Presenilin-dependent γ-Secretase Processing Regulates Multiple ERBB4/HER4 Activities*

Received for publication, November 3, 2004, and in revised form, March 2, 2005
Published, JBC Papers in Press, March 2, 2005, DOI 10.1074/jbc.M412457200

Gregory A. Vidal‡, Anjali Naresh§, Luis Marrero¶, and Frank E. Jones§§

From the ‡Departments of Structural and Cellular Biology and §Biochemistry, Tulane University Health Sciences Center, Tulane Cancer Center, New Orleans, Louisiana 70112-2699 and the ¶Louisiana State University Health Sciences Center, Gene Therapy Program, The Morphology and Imaging Core Laboratory, New Orleans, Louisiana 70112

Transmembrane receptors typically transmit cellular signals following growth factor stimulation by coupling to and activating downstream signaling cascades. Reports of proteolytic processing of cell surface receptors to release an intracellular domain (ICD) has raised the possibility of novel signaling mechanisms directly mediated by the receptor ICD. The receptor tyrosine kinase ERBB4/HER4 (referred to here as ERBB4) undergoes sequential processing by tumor necrosis factor-α-converting enzyme and presenilin-dependent γ-secretase to release the ERBB4 ICD (4ICD). Our recent data suggests that regulation of gene expression by the ERBB4 nuclear protein and the proapoptotic activity of ERBB4 involves the γ-secretase release of 4ICD. To determine the role γ-secretase processing plays in ERBB4 signaling, we generated an ERBB4 allele with the transmembrane residue substitution V673I (ERBB4-V673I). We demonstrate that ERBB4-V673I fails to undergo processing by γ-secretase but retains normal cell surface signaling activity. In contrast to wild-type ERBB4, however, ERBB4-V673I was excluded from the nuclei of transfected cells and failed to activate STAT5A stimulation of the β-casein promoter. These results support the contention that γ-secretase processing of ERBB4 is necessary to release a functional 4ICD nuclear protein which directly regulates gene expression. We also demonstrate that 4ICD failed to accumulate within mitochondria of ERBB4-V673I transfected cells and the potent proapoptotic activity of ERBB4 was completely abolished in cells expressing ERBB4-V673I. Our results provide the first formal demonstration that proteolytic processing of ERBB4 is a critical event regulating multiple receptor signaling activities.

Activated single transmembrane cell surface receptors typically transmit extracellular signals through the recruitment of membrane and cytosolic signal transduction proteins. These complex cascades of protein:protein interactions and post-translational modifications culminate in the nucleus where the activation of specific target genes regulates diverse cellular responses including proliferation, differentiation, migration, and apoptosis. Recent biochemical and genetic evidence suggests that the presenilin-dependent γ-secretase processing of cell surface receptors contributes to cellular signaling through novel pathways involving an active receptor intracellular domain (ICD)1 (1, 2). For example, the presenilin-dependent γ-secretase processing of the transmembrane receptor Notch results in release of the Notch ICD (NICD) and subsequent NICD transcription factor activity in the nucleus (3–5).

The receptor tyrosine kinase ERBB4 also undergoes γ-secretase processing releasing the ERBB4 intracellular domain (4ICD); however, the contribution of this event to ERBB4 signaling remains to be determined. ERBB4 is a member of the ERBB-family of receptor tyrosine kinases, which also includes the epidermal growth factor receptor, ERBB2/HER2/Neu, and ERBB3. This receptor family controls several cell fate decisions during multiple stages of embryonic and postnatal development (6). ERBB4 activation has been associated with diverse cellular responses including proliferation (7), cell migration (8), and differentiation (9, 10).

The biochemical details of ERBB4 proteolytic processing resulting in ERBB4 ectodomain shedding and nuclear accumulation of 4ICD have been elucidated. Activated ERBB4 is first cleaved within the juxtamembrane region through the activity of tumor necrosis factor-α-converting enzyme (TACE) (12). This cleavage event results in shedding of the 120-kDa ERBB4 ectodomain and membrane association of the m80 ERBB4 transmembrane region and endodomain. Subsequent cleavage of TACE processed ERBB4 by γ-secretase releases 4ICD from cellular membranes. ERBB4 proteolytic processing results in nuclear accumulation of ERBB4 (13–15) and our recent biochemical data indicates that 4ICD is the predominant form of nuclear ERBB4 (15).

