Ubiquitination contributes to functional diversity in cell signalling

Alternative splicing, alternative cleavage, alternative fate and alternative functions of growth factor receptors.

ERBB is a family of Tyrosine Kinase Receptors composed of four members ERBB1 (EGFR, HER1) - ERBB2 (HER2, neu) - ERBB3 (HER3) - ERBB4 (HER4).

The name derives from the oncogene of the avian Erithroblastosis B virus.

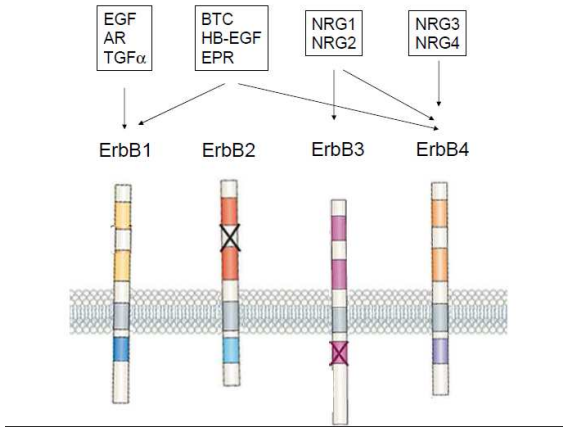
ERBB receptors deserve signalling from a variety of ligands and play important roles in development and cancer.

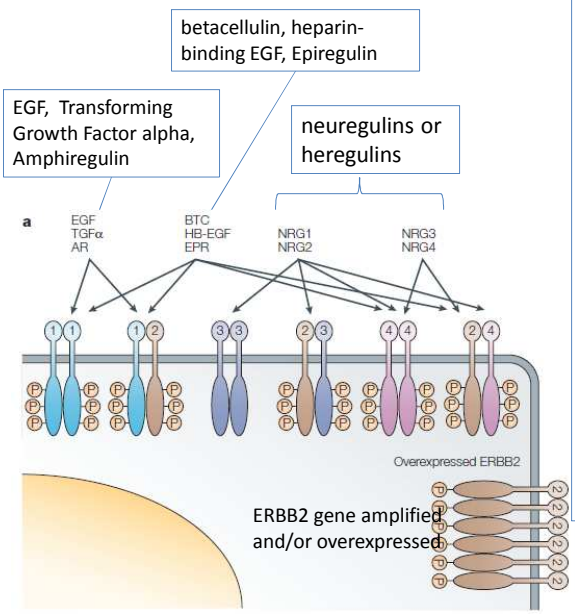
Expression in various tissues of epithelial, mesenchymal and neuronal origin.

Family specialty is formation of all kinds of homo- and heterodimeric arrangements.

Due to their role in variouos kind of human malignancies, two important types of ERBB inhibitor are in clinical use: humanized antibodies directed against the extracellular domain of EGFR or ERBB2, and small-molecule tyrosine-kinase inhibitors (TKIs) that compete with ATP in the tyrosine-kinase domain of the receptor.

Specialized functions of ERBB TKRs





Members of the epidermal growth factor (EGF) family of growth factors are ligands for the ERBB receptors. Ligand binding to ERBB receptors induces the formation of receptor homo- and heterodimers and the activation of the intrinsic kinase domain, resulting in phosphorylation on specific tyrosine residues within the cytoplasmic tail. These phosphorylated residues serve as docking sites for a range of proteins, the recruitment of which leads to the activation of intracellular signalling pathways. None of the ligands bind ERBB2, but ERBB2 is the preferred dimerization partner for all the other ERBB receptors. ERBB3 has impaired kinase activity and only acquires signalling potential when it is dimerized with another ERBB receptor, such as ERBB2.
Overexpression of ERBB2 in tumours leads to constitutive activation of ERBB2, presumably because of increased receptor concentrations at the plasma membrane. Many of these tumours contain phosphorylated ERBB3, which couples ERBB2 to the phosphatidyl-inositol 3-kinase (PI3K)–AKT pathway128

No ligand

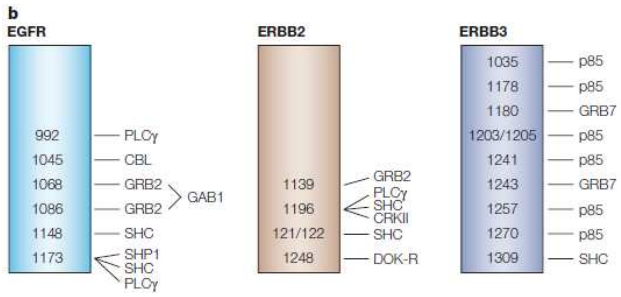
Hynes & Lane, 2005, Nat Rev Cancer 5:341-354

