FEBS Letters 587 (2013) 2591–2596

FEBS Letters

journal homepage: www.FEBSLetters.org

Extracellular cleavage of the p75 neurotrophin receptor is implicated in its pro-survival effect in breast cancer cells

Stéphanie Verbeke [a,b,1], Elisa Tomellini [a,b,1], Fatima Dhamani [a,c], Samuel Meignan [a,b], Eric Adriaenssens [a,c], Le Bourhis Xuefen [a,b,*]

[a] Université Lille 1, F-59650 Villeneuve d'Ascq, France
[b] INSERM U908, F-59650 Villeneuve d'Ascq, France
[c] CNRS UMR 8161, Institut Pasteur de Lille, F-59800 Lille, France

ARTICLE INFO

Article history:
Received 24 April 2013
Revised 14 June 2013
Accepted 24 June 2013
Available online 4 July 2013

Edited by Lukas Huber

Keywords:
$p75^{NTR}$
Regulated proteolysis
Survival
Breast cancer cell

ABSTRACT

The p75 neurotrophin receptor ($p75^{NTR}$) undergoes sequential proteolytic cleavages leading to the generation of a carboxyl-terminal fragment ($p75^{NTR}$-CTF) and an intracellular domain ($p75^{NTR}$-ICD) in many cellular models. We have previously shown that $p75^{NTR}$ is involved in the survival of breast cancer cells. Here, we demonstrated that $p75^{NTR}$ cleavage occurs also in these cells. Surprisingly, $p75^{NTR}$-CTF increased cell survival, whereas $p75^{NTR}$-ICD had no effect. The pro-survival effect of $p75^{NTR}$-CTF was associated with a decrease of TNF-related apoptosis-inducing ligand (TRAIL)-induced PARP and caspase 3 cleavages. Finally, our findings indicate that $p75^{NTR}$ could favor cell survival via its carboxyl-terminal fragment, independently of PI3-kinase, NF-κB, or MAP kinase signaling pathways.

1. Introduction

p75 Neurotrophin receptor ($p75^{NTR}$) is the common receptor for all the neurotrophins (NGF, BDNF, NT-3 and NT-4) and their precursors. It is a member of the TNF receptor superfamily that includes the Fas (CD95) antigen, CD30, and CD40. This family of receptors is distinguished with multiple cysteine-rich domains for ligand binding, a single transmembrane sequence, and a non-catalytic cytoplasmic domain containing a death domain sequence [1]. Since its identification and cloning in the 1980s, $p75^{NTR}$ has been reported to be involved in diverse and often contradictory functions such as survival, apoptosis, neurite outgrowth, differentiation, proliferation and migration, depending on the cell type and cellular context [1,2]. In some cases, $p75^{NTR}$ is an inducer of apoptosis, even without NGF stimulation [3], whereas in other cases the activation of $p75^{NTR}$ by NGF results in a protection from cell death [4]. $p75^{NTR}$, through a neurotrophin-dependent mechanism, dramatically enhances migration and invasion of melanoma and glioma cells [5,6]. We have shown that $p75^{NTR}$ favors survival of breast cancer cells [7–9].

$p75^{NTR}$ exerts its diverse effects through several signaling pathways including Ras homolog gene family, member A (RhoA), Jun N-terminal kinase (JNK), mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NFκB) [2]. These pathways are shown to be activated by upstream proteins that directly associate with various regions of the $p75^{NTR}$ intracellular domain. For example, guanine nucleotide dissociation inhibitor, ribosome-inactivating protein-2 (RIP-2), is able to associate with the death domain of $p75^{NTR}$; tumor necrosis factor (TNF) receptor-associated factor (TRAF), can associate with the juxtamembrane region of $p75^{NTR}$; PDZ-containing protein Fas-associated phosphatase-1 (FAP-1) can associate with the C-terminal Ser-Pro-Val [10]. In addition to associating with signaling molecules, $p75^{NTR}$, like an increasing number of proteins including β-amyloid precursor protein (APP) and Notch, undergoes regulated two-step proteolysis. Full-length $p75^{NTR}$ is initially cleaved in its extracellular juxtamembrane domain, leading to the release of the neurotrophin-binding extracellular domain and the production of a membrane-bound carboxyl-terminal fragment (CTF) [11]. The enzyme responsible for this cleavage is the tumor necrosis factor-α-converting enzyme