Similar to Notch, proteolytic processing of ERBB4 may contribute to novel ERBB4 signaling properties. For example, ERBB4 is essential for lactation initiation and milk-gene expression in the mouse mammary gland at parturition (9, 16). Moreover, ERBB4 regulates milk-gene expression through the direct association of nuclear 4ICD with a transcription complex at the endogenous β-casein promoter, which includes the signal transducer and activator of transcription family member STAT5A (15). Indeed, nuclear accumulation of 4ICD is necessary for STAT5A stimulation of the β-casein promoter suggesting that 4ICD regulates gene expression by functioning as a nuclear chaperone.

* This work was supported by NCI, National Institutes of Health Grants RO1CA95783 and RO1CA96717 (to F. E. J.), United States Army Medical Research and Material Command Grant DAMD17-03-1-0395 (to G. A. V.), and funds generously supplied through the National Cancer Coalition and the Tulane Cancer Center. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed. Tel.: 504-988-6585; Fax: 504-584-2739; E-mail: jones@tulane.edu.

1 The abbreviations used are: ICD, intracellular domain; 4ICD, ERBB4 ICD; APP, β-amyloid precursor protein; EGFP, enhanced green fluorescent protein; HRG, heregulin β1; NICD, Notch ICD; NLS, nuclear localization signal; PS1, presenilin 1; STAT5A, signal transducer and activator of transcription 5A; TACE, tumor necrosis factor-α-converting enzyme; TPA, tetradecanoylphorbol-13-acetate; DAPI, 4',6-diamidino-2-phenylindole; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositols 3-kinase.

From guest on July 23, 2018http://www.jbc.org/Downloaded from


**EXPERIMENTAL PROCEDURES**

**ERBB4 cDNA**—The human ERBB4 cDNA used in these experiments has been sequenced in its entirety (17) and represents the JM-a isoform (18). This isoform retains both TACE and γ-secretase recognition sequences and is therefore an ERBB4 isoform that undergoes complete proteolytic processing at the cell surface following ligand stimulation.

**Plasmid Constructs**—PCR-mediated site-directed mutagenesis to generate the V673I residue substitution in ERBB4 with a unique NruI recognition sequence was performed in the ERBB4 subclone pBHERBB4/SB and mutated the unique BclI recognition sequence was performed in the ERBB4 subclone pBHERBB4/SB with the SpeI/BclI-digested PCR fragment. The plasmids pERBB4-Flag (15) and pERBB4-V673I-EGFP were generated by replacing the SpeI/BclI fragment of pERBB4-Flag (15) and pERBB4-V673I-EGFP (15), respectively, with an upstream primer corresponding to the T3 promoter and downstream primer containing the specific recognition sequence was performed in the ERBB4 subclone pBlERBB4

**Preparation of Membrane and Cytosolic Cell Fractions**—Membrane and cytosolic fractions were isolated from COS-7 cells transfected with 100 ng/ml of tetradecanoylphorbol-13-acetate (TPA) for 30 min at 37 °C using a modification (15) of a protocol published elsewhere (13). Cytosolic and membrane fractions were subjected to ERBB4 immunoprecipitation and Western blot analysis.

**Western Blot Analysis**—Fractionated cell lysates or total cell lysates were separated on a 7.5% polyacrylamide gel, transferred to Hybond ECL (Amersham Biosciences) membrane, and analyzed by Western blot as described previously (10). Primary antibodies were anti-FLAG M2 (Sigma), anti-ERBB4 (Santa Cruz), anti-STAT5A (Santa Cruz), anti-F-STAT5 (Zymed Laboratories Inc.), anti-phospho-Akt (S473) (Cell Signaling Technology), anti-Akt (Cell Signaling Technology), anti-phospho-p44/42 MAPK (Erk1/2) (Cell Signaling Technology, anti-p44/42 MAPK (Erk1/2) (Cell Signaling Technology), anti-α-tubulin (Upstate) and anti-phosphoeyrocin (Santa Cruz).

**Deconvolution Microscopy**—Transfected MCF-7B cells cultured on coverslips in a 35-mm tissue culture dish were mock-stimulated or stimulated with 50 ng/ml of heregulin. Coverslips in a 35-mm tissue culture dish were mock-stimulated or stimulated with 50 ng/ml of heregulin and anti-phosphotyrosine (Santa Cruz).

**Membrane and Cytoplasmic Fractions**—The SKBr3 breast cancer cell line was transfected with pEGFP vector control, ERBB4-EGFP, or ERBB4-V673I-EGFP, and at 40 h post-transfection subcellular fractions including cytosolic, endoplasmic reticulum/endosomal, and mitochondrial were isolated exactly as described previously (25). Fifty μg of each fraction was analyzed for ERBB4/4ICD expression by Western blot.