ERBB are implicated in various kind of human cancers

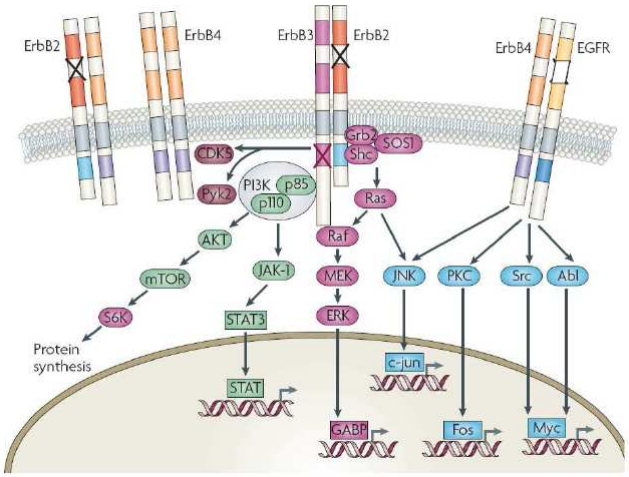
ERBB1/EGFR
Gene amplification → overexpression
Growth factors – autocrine or paracrine
Structural rearrangements (gliomas)
Mutations in the tyrosine kinase domain (NSCLC)

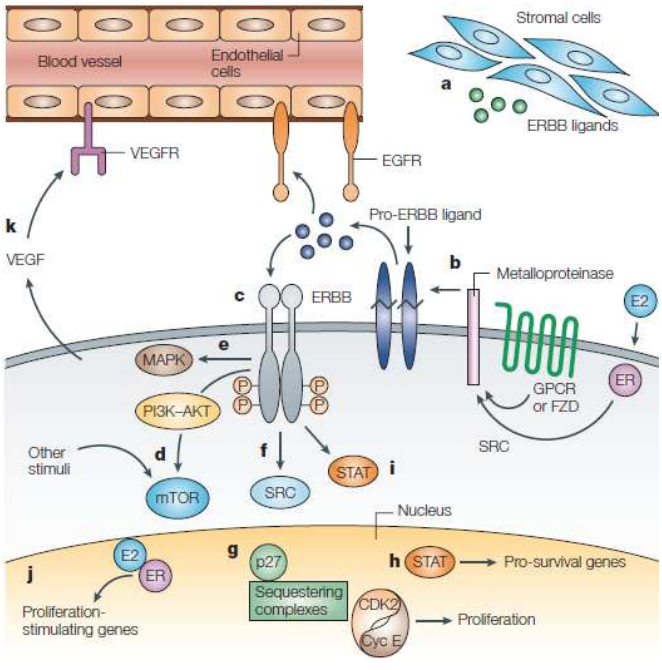
ERBB2
Gene amplification → overexpression (breast, ovarian, gastric, salivary)
mutations (NSCLC)

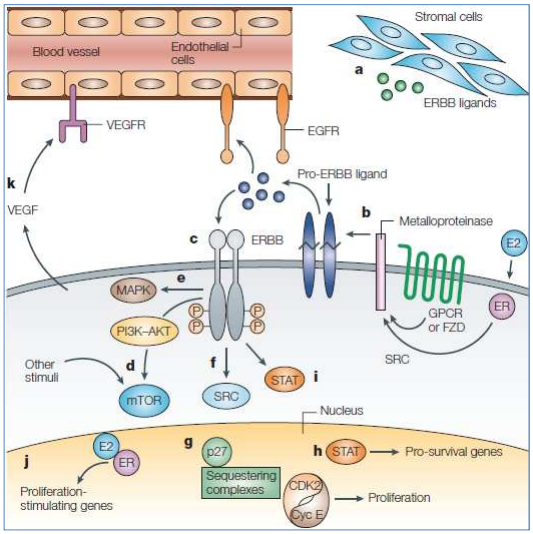
Hynes & Lane, 2005, Nat Rev Cancer 5:341-354



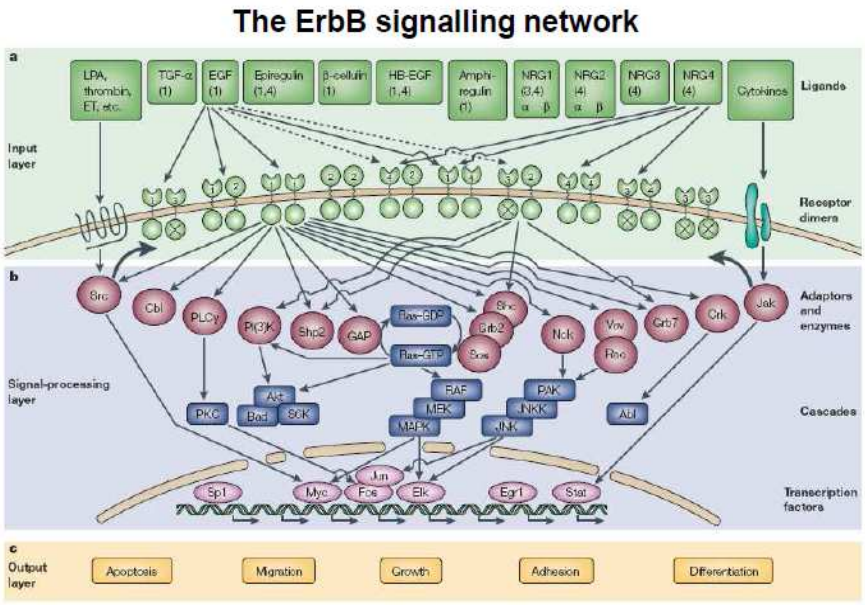
Schematic representation of the main autophosphorylation sites in EGF receptor (EGFR), ERBB2 and ERBB3 and of the signalling molecules associated with these sites. Despite extensive overlap in the molecules recruited to the active receptors, there is some preferential modulation of signalling pathways. Tumour cells that express EGFR with kinase-domain mutations preferentially activate the pro-survival PI3K–AKT and signal transducer and activator of transcription (STAT) pathways. Although EGFR has no consensus sequence for the p85 adaptor subunit of PI3K, it couples to this pathway through GAB1, which binds growth-factor receptor- bound protein 2 (GRB2). Although no direct binding data have been published, STATs have been proposed to couple to EGFR through tyrosine-1068 and tyrosine-1086. Additional EGFR binding partners exist. ERBB2 couples to the mitogen-activated protein kinase pathway through GRB2, SHC, downstream of kinase related (DOK-R) and CRK; phospholipase Cγ (PLCγ) binding has recently been described. Although ERBB3 is able to bind neuregulins (NRGs), it has impaired kinase activity owing to substitutions in crucial residues in the tyrosine-kinase domain. Therefore, ERBB3 only becomes phosphorylated and functions as a signalling entity when it is dimerized with another ERBB receptor, ERBB2 being its preferred partner. ERBB3 contains six docking sites for the p85 adaptor subunit of PI3K and couples very efficiently to this pathway. AR, amphiregulin; BTC betacellulin; EPR epiregulin; HB-EGF, heparin binding EGF; NRGs neuregulins; TGFα transforming growth factor-α.



