kinazë ir sudaromos prielaidos specifinëms signalinëms molekulëms sàveikauti su aktyvuotu receptoriumi. Ðiame darbe mes nustatëme, kad krûties karcinomos làstelës, paimtos ið skirtingø ligoniø, ekspresuoja PDGF receptoriø. Be to, PDGF receptorius koimunoprecipituoja fosfotidilinozitol 3'-kinazæ ir Ras GTPazæ aktyvuojantá baltymà. Tai rodo, kad krûties karcinomos làstelëse PDGF β receptorius yra aktyvuotas ir gali bûti svarbus krûties kancerogenezëje.