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Photic injury promotes cleavage of p75NTR by TACE and nuclear trafficking of the p75 intracellular domain

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The p75 neurotrophin receptor (p75NTR) is a member of the tumor necrosis factor receptor superfamily that paradoxically mediates neuronal survival and differentiation or apoptotic cell death. Cleavage of p75NTR by a constitutively active metalloprotease could result in shedding of its extracellular domain (p75ECD) and generation of a pro-apoptotic intracellular domain (p75ICD). In this study, we established that exposure of a transgenic mouse photoreceptor cell line to intense light upregulated the expression of p75NTR and of the disintegrin metalloprotease tumor necrosis factor-converting enzyme (TACE) and resulted in apoptotic cell death. Light damage promoted TACE cleavage of p75NTR resulting in shedding of the soluble p75ECD and nuclear translocation of the p75ICD. Overexpression of TACE and p75NTR-induced p75NTR cleavage and secretion of p75ECD, but not nuclear transport of p75ICD. Light-induced cleavage of p75NTR, nuclear localization of p75ICD, and apoptosis were inhibited by IC-3, a metalloprotease inhibitor. Increased levels of p75NTR and TACE were observed in photoreceptor cells of animals with photic injury. Our findings support a role for TACE in the proteolytic cleavage of p75NTR and light-induced apoptosis.

Keywords: p75 neurotrophin receptor; p75ICD; p75NTR; Intracellular domain; Tumor necrosis factor-α converting enzyme; Photic injury; Apoptosis; Photoreceptor cells

Introduction

p75NTR[1] is a member of tumor necrosis factor receptor (TNFR) superfamily that includes CD27, CD30, CD40, OX40, Fas/CD95, DR3, DR4, DR5, and TNFR-1 and TNFR-2 (Baker and Reddy, 1996, 1998; Miller and Kaplan, 1998) and can induce cell death both in vitro and in vivo (Barrett and Bartlett, 1994; Casaccia-Bonnefil et al., 1996; Frade et al., 1996; Majdan et al., 1997; Miller and Kaplan, 1998; Frade and Barde, 1999; Coulson et al., 2000). p75NTR contains a cysteine-rich extracellular domain (p75ECD), and an intracellular domain (p75ICD) containing a type II death domain that does not aggregate or self-associate like the Fas/CD95 death domain nor bind other death domain-containing proteins in order to induce cell death (Liepinsh et al., 1997; Barker, 1998; Wang et al., 2001; Roux and Barker, 2002). Instead, studies show that overexpression of p75ICD, which is highly conserved among animal species (Heuer et al., 1990; Liepinsh et al., 1997), induces neuronal cell death within the central and peripheral nervous systems (Frade et al., 1996; Majdan et al., 1997). The full-length p75NTR has been reported to be cleaved by a constitutively active membrane-bound metalloprotease to generate a soluble p75ECD and a membrane-bound receptor fragment containing the p75ICD (DiStefano and Johnson, 1988; Zupan et al., 1989; Barker et al., 1991; DiStefano et al., 1993). Elevated levels of p75ICD such as those generated by cleavage of full-length p75NTR could thus promote cell death.

Tumor necrosis factor-α-converting enzyme (TACE) is a member of A disintegrin and metalloprotease (ADAM) family (Schlondorff and Blobel, 1999) of transmembrane glycoproteins that contain both a disintegrin and a metalloprotease domain (Black and White, 1998). These glycoproteins have been implicated in various cellular processes such as matrix degradation, cell migration, cell–cell interaction, and shedding of

Abbreviations: TNFR, Tumor necrosis factor receptor; p75NTR, p75 neurotrophin receptor; p75ECD, p75NTR extracellular domain; p75ICD, p75NTR intracellular domain; TACE, Tumor necrosis factor-converting enzyme; IC-3, Immunex compound-3; PI, Propidium iodide; H-33342, Hoechst 33342.