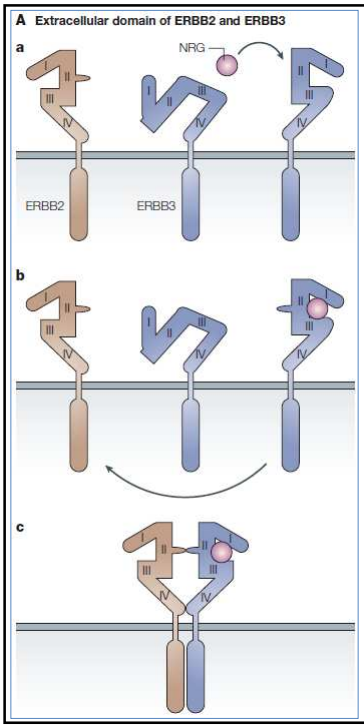




Active ERBB receptors and downstream signalling pathways in a tumour setting. In tumour cells, ERBB receptor tyrosine kinases are activated by various mechanisms, including mutation, overexpression, and autocrine or paracrine production of epidermal growth factor (EGF) family ligands. **a |** Paracrine ERBB ligands (green circles) are released from stromal cells. **b |** Autocrine ligand (blue circles) production results from the activation of G-protein-coupled receptors (GPCRs), Frizzled (FZD) or oestrogen receptor (ER), which causes the metalloproteinase-mediated cleavage and release of pro-EGF-related ligands (a process known as ectodomain shedding). The mechanisms controlling ectodomain shedding are still largely unknown, although SRC kinase has been implicated. **c |** Active ERBB receptors stimulate numerous signalling pathways by recruiting proteins to specific phosphorylated tyrosine residues in their carboxy-terminal domain. **d |** The phosphatidylinositol 3-kinase (PI3K)–AKT pathway is stimulated through recruitment of the p85 adaptor subunit of PI3K to the receptor. Mammalian target of rapamycin (mTOR) acts as a central sensor for nutrient/energy availability, and can also be modulated by PI3K–AKT-dependent mechanisms. **e |** The mitogen-activated protein kinase (MAPK) pathway is activated by recruitment of growth-factor-receptor-bound protein 2 (GRB2) or SHC to the receptor. **f |** SRC kinase is activated by ERBB receptors and by GPCRs (b) and ER. There are many nuclear effectors of ERBBs in tumour cells. **g |** One of these is the cyclin dependent kinase inhibitor p27 (also known as KIP1), which has an important role in the control of proliferation. In tumour cells with overexpressed ERBB2, p27 is sequestered from cyclin E (Cyc E)–CDK2 complexes and cells progress through the cell cycle. **h |** Signal transducer and activator of transcription (STAT) is another nuclear effector. **i |** Binding of STAT to ERBB leads to its tyrosine phosphorylation, dimerization and nuclear entry, resulting in STAT binding to specific DNA sequences in promoter regions of target genes encoding, for example, pro-survival factors (h). **j |** Nuclear ER and oestradiol (E2) controls transcription of cell-cycle regulators that are particularly important for breast cancer cell proliferation. **k |** ERBB receptors also stimulate transcription of vascular endothelial growth factor (VEGF) through the MAPK pathway. VEGF has a role in induction of tumour-associated angiogenesis. Active EGFR receptors have been detected on tumour-associated endothelial cells, which has been proposed to result from tumour release of ERBB ligands. EGFR, EGF receptor; VEGFR, VEGR receptor.



from Yosef Yarden and Mark X. Sliwkowski



ERBB-receptor ectodomain structures.
A | The extracellular region of each ERBB receptor consists of four domains (I–IV). It has been proposed that in the absence of ligand, ERBB3 and epidermal growth factor receptor (EGFR; not shown) assume a tethered structure (**a**). Domains I and III are involved in neuregulin (NRG) binding and, following this, the dimerization arm in domain II is exposed (**b**) and promotes receptor–receptor interaction (**c**). ERBB2 has a fixed conformation that resembles the ligand-activated state of EGFR and ERBB3.
B | The ERBB2-directed antibodies trastuzumab and pertuzumab bind domains IV and II, respectively.