**RESULTS**

**A Potential γ-Secretase Cleavage Site in the ERBB4 Transmembrane—Presenilin-dependent γ-secretase activity catalyzes the intramembrane cleavage of several single domain cell surface proteins including ERBB4, Notch1, and β-amyloid precursor protein (APP) (21). Notch1 is first cleaved by a furin-like protease (22, 23), and both ERBB4 and Notch are cleaved by TACE (12, 24, 25) followed by presenilin-dependent γ-secretase processing, which results in the membrane release and nuclear accumulation the ICD of the receptor (3, 5, 13–15). The 4ICD harbors a nuclear localization signal (NLS) (15) and a pro-apoptotic BH3-domain (Fig. 1A) (15).

Although γ-secretase cleaves APP with a relatively relaxed specificity (26) a single base substitution described for Notch1 in vivo (27). To determine whether γ-secretase cleavage of ERBB4 has an impact on ERBB4 function we incorporated the base substitution V673I within the ERBB4 transmembrane, which is positioned similar to both the inactivating base substitution described for Notch1 (28) and a valine critical for γ-secretase processing of APP (26, 29) (Fig. 1B, asterisks).

**A Point Mutation (V673I) within the ERBB4 Transmembrane Abolishes γ-Secretase Processing**—We used Western blot analysis of membrane and cytosolic fractions from transiently transfected COS-7 cells to confirm that the ERBB4-V673I base substitution abolished ERBB4 γ-secretase processing and subsequent cytosolic accumulation of 4ICD. In the absence of TPA, Western blot analysis of ERBB4 immunoprecipitates revealed the membrane-associated 180-kDa holoreceptor and the TACE-processed ERBB4 m80 fragment (Fig. 2, lane 2). Detection of the m80 fragment in ERBB4-V673I immunoprecipitates indicates that the V673I mutation does not impact TACE processing or ERBB4 ectodomain shedding (Fig. 2, lane 3). The treatment of ERBB4-transfected cells with TPA stimulates...
gamma-Secretase Processing Regulates ERBB4 Signaling

γ-Secretase Processing Regulates ERBB4 Signaling, which was previously demonstrated by Western blot analysis of separated membrane and cytosolic cellular fractions. Transfected COS-7 cells were mock-treated (→ TPA; lanes 1–6) or stimulated with 100 ng/ml of TPA (+ TPA; lanes 7–12) for 30 min at 37 °C. Separate membrane and cytosolic cellular fractions were prepared as described under “Experimental Procedures,” and ERBB4 was immunoprecipitated from equivalent amounts of protein from each lysate. Immunoprecipitates were resolved by SDS-PAGE with a 12% resolving gel and transferred to membrane for anti-FLAG Western blot analysis. The 180-kDa ERBB4 holoreceptor (lanes 2, 3, 8, and 9), membrane-bound m80 (+) (lanes 2, 3, 8, and 9), and the 80-kDa cytosolic 4ICD (++) (lane 11) are indicated.

Fig. 1. Alignment of transmembrane domains from ERBB4, Notch1, and APP. A, schematic of ERBB4 functional domains. The ERBB4 ectodomain consists of an amino-terminal ligand binding region composed of two cysteine-rich regions (cys1, cys2). Proteolytic processing by TACE results in ectodomain cleavage. Subsequent presenilin-dependent γ-secretase processing is predicted to occur at Val-673 (V763) and results in membrane release of the 4ICD (residues 673–1309). We have identified functional domains harbored within 4ICD including a NLS (residues 676–684) (15) and a BH3 domain (residues 896–992). B, alignment of transmembrane domains from ERBB4, Notch1, and APP reveals a conserved valine residue (asterisks) with similar transmembrane positions. This valine (Val-1743) is essential for Notch1 cleavage by γ-secretase, and we predict that a similar base substitution in ERBB4 (V673) will abolish γ-secretase processing of this receptor. APP is processed by γ-secretase with relaxed specificity at two residues (underlined) in addition to the conserved valine. The ERBB4 NLS is located immediately downstream of the transmembrane domain (underlined).

γ-secretase processing of ERBB4 and subsequent accumulation of cytosolic 4ICD (14) (Fig. 2, lane 11). In contrast, TPA treatment of ERBB4-V673I-transfected cells failed to liberate 4ICD from cellular membranes, confirming that this base substitution abolishes γ-secretase processing of the ERBB4 m80 fragment (Fig. 2, lane 12).