Abbreviations: FBS, fetal bovine serum; CTF, carboxyl-terminal fragment; ICD, intracellular domain; RIP, regulated intramembrane proteolysis; PS, presenilin; ADAM, a disintegrin and metalloprotease; TAPI, tumor necrosis factor-α protease inhibitor; TRAIL, TNF-related apoptosis-inducing ligand

* Corresponding author. Address: INSERM U908, Batiment SN3, University of Lille, 59650 Villeneuve d'Ascq, France. Fax: +33 (0)3 20 43 40 38.
E-mail address: xuefen.lebourhis@univ-lille1.fr (L.B. Xuefen).

[1] These authors contributed equally to this work.

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.06.039

(TACE/ADAM-17), a disintegrin metalloprotease [12]. Following the first cleavage, the transmembrane domain is then cleaved by presenilin-dependent γ-secretase activity, yielding a soluble intracellular fragment (ICD) [11]. These cleavages may regulate the abilities of the membrane bound C-terminal fragment and the intracellular domain to interact with downstream partners, explaining the diversity of functions mediated by $p75^{NTR}$ [13].

In this work, we first determined if $p75^{NTR}$ underwent regulated proteolysis in breast cancer cells and then evaluated the impact of $p75^{NTR}$ proteolysis in $p75^{NTR}$-mediated cellular survival. Our results indicate that $p75^{NTR}$ proteolysis does occur in breast cancer cells. Moreover, the product of first cleavage (CTF) is involved in $p75^{NTR}$-mediated survival while the product of second cleavage (ICD) has no effect.

2. Materials and methods

2.1. Reagents

Tumor necrosis factor-α protease inhibitor 1 (TAPI-1), compound E, LY294002, PD98059, BAY117082 and MG132 were purchased from Calbiochem. TNF-related apoptosis-inducing ligand (TRAIL) and hepatocyte growth factor (HGF) were purchased from R&D system.

2.2. Cell culture and transfection

MDA-MB-231 and BT-20 breast cancer cell lines were obtained from the American Type Culture Collection. Cells were routinely maintained in monolayer cultures in minimal essential medium (MEM) (Gibco®) supplemented with 10% fetal bovine serum (FBS; Perbio). The inducible $p75^{NTR}$ overexpressing MDA-MB-231 cells were established as described previously [8], and $p75^{NTR}$ overexpression was induced by treating cells with ponasterone A (PA; Sigma Aldricht®) 10 μM for 24 h. To test the effect of $p75^{NTR}$ cleavages, breast cancer cells were transfected by nucleofection (Amaxa®) with siRNA against ADAM17 5′-GGAAGCUGACCUGGUUACAACUCAU-3′, or presenilin 1 (PS1) 5′-AAGGUCCACUUCGUAUGCUGGTT-3′, or control siRNA 5′-GCUGACCCUGAAGUUCAUC-3′ according to manufacturer's instructions. Cells were then cultured for 24 h before PA induction. For transient expression experiments, MDA-MB-231 cells (3×10^5 cells/well) were seeded in six-well dishes. Transfections were performed using Lipofectamine™ 2000 Transfection Reagent (Invitrogen) according to manufacturer's instructions. For luciferase activity assay, 100 ng of κB luciferase reporter construct and 25 ng of normalizing vector pRLnull was cotransfected with 1 μg of DNA of interest. Cells were incubated with the reagents for 5 h at 37 °C before the culture medium was replaced with fresh one for further culture. To measure the reporter activity, cells were harvested 24 h after transfection using a passive lysis buffer (Promega) and lysates were clarified by centrifugation. Luciferase assay was performed using a Luciferase assay system (Promega) and Luciferase activities were measured using a lumat LB 9501 (Berthold). Triplicate samples were performed in each experiment, and standard deviations (S.D.) are shown.