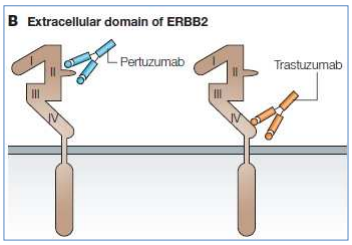
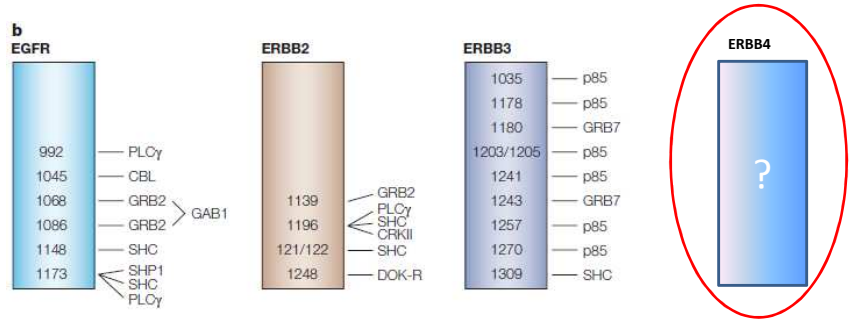


Table 1 | **ERBB-targeted therapeutics in clinical use**

Compound	Type	Target	Company	Status and comments
Trastuzumab (Herceptin)	Humanized mAb	ERBB2	Genentech/Roche	Approved for the treatment of ERBB2-overexpressing breast cancer; ongoing trials for use in combination with various other drugs
Pertuzumab (Omnitarg)	Humanized mAb	ERBB2	Genentech	Phase II trials to treat ovarian cancer, breast cancer, prostate cancer and NSCLC; based on its ability to block ERBB2 dimerization, trials are ongoing in cancer that express low ERBB2 levels
Cetuximab (Erbitux)	Chimeric mAb	EGFR	ImClone/Merck KgaA Bristol-Myers Squibb	Approved for the treatment of CRC; ongoing trials in combination with various drugs for treatment of pancreatic cancer, HNSCC and NSCLC
Matuzumab	Humanized mAb	EGFR	Merck KGaA	Phase II trials for NSCLC, gynaecological cancer, pancreatic cancer and oesophageal cancer
Panitumumab	Humanized mAb	EGFR	Abgenix	Trials are ongoing for CRC, RCC and NSCLC
Gefitinib (Iressa)	TKI	EGFR	AstraZeneca	Approved for the treatment of NSCLC after failure on other available treatments; ongoing trials in HNSCC, gastrointestinal cancer and breast cancer
Erlotinib (Tarceva)	TKI	EGFR	Genentech/OSI Pharmaceuticals	Approved for the treatment of NSCLC after failure on other available treatments; ongoing trials in many cancer types
Lapatinib	TKI	EGFR/ERBB2	GlaxoSmithKline	Phase III trial underway on breast cancer patients who are refractory to trastuzumab and chemotherapy
AEE788	TKI	EGFR/ERBB2/ VEGFR	Novartis	Phase I trials underway — first multifunction EGFR/ERBB2/VEGFR inhibitor, and there are many potential indications
CI-1033	Irreversible TKI	EGFR/ERBB2	Pfizer	Phase II trials underway in breast and NSCLC
EKB-569	Irreversible TKI	EGFR/ERBB2	Wyeth-Ayerst	Phase II trials underway in NSCLC
EXEL 7647/EXEL 0999	TKI	EGFR/ERBB2/VEGFR	EXELIXIS	Phase I trials underway

CRC, colorectal cancer; EGFR, epidermal growth factor receptor; HNSCC, nead and neck squamous-cell cancer; mAb, monoclonal antibody; NSCLC, non-small-cell lung cancer; RCC, renal-cell cancer; TKI, tyrosine-kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.