Fig. 2. A single base substitution within the ERBB4 transmembrane domain (V673I) abolishes γ-secretase processing of ERBB4. TPA-induced processing of ERBB4 and ERBB4-V673I by γ-secretase to release the 80-kDa 4ICD was assessed by Western blot analysis of separated membrane and cytosolic cellular fractions. Transfected COS-7 cells were mock-treated (→ TPA; lanes 1–6) or stimulated with 100 ng/ml of TPA (+ TPA; lanes 7–12) for 30 min at 37 °C. Separate membrane and cytosolic cellular fractions were prepared as described under “Experimental Procedures,” and ERBB4 was immunoprecipitated from equivalent amounts of protein from each lysate. Immunoprecipitates were resolved by SDS-PAGE with a 12% resolving gel and transferred to membrane for anti-FLAG Western blot analysis. The 180-kDa ERBB4 holoreceptor (lanes 2, 3, 8, and 9), membrane-bound m80 (+) (lanes 2, 3, 8, and 9), and the 80-kDa cytosolic 4ICD (++) (lane 11) are indicated.

ERBB4-V673I Retains Normal Signaling Activity—Growth factor stimulation of the wild-type ERBB4 receptor results in receptor tyrosine phosphorylation and activation of downstream signaling pathways including co-transfected STAT5A (10), MAPK (Erk1/2), and PI3K/Akt (8). To determine the impact of the transmembrane mutation in ERBB4-V673I on downstream signaling pathways including co-transfected STAT5A (see Fig. 5), Akt, and Erk1/2.

Western blot analysis of lysates prepared from transiently transfected COS-7 cells indicated similar levels of ERBB4 and ERBB4-V673I tyrosine phosphorylation in response to HRG (Fig. 3, IP: ERBB4 and IB: P-Tyr). Likewise, despite lower levels of ERBB4-V673I expression, HRG treatment of cells expressing ERBB4-V673I or ERBB4-activated equivalent levels of Akt and Erk1/2 phosphorylation (Fig. 3). In another experiment, ERBB4-V673I regulated phosphorylation of co-expressed STAT5A at the regulatory Tyr-694 (Fig. 5A). Taken together these results indicate that the residue substitution in ERBB4-V673I does not impact signal transduction mediated by the ERBB4 holoreceptor.

γ-Secretase Processing Is Required for ERBB4 Nuclear Translocation—We have previously demonstrated that ERBB4 nuclear translocation is mediated by a NLS harbored within 4ICD (residues 676–684) and positioned immediately downstream of the potential γ-secretase cleavage site (15). Other investigators have demonstrated that pharmacological inhibitors of γ-secretase abolish ERBB4 nuclear localization (14), presumably because 4ICD was tethered at cellular membranes. Here we have shown that ERBB4-V673I fails to generate a cytosolic 4ICD fragment (Fig. 2). We therefore reasoned that a mutation of the γ-secretase cleavage site within ERBB4 would abolish ERBB4 nuclear translocation. In this experiment, we used deconvolution microscopy to visualize nuclear accumulation of ERBB4 proteins fused to the carboxyl terminus with EGFP. Consistent with our recent observations (15), wild-type ERBB4 expressed on the cell surface of transiently transfected MCF-7B cells (Fig. 4A, arrowhead), was translocated to the perinuclear region (Fig. 4B, arrow) and nucleus (Fig. 4, B N, and E) following HRG stimulation. Similar to other reports, HRG stimulation of ERBB4 in the presence of the γ-secretase inhibitor, Compound E, prevented nuclear translocation of ERBB4 (14, 30) and resulted in the retention of ERBB4 at the cell membrane (Fig. 4C, arrowhead) and also within perinuclear structures resembling late endosomes (Fig. 4C, arrow).
ERBB4-V673I was also observed at the cell surface following HRG stimulation (Fig. 4D, arrowhead). Significantly, HRG-stimulated ERBB4-V673I was excluded from the nucleus (Fig. 4D and E) and accumulated within perinuclear endosome-like structures (Fig. 4D, arrow) identical to those observed in Compound E-treated cells transfected with wild-type ERBB4 (Fig. 4C, arrow). Taken together these results strongly imply that the mutant ERBB4-V673I fails to undergo γ-secretase processing at the cell membrane thereby preventing nuclear translocation of 4ICD.