2.3. Plasmids

Vectors containing full length $p75^{NTR}$ ($p75^{NTR}$ wt), a chimeric receptor in which the transmembrane domain of the FasR was introduced into $p75^{NTR}$ (P3) were generous gifts of Dr. Moses V. Chao [11]. The $p75^{NTR}$-ICD encoding vector was a generous gift of Dr. Philip A Barker [14]. $p75^{NTR}$-CTF was performed as following: CTF fragment was fused to GFP (green fluorescent protein) in pEGFP C1 vector (Promega) and GFP-CTF was cloned in phase with transmembrane domain of $p75^{NTR}$ in PCDNA3 vector. The normalizing vector pRLnull has no promoter sequence to drive expression of the Renilla luciferase gene and was purchased from Promega. The κB luciferase reporter construct has five NF-κB-responsive elements in tandem and was purchased from Stratagene.

2.4. Western blot

Cells were lysed in lysis buffer (20 mM Tris–HCl pH 7.4, 50 mM NaCl, 5 mM EDTA, 1% Triton X-100) and western blot was performed as previously described [8]. Anti-actin (sc-47778), anti-ERK2 (sc-154), anti-PARP (sc-7150) antibodies were purchased from Santa Cruz Biotechnology. Anti-PS1 (#3622), anti-TACE/ADAM17 (#3976), anti-cleaved caspase 3 (#9664), anti-AKT (#2920), anti-phosphoAKT (#4060), anti-phophoERK (#91065) antibodies were purchased from Cell Signalling Technology. Anti-$p75^{NTR}$ (G323A) antibody was purchased from Promega.

2.5. Measurement of cell survival or cell death

(i) Hoechst staining. After treatment by TRAIL (4 ng/ml for 4 h) or transfection, cells were fixed with ice-cold methanol (−20 °C) for 10 min and washed twice with phosphate-buffered saline (PBS) before incubation with 1 mg/ml Hoechst 33258 for 10 min at room temperature in the dark. Cells were then washed with PBS and mounted with coverslips using Glycergel (Dako). The apoptotic cells exhibiting condensed and fragmented nuclei were counted under an Olympus-BH2 fluorescence microscope in randomly selected fields. (ii) Flow cytometry analysis. 48 h after transfection of $p75^{NTR}$ derived-vectors, cells were treated with TRAIL, washed and stained with 50 μg/ml propidium iodide (PI) (Sigma) according to the manufacturer's instructions. Cell death was

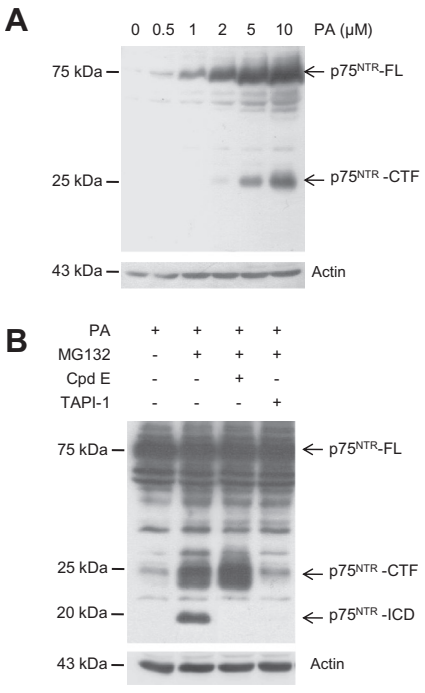

Fig. 1. Western blot detection of $p75^{NTR}$ proteolytic processing in MDA-MB-231 breast cancer cells. (A) Cells were treated with different concentrations of pronasterone A (PA) during 24 h for the detection of $p75^{NTR}$-FL and $p75^{NTR}$-CTF. (B) Effect of MG132 (proteasome inhibitor), compound E (γ-secretase inhibitor) and TAPI-1 (MMPs/ADAMs inhibitor) on $p75^{NTR}$ processing. Cells were treated with 10 μM PA for 24 h and then treated for 1 h with 5 μM MG132, 1 μM compound E, or 10 μM TAPI-1. Actin was used as a loading control.