Hynes & Lane, 2005, Nat Rev Cancer 5:341-354



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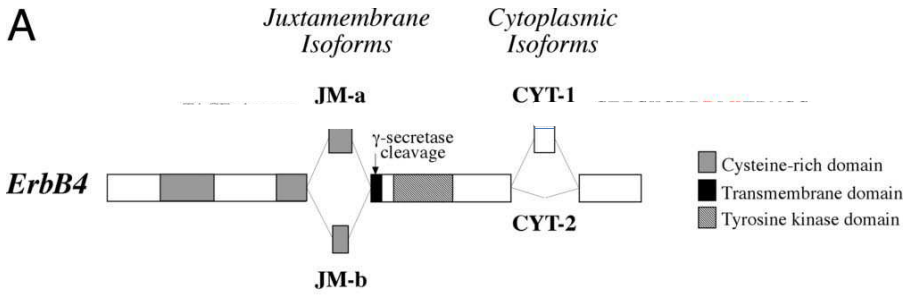
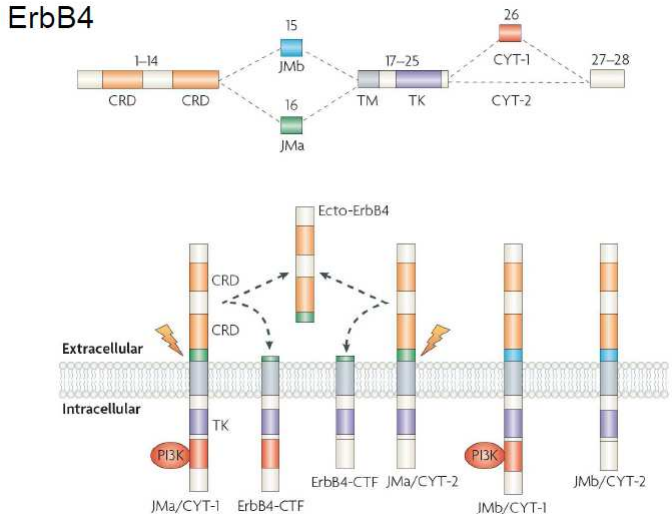
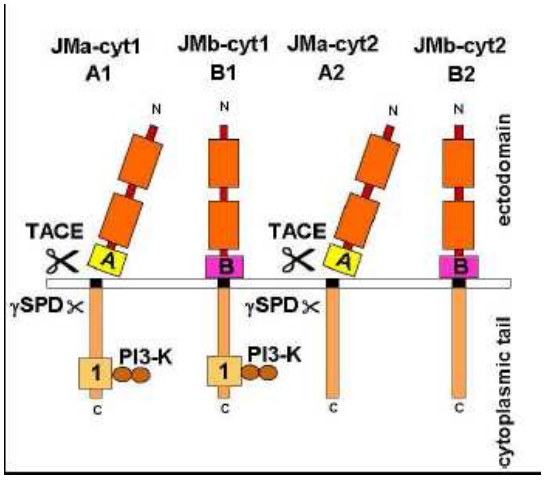
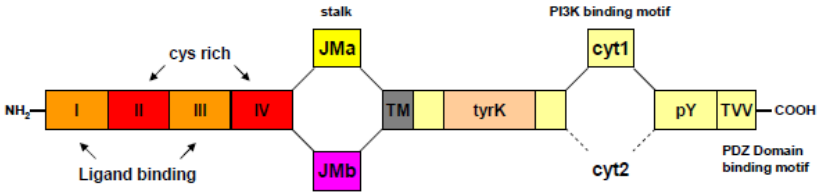
A number of contradictory reports on ERBB4 signalling and function in different model systems are possibly explained by the fact that ERBB4 is not one protein

but alternative splicing creates **four** different proteins



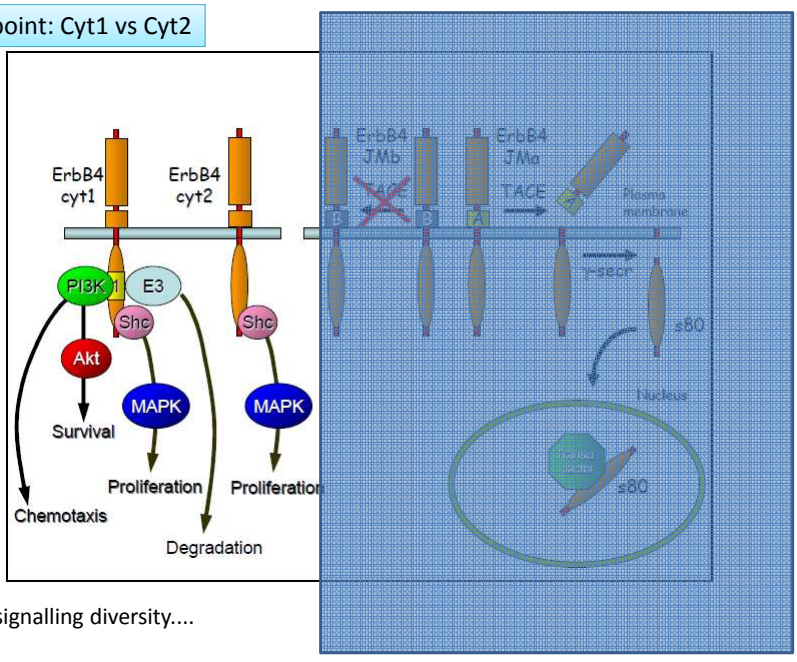






Quale tecnica si può utilizzare per sapere quali isoforme sono presenti nelle cellule/tessuto che stiamo studiando?

First point: Cyt1 vs Cyt2



signalling diversity....

Isoform-specific monoubiquitination, endocytosis, and degradation of alternatively spliced ErbB4 isoforms

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Endocytosis and subsequent lysosomal degradation serve as a well characterized mechanism to fine-tune and down-regulate EGFR signaling. However, other members of the EGFR/ErbB receptor family have been reported to be endocytosis-impaired. Here we demonstrate that endocytosis of ErbB4 is regulated in an isoform-specific manner: CYT-1 isoforms were efficiently endocytosed whereas CYT-2 isoforms were endocytosis-impaired. CYT-1 isoforms in endocytic vesicles colocalized with Rab5 and Rab7 indicating trafficking via early endosomes to late endosomal/lysosomal structures. A PPXY motif within the CYT-1-specific sequence that lacks from CYT-2 was necessary both for ubiquitination and endocytosis of CYT-1 isoforms and provided a binding site for a WW domain-containing ubiquitin ligase Itch. Itch catalyzed ubiquitination of ErbB4 CYT-1, promoted its localization into intracellular vesicles, and stimulated degradation of ErbB4 CYT-1. Dominant negative Itch suppressed ErbB4 CYT-1 endocytosis and degradation. These data indicate that ErbB4 isoforms differ in endocytosis and degradation by a mechanism mediated by CYT-1-specific PPXY motif interacting with a WW domain-containing E3 ubiquitin ligase.