** FIG. 4.** The γ-secretase processing mutant ERBB4-V673I fails to translocate to the nucleus. A–D, deconvolution microscopy of ERBB4 or ERBB4-V673I-EGFP fusions transfected into MCF-7B cells. Transfected ERBB4-EGFP cells were mock-stimulated (A) or stimulated with 50 ng/ml of HRG for 30 min at room temperature without (B) or with (C) overnight pre-treatment with 10 μM Compound E. D, transfected ERBB4-V673I-EGFP cells were stimulated with 50 ng/ml of HRG for 30 min at room temperature. Treated cells were fixed, counterstained with DAPI, coverslipped, and observed by fluorescent deconvolution microscopy. E, the mean percentage of EGFP fluorescence in the nuclear compartment for each treatment was determined by analyzing EGFP intensities from 10 transfected cells. Arrowheads indicate membrane localization of ERBB4, and arrows indicate endosomal accumulation of ERBB4.

** FIG. 5.** γ-Secretase processing of ERBB4 is required for STAT5A stimulation of the β-casein promoter. A, Western blot (IB) analysis of STAT5A immunoprecipitations (IP) from MCF-7B cell lysates prepared from transfections with the indicated cDNAs. B, MCF-7B cells were co-transfected with the bovine β-casein promoter fused to luciferase and plasmids expressing the indicated cDNAs. Cell lysates were prepared at 2 days post-transfection, and luciferase activity was determined using standard methods. Results are reported as the fold increase in luciferase activity relative to β-casein promoter luciferase co-transfected with empty vector controls. Each treatment was performed in duplicate, and the entire experiment was repeated three times.

ERBB4-V673I was also observed at the cell surface following HRG stimulation (Fig. 4D, arrowhead). Significantly, HRG-stimulated ERBB4-V673I was excluded from the nucleus (Fig. 4, D and E) and accumulated within perinuclear endosome-like structures (Fig. 4D, arrow) identical to those observed in Compound E-treated cells transfected with wild-type ERBB4 (Fig. 4C, arrow). Taken together these results strongly imply that the mutant ERBB4-V673I fails to undergo γ-secretase processing at the cell membrane thereby preventing nuclear translocation of 4ICD.

** ERBB4-V673I Activates STAT5A but Fails to Stimulate β-Casein Promoter Activity—**We have previously demonstrated that ERBB4 activates STAT5A (10), and in the developing breast the ERBB4/STAT5A signaling pathway is essential for expression of critical milk genes during lactation (9, 16, 31). Furthermore, ERBB4 regulates STAT5A transcriptional activation of the β-casein promoter by functioning as a STAT5A nuclear chaperone (15). We therefore predict that ERBB4-V673I, which remains tethered to cellular membranes, thereby preventing nuclear accumulation of 4ICD, would lack the ability to induce STAT5A stimulation of a bovine β-casein promoter fused to luciferase. Co-transfection of STAT5A with ERBB4 or ERBB4-V673I resulted in significant levels of STAT5A phosphorylation at the regulatory Tyr-694 (Fig. 5A). In concordance with our previous observations (15), co-transfection of STAT5A and ERBB4 resulted in significant stimulation of the β-casein promoter leading to luciferase expression (Fig. 5B). In contrast, ERBB4-V673I failed to induce STAT5A stimulation of the β-casein promoter (Fig. 5B). The inability of ERBB4-V673I to cooperate with STAT5A and stimulate gene expression provides additional evidence that γ-secretase processing of ERBB4 is required for nuclear translocation of the functionally active 4ICD. Furthermore, these results corroborate our recent observations demonstrating that 4ICD nuclear localization is necessary to induce STAT5A stimulation of gene expression (15).

** Cytosolic/Mitochondrial 4ICD Is Necessary and Sufficient for ERBB4 Apoptotic Activity—**We have demonstrated that ERBB4 induces apoptosis of malignant cell lines. Here we have determined the apoptotic activity of ERBB4-V673I fused to EGFP in transiently transfected COS-7 cells. Consistent with our unpublished observations,2 ectopic ERBB4 and 4ICD induced significant levels of COS-7 cell killing (Fig. 6B). Interestingly, ERBB4 lacking an intact NLS (ERBB4muNLS) harbored apoptotic activity equivalent to wild-type ERBB4 (Fig. 6B). This result indicates that nuclear translocation of 4ICD is
In order to determine whether the ERBB4 cell-killing activity depends the mitochondrial accumulation of 4ICD, we performed an experiment to determine whether the mitochondrial accumulation of 4ICD was necessary for ERBB4-induced apoptosis. We employed a genetic approach to investigate the contribution of ERBB4 signaling to the mitochondrial accumulation of 4ICD. We have recently identified the contribution of ERBB4 signaling to the mitochondrial accumulation of 4ICD during lactation (9, 31) and the beta-casein promoter in a luciferase reporter assay (15).

FIG. 6. *γ*-Secretase processing is necessary for ERBB4-induced apoptosis and mitochondrial accumulation of 4ICD. A, ERBB4-transfected cells with the *γ*-secretase inhibitor, Compound E, dramatically reduced ERBB4 cell-killing activity (Fig. 6B). Moreover, apoptosis was essentially abolished in cells transfected with ERBB4-V673I, clearly demonstrating that proteolytic processing of ERBB4 by *γ*-secretase is necessary for ERBB4 apoptotic activity. (Fig. 6B). Ectopic overexpression of ERBB4 and ERBB4-V673I results in the generation of the membrane-bound TACE catalyzed ERBB4 cleavage product, m80. The m80 ERBB4 product, however, lacks apoptotic activity as demonstrated by the lack of cell killing induced by ERBB4 in the presence of Compound E or ERBB4-V673I. Equivalent levels of ERBB4 and ERBB4-V673I expression (Fig. 6A) indicate that cell-killing activity of ERBB4 is regulated by proteolytic processing. Interestingly, the apoptotic activity of 4ICD was significantly greater (p < 0.01) than the ERBB4 holoreceptor suggesting that proteolytic processing of ERBB4 to release 4ICD represents the limiting step during ERBB4-induced cell killing.

Our unpublished results indicate that mitochondrial accumulation of 4ICD regulates ERBB4 cell-killing activity. We therefore performed an experiment to determine whether the introduction of the V673I mutation into full-length ERBB4 completely abolished *γ*-secretase processing to recently demonstrated that proteolytic processing remains to be established. We have employed a genetic approach to investigate the contribution of ERBB4 *γ*-secretase processing to recently described ERBB4-signaling activities including activation of gene expression and induction of apoptosis.

A valine residue within the Notch1 transmembrane was shown to be essential for *γ*-secretase processing of this receptor (27). We predicted that a similarly positioned ERBB4 transmembrane base substitution (V673I) would abolish *γ*-secretase processing of ERBB4. Several lines of experimental evidence confirmed that the mutant receptor, ERBB4-V673I, was no longer a substrate for *γ*-secretase processing. For example, the introduction of the V673I mutation into full-length ERBB4 completely abolished TPA-induced accumulation of 4ICD in cytosolic cell fractions and prevented mitochondrial accumulation of 4ICD in cells ectopically expressing ERBB4-V673I. Furthermore, we show that ERBB4-V673I failed to translocate to the nucleus and accumulated within cytosolic endosome-like structures. Similar endosomal ERBB4 accumulations were observed in cells transfected with wild-type ERBB4 and treated with the *γ*-secretase inhibitor Compound E.

Using the ERBB4-V673I mutant, we investigated the impact of impaired ERBB4 proteolytic processing on the ERBB4 regulation of gene expression. We have recently identified the in vivo contribution of ERBB4 signaling to STAT5A stimulation of the beta-casein and whey acidic protein genes during lactation (9, 31) and the beta-casein promoter in a luciferase reporter assay (15). Interestingly, ERBB4 functions as a STAT5A nuclear chaperone and ERBB4-induced STAT5A stimulation of the beta-casein promoter required an intact ERBB4 NLS harbored
of 4ICD is dispensable for 4ICD apoptotic activity. Taken together these results support a model of ERBB4-induced apoptosis where TACE and γ-secretase processing of activated ERBB4 at the cell surface is necessary to release the proapoptotic BH3-only protein 4ICD (Fig. 7).

An active ERBB4 intrinsic tyrosine kinase is required for both ERBB4 nuclear localization with subsequent regulation of gene expression and ERBB4-induced apoptosis. Kinase activity is dispensable, however, for nuclear translocation and the cell-killing activity of independently expressed 4ICD (15). Although we have shown that ERBB4-V673I activates downstream signal transduction pathways, this mutant receptor failed to induce apoptosis or regulate STAT5A stimulation of the β-casein promoter. These results imply that canonical signaling pathways regulated by the holoreceptor are dispensable for these ERBB4 activities. Moreover, our results imply that the intrinsic kinase activity of ERBB4 contributes to 4ICD signaling, in part, by regulating proteolytic processing of ERBB4. Indeed, an interaction between 4ICD and presenilin 1, which harbors γ-secretase activity, has been reported (32). Likewise, presenilin 1 directly interacts with the substrates Notch1 (36), APP (37), and β-catenin (38, 39). Furthermore, Notch1 actively recruits presenilin 1 to the cell surface establishing the proteolytic complex (4). We are currently investigating the ability of activated ERBB4 to promote the formation of stable proteolytic complexes containing presenilin 1 and the ERBB4 substrate. Alternatively, ERBB4 kinase activity may stimulate signaling cascades that regulate proteolytic processing at the cell surface. For example, enhanced TACE activity is associated with cellular growth stimulation and intracellular kinase activity (11, 40).