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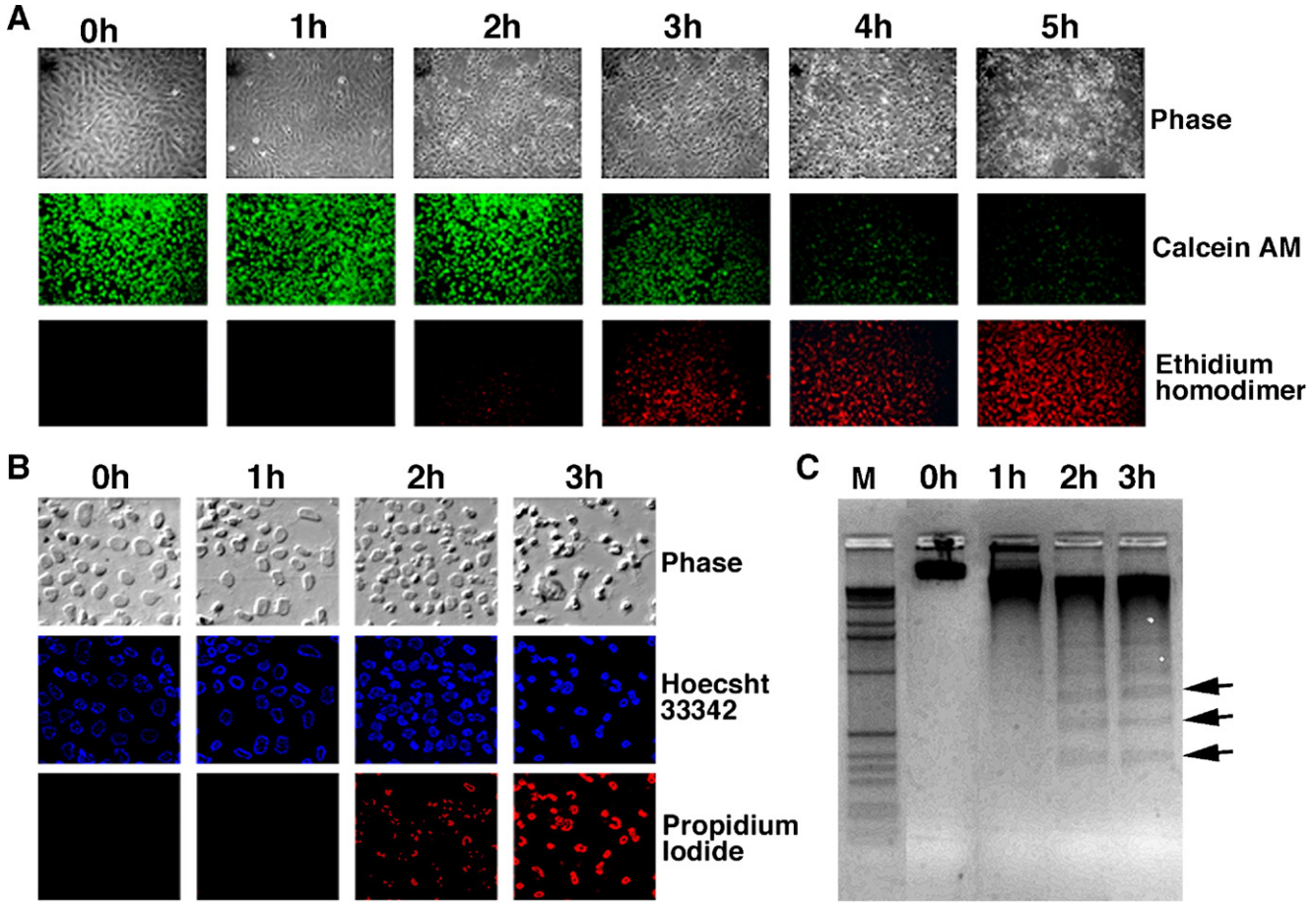

Fig. 1. Light exposure induces photoreceptor apoptosis. 661 W cells were pulsed with 1400fc of light for 0–5 h and then chased for 20 h in the dark prior to assays. (A) Cells were labeled with 2.0 μM calcein AM (CA) and 4.0 μM ethidium homodimer (EH) for 45 min to test for cell survival. 661 W cells exposed to light for 0–1 h stained intensely for CA (green) but not with EH (red). Light exposure from 2 to 5 h resulted in decreasing amount of staining for CA and increased nuclear staining with EH. (B) 661 W cells were double-stained with 500 nM propidium iodide (PI) and 500 nM Hoechst 33342 for 10 min to test for apoptosis. 661 W cells exposed to light for 2–3 h, exhibited intense nuclear staining for PI (red) while cells exposed for 0–1 h were negative. (C) Ethidium bromide-labeled bands (arrows) in multiples of ~180 bp were observed in 661 W cells exposed to light for 2–3 h. Genomic DNA from cells exposed to light for 0–1 h barely migrated in the agarose gels. M, molecular sizes.

cytokines and growth factors from membrane-bound precursors (Schlondorff and Blobel, 1999). TACE, which is expressed constitutively in many tissues (Black et al., 1997), is a major proteolytic enzyme for tumor necrosis factor-α (Black et al., 1997; Moss et al., 1997) and also promotes cleavage of a diverse group of transmembrane proteins including transforming growth factor-α (Peschon et al., 1998), L-selectin (Peschon et al., 1998), p75 TNFR (Peschon et al., 1998), growth hormone receptor (Zhang et al., 2000), amyloid precursor protein (APP) (Buxbaum et al., 1998), and prions (Vincent et al., 2001). Cleavage by TACE results in shedding of the soluble extracellular domain of the affected substrates and, in the case of Notch, APP, and the ErbB-4 receptor, initiates the release and nuclear translocation of their intracellular domains in a process called regulated intramembrane proteolysis (Heldin and Ericsson, 2001). Due to its high homology to TNFR, p75NTR appears to be a suitable substrate for TACE-mediated cleavage that may both lead to shedding of a soluble p75ECD and generation of a pro-apoptotic p75ICD. In the following study, we investigated the effects of intense light exposure on expression of p75NTR and TACE; the ability of TACE to promote cleavage and nuclear translocation of p75ICD; and the role of p75ICD in light-induced apoptosis.

Fig. 2. Increased expression and cleavage of p75NTR in light-exposed photoreceptor cells. (A) 661 W cells were exposed to light for 0–3 h and labeled with anti-p75ICD antiserum at 1:200 dilution. After 3 h of exposure, cells were rounded and their nuclei (stained with DAPI) much smaller in size suggestive of cell death. Increased staining for p75NTR (green) was observed with increased light exposure and appeared to co-localize with DAPI (blue) in the nuclear areas. (B) Western blot of 661 W lysates using 1:2000 dilutions of the anti-p75ICD or the anti-p75ECD antisera both showed ~75-kDa bands, while ~50-kDa bands reacted only with the anti-p75ICD antiserum. β-Tubulin was used to normalize protein loading. (C) Subcellular fractionation followed by Western blots with both antisera showed increased intensity of the ~75-kDa bands in both membrane and cytosolic fractions, but not in the nuclear extracts. Bands ~50-kDa reactive for p75ICD, but not p75ECD, were absent in the membrane fractions but appeared in the cytosolic fractions as early as 1 h of light exposure and in the nuclear fractions after 2–3 h light exposure. The purity of the fractions was verified by probing for β-actin (membrane), splicing factor (nuclear), or Akt (cytosolic). Scale bar=50 μm.