articolo da studiare

Proc Natl Acad Sci US(2008), 105:4165-74

Materiale per uso didattico

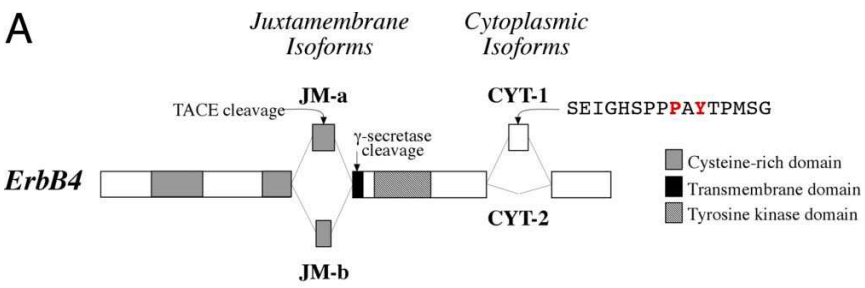
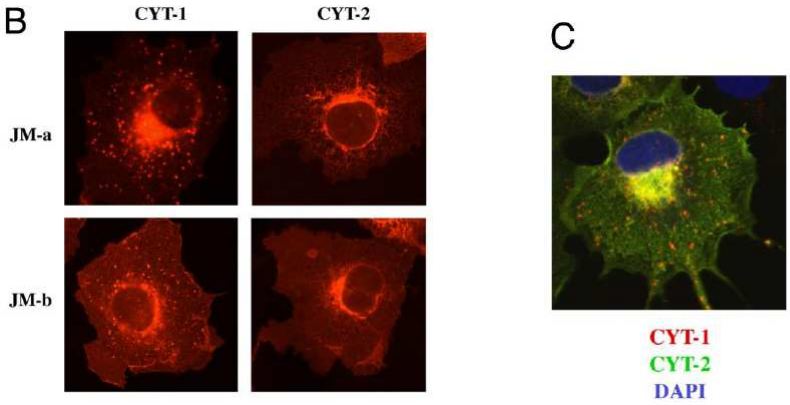
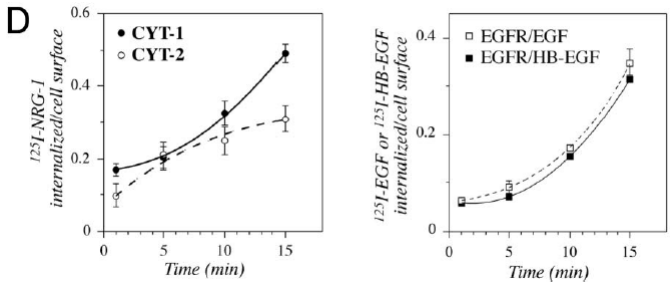


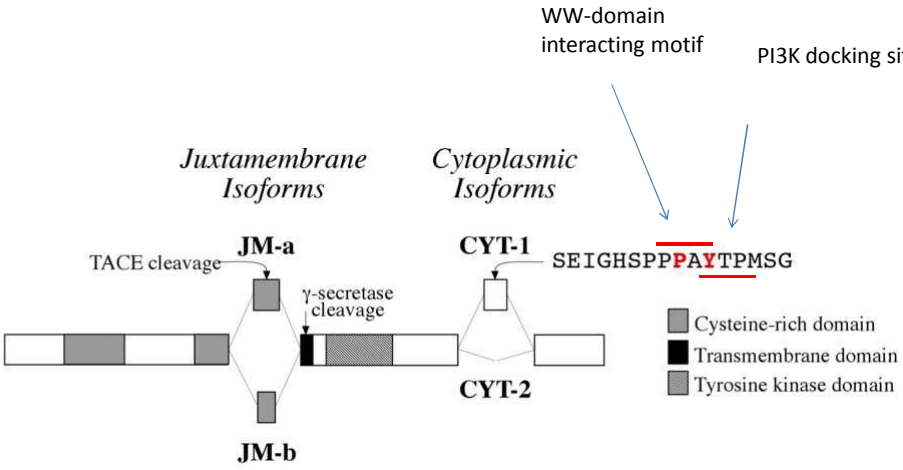
Fig. 1. Subcellular localization and internalization of ErbB4 isoforms. (*A*) Alternative splicing generates four ErbB4 isoforms that differ at juxtamembrane (JM) or cytoplasmic (CYT) domains. JM-a isoforms, unlike JM-b isoforms, include a proteolytic cleavage site for TACE and -secretase. CYT-1 isoforms, unlike CYT-2 isoforms, contain a 16-aa sequence that includes a PI3-K binding site (YTPM) as well as a proline-rich protein interaction motif (PPAY). P1054 and Y1056 are shown in red.