In summary, we have generated the ERBB4 allele, ERBB4-V673I, with a mutated γ-secretase cleavage site that effectively abolishes two ERBB4 functional activities, 4ICD nuclear translocation with subsequent regulation of gene expression and the independent proteolytic activity of 4ICD. Interestingly, ERBB4-V673I retains canonical signal transduction activities; however, signal transduction through the holoreceptor appears to be dispensable for both ERBB4 regulation of gene expression and ERBB4-induced apoptosis. Our results represent the first demonstration of a physiological function for ERBB4 proteolytic cleavage and underscore the essential contributions of transmembrane receptor proteolytic processing to novel signal transduction mechanisms.

Acknowledgments—We thank Diane Clark for excellent laboratory management and members of the Jones laboratory for stimulating discussion and helpful insight. We thank Amy Notwick for editing this manuscript.

REFERENCES
1. De Strooper, B., and Annaert, W. (2001) Nat. Cell Biol. 3, 221–225
2. Steiner, H., and Haass, C. (2000) Nat. Rev. Mol. Cell. Biol. 1, 217–224
3. De Strooper, B., Annaert, W., Cuppers, P., Saftig, P., Craessaerts, K., Mumm, J. S., Schroeter, E. H., Schrijvers, V., Wolfe, M. S., Ray, W. J., Goate, A., and Kopan, R. (1999) Nature 398, 518–522
4. Ray, W. J., Yao, M., Mumm, J., Schroeter, E. H., Saftig, P., Wolfe, M., Selkoe, D. J., Kopan, R., and Goate, A. M. (1999) J. Biol. Chem. 274, 36801–36807
5. Struhl, G., and Greenwald, I. (1999) Nature 398, 522–525
6. Alroy, I., and Yarden, Y. (1997) FEBS Lett. 410, 83–86
7. Weiss, P. U., Wallasch, C., Campiglio, M., Issing, W., and Ullrich, A. (1997) J. Cell. Biol. 173, 187–195
8. Kainulainen, V., Sundvall, M., Maatta, J. A., Santiestevan, E., Klagsbrun, M., and Elenius, K. (2000) J. Biol. Chem. 275, 8641–8649
9. Long, W., Wagner, K.-U., Lloyd, K. C. K., Binart, N., Shillingford, J. M., Hennighausen, L., and Jones, F. E. (2003) Development (Camb.) 130, 5257–5268
10. Jones, F. E., Welte, T., Fu, X.-Y., and Stern, D. F. (1999) J. Cell Biol. 147, 77–87
11. Black, R. A., Doedens, J. R., Mahimkar, R., Johnson, R., Guo, L., Wallace, A., Virca, D., Eisenman, J., Slack, J., Castner, B., Sunnarborg, S. W., Lee, D. C., Cowling, R., Jin, G., Charrier, K., Peschon, J. J., and Paxton, R. (2003) Biochem. Soc. Symp. 39–52
12. Rio, C., Busbaum, J. D., Peschon, J. J., and Corfas, G. (2000) J. Biol. Chem. 275, 10379–10387
13. Kainulainen, V., Sundvall, M., Maatta, J. A., Santiestevan, E., Klagsbrun, M., and Elenius, K. (2000) J. Biol. Chem. 275, 8641–8649
14. Long, W., Wagner, K.-U., Lloyd, K. C. K., Binart, N., Shillingford, J. M., Hennighausen, L., and Jones, F. E. (2003) Development (Camb.) 130, 5257–5268
15. Black, R. A., Doedens, J. R., Mahimkar, R., Johnson, R., Guo, L., Wallace, A., Virca, D., Eisenman, J., Slack, J., Castner, B., Sunnarborg, S. W., Lee, D. C., Cowling, R., Jin, G., Charrier, K., Peschon, J. J., and Paxton, R. (2003) Biochem. Soc. Symp. 39–52
16. Rio, C., Busbaum, J. D., Peschon, J. J., and Corfas, G. (2000) J. Biol. Chem. 275, 10379–10387
13. Lee, H. J., Jung, K. M., Huang, Y. Z., Bennett, L. B., Lee, J. S., Mei, L., and Kim, T. W. (2002) *J. Biol. Chem.* **277**, 6318–6323
14. Ni, C.-Y., Murphy, M. P., Golde, T. E., and Carpenter, G. (2001) *Science* **294**, 2179–2181
15. Williams, C. C., Allison, J. G., Burow, M. E., Beckman, B. S., Marrero, L., and Jones, F. E. (2004) *J. Cell Biol.* **167**, 469–478