(*B)* COS-7 cells expressing each of the HA-tagged ErbB4 isoforms were stained with anti-HA antibody (red) and photographed under a fluorescence microscope.
(*C)* COS-7 cells simultaneously expressing HA-tagged ErbB4 JM-a CYT-1 and Myc-tagged ErbB4 JM-a CYT-2 were stained with anti-HA (red) and anti-Myc (green) antibodies. The nuclei were stained with DAPI (blue).



(*D)* NIH 3T3-7d transfectants stably expressing ErbB4 JM-b CYT-1, ErbB4 JM-b CYT-2, or EGFR were incubated for 1, 5, 10, or 15 min with ^{125}I-NRG-1, ^{125}I -EGF, or ^{125}I-HB-EGF at 37°C. Cells were washed once with PBS and twice with an acidic buffer and lysed with NaOH. Radioactivity removed in acid washes containing surface-bound growth factors was compared with radioactivity in cell lysates containing internalized growth factors.



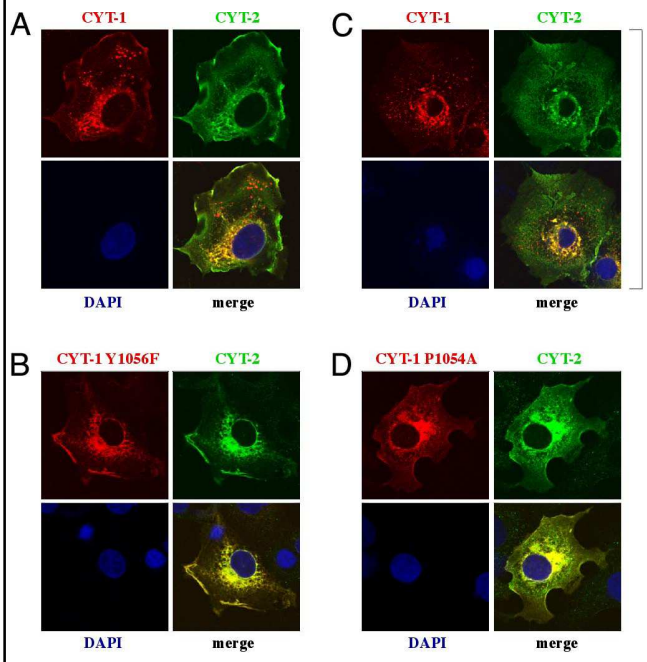
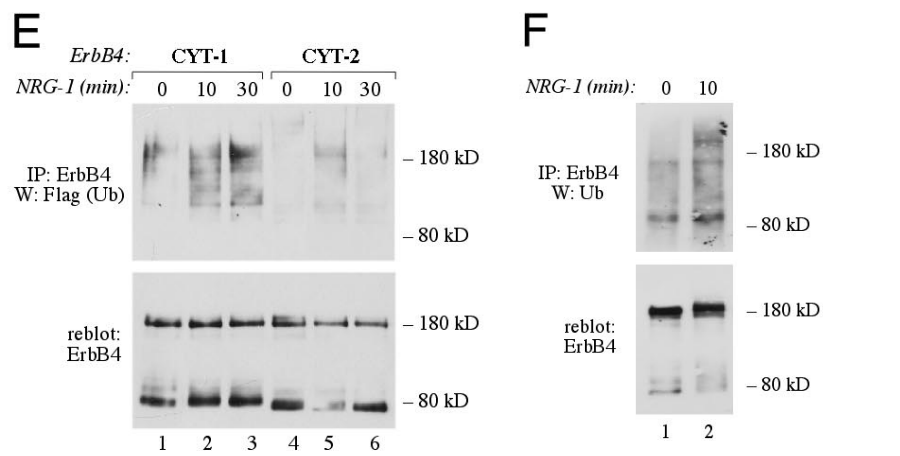
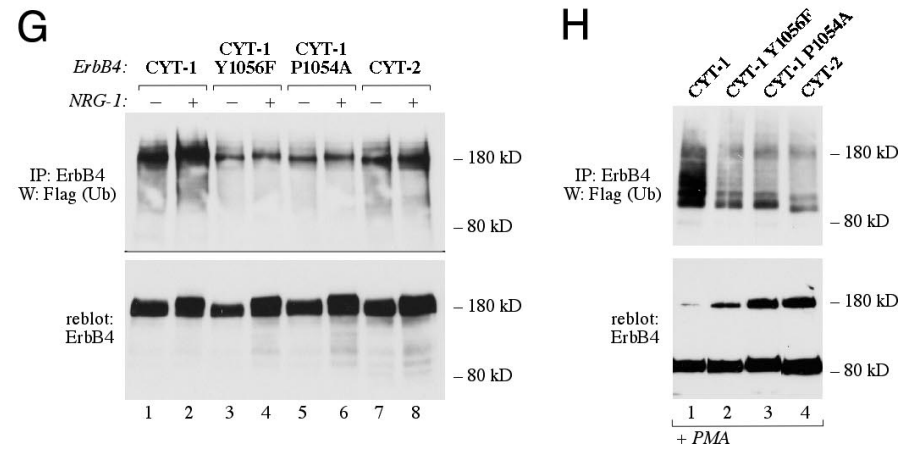


Fig. 2. PPXY motif in regulation of differential endocytosis and ubiquitination of ErbB4 CYT-1 and CYT-2. COS-7 cells were transfected with constructs encoding wild-type Myc-tagged ErbB4 JM-a CYT-2 and wild-type HA-tagged ErbB4 JM-a CYT-1 (A and C), HA-tagged ErbB4 JM-a CYT-1 **Y1056F** (B), or HA-tagged ErbB4 JM-a CYT-1 P1054A (D). Cells expressing Myc-tagged ErbB4 JM-a CYT-2 and HA-tagged ErbB4 JM-a CYT-1 were treated for 4 h without (A) or with (C) 20 M LY294002 to inhibit PI3-K activity. Cells were stained with anti-HA (red) and anti-Myc (green) antibodies. The nuclei were stained with DAPI (blue).



(*E) COS-7 cells* expressing Myc-tagged ErbB4 JM-a CYT-1 or ErbB4 JM-a CYT-2 and Flag-tagged ubiquitin were stimulated for 0, 10, or 30 min with 50 ng/ml NRG-1. Lysates were immunoprecipitated with anti-ErbB4 antibody (HFR-1) followed by Western blotting with anti-Flag antibody. Membranewas reblotted with anti-ErbB4 antibody (sc-283). (*F) OVCAR-3 cells were stimulated for 0 or 10 min with 50 ng/ml NRG-1. Lysates were immunoprecipitated with anti-ErbB4 (HFR-1) followed by Western* blotting with anti-ubiquitin (P4D1). Membrane was reblotted with anti-ErbB4 antibody (Abcam).



(*G) COS-7 cells expressing HA-tagged ErbB4 JM-b CYT-1, ErbB4* JM-b CYT-1 Y1056F, ErbB4 JM-b CYT-1 P1054A, or ErbB4 JM-b CYT-2 and Flag-tagged ubiquitin were stimulated for 0 or 30 min with 50 ng/ml NRG-1. Lysates were immunoprecipitated with anti-ErbB4 antibody (HFR-1) followed by Western blotting with anti-Flag antibody. Membrane was reblotted with anti-ErbB4 antibody (Abcam). (*H) COS-7 cells expressing ErbB4 JM-a CYT-1, ErbB4 JM-a CYT-1 Y1056F, ErbB4 JM-a CYT-1 P1054A, or ErbB4 JM-a CYT-2 and Flag-tagged* ubiquitin were stimulated for 0 or 30 min with 100 ng/ml PMA. Lysates were immunoprecipitated with anti-ErbB4 antibody (HFR-1) followed by Western blotting with anti-Flag antibody. Membrane was reblotted with anti-ErbB4 antibody (sc-283).

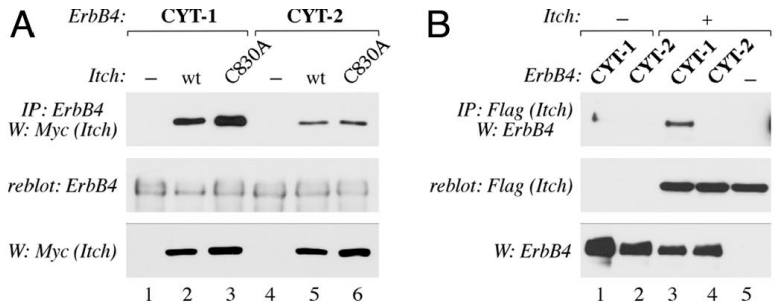
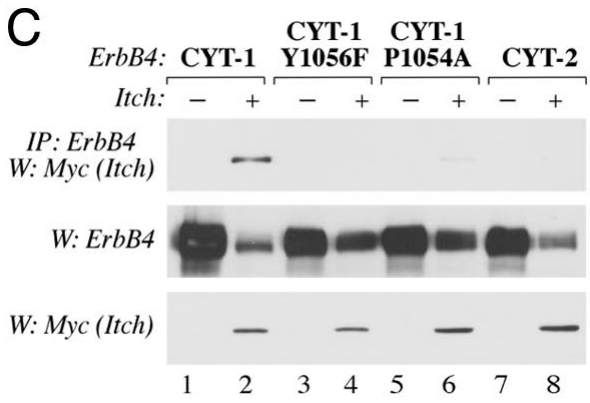
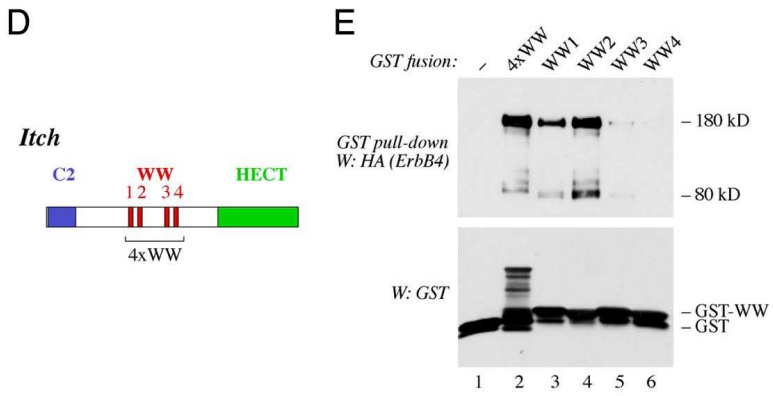