16. Tidcombe, H., Jackson-Fisher, A., Mathers, K., Stern, D. F., Gassmann, M., and Golding, J. P. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100**, 8281–8286
17. Plowman, G. D., Culouscou, J.-M., Whitney, G. S., Green, J. M., Carlton, G. W., Foy, L., Neubauer, M. G., and Shoyab, M. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 1746–1750
18. Elenius, K., Corfas, G., Paul, S., Choi, C. J., Bao, C., Plowman, G. D., and Klagsbrun, M. (1997) *J. Biol. Chem.* **272**, 26761–26768
19. Plowman, G. D., Whitney, G. S., Neubauer, M. G., Green, J. M., McDonald, V. L., Todaro, G. J., and Shoyab, M. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87**, 4905–4909
20. Burow, M. E., Weldon, C. B., Tang, Y., McLachlan, J. A., and Beckman, B. S. (2001) *J. Steroid Biochem. Mol. Biol.* **78**, 409–418
21. De Strooper, B., Saftig, P., Craessaerts, K., Vanderstichele, H., Guhde, G., Annaert, W., Von Figura, K., and Van Leuven, F. (1998) *Nature* **391**, 387–390
22. Blaumueller, C. M., Qi, H., Zagouras, P., and Artavanis-Tsakonas, S. (1997) *Cell* **90**, 281–291
23. Logeat, F., Bessaia, C., Brou, C., LeBail, O., Jarriault, S., Seidah, N. G., and Israel, A. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 8108–8112
24. Brou, C., Logeat, F., Gupta, N., Bessaia, C., LeBail, O., Doedens, J. R., Cumano, A., Roux, P., Black, R. A., and Israel, A. (2000) *Mol. Cell* **5**, 207–216
25. Mumm, J. S., Schroeter, E. H., Saxena, M. T., Griesemer, A., Tian, X., Pan, D. J., Ray, W. J., and Kopan, R. (2000) *Mol. Cell* **5**, 197–206
26. Lichtenthaler, S. F. Wang, R., Grimm, H., Uljum, S. N., Masters, C. L., and Beyreuther, K. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 3053–3058
27. Huppert, S. S., Le, A., Schroeter, E. H., Mumm, J. S., Saxena, M. T., Milner, L. A., and Kopan, R. (2000) *Nature* **405**, 966–970
28. Schroeter, E. H., Kissingler, J. A., and Kopan, R. (1998) *Nature* **393**, 382–386
29. Sastre, M., Steiner, H., Fuchs, K., Capell, A., Multhaup, G., Condron, M. M., Teplow, D. B., and Haass, C. (2001) *EMBO Rep.* **2**, 835–841
30. Lai, C., and Peng, L. (2004) *Biochem. Biophys. Res. Commun.* **314**, 535–542
31. Jones, F. E., Golding, J. P., and Gassmann, M. (2003) *Cell Cycle* **2**, 555–559
32. Ni, C. Y., Yuan, H., and Carpenter, G. (2003) *J. Biol. Chem.* **278**, 4561–4565
33. Jarriault, S., Brou, C., Logeat, F., Schroeter, E. H., Kopan, R., and Israel, A. (1995) *Nature* **377**, 355–358
34. Adams, J. M. (2003) *Genes Dev.* **17**, 2481–2495
35. Bouillet, P., and Strasser, A. (2002) *J. Cell Sci.* **115**, 1567–1574
36. Ray, W. J., Yao, M., Nowotny, P., Mumm, J., Zhang, W., Wu, J. Y., Kopan, R., and Goate, A. M. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 3263–3268
37. Xia, W., Zhang, J., Perez, R., Koo, E. H., and Selkoe, D. J. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 8208–8213
38. Murayama, M., Tanaka, S., Palacino, J., Murayama, O., Honda, T., Sun, X., Yasuike, K., Nihonmatsu, N., Wolozin, B., and Takashima, A. (1998) *FEBS Lett.* **433**, 73–77
39. Soriano, S., Kang, D. E., Fu, M., Pestell, R., Chevallier, N., Zheng, H., and Koo, E. H. (2001) *J. Cell Biol.* **152**, 785–794
40. Black, R. A. (2002) *Int. J. Biochem. Cell Biol.* **34**, 1–5

**γ-Secretase Processing Regulates ERBB4 Signaling**

19783

by guest on July 23, 2018http://www.jbc.org/Downloaded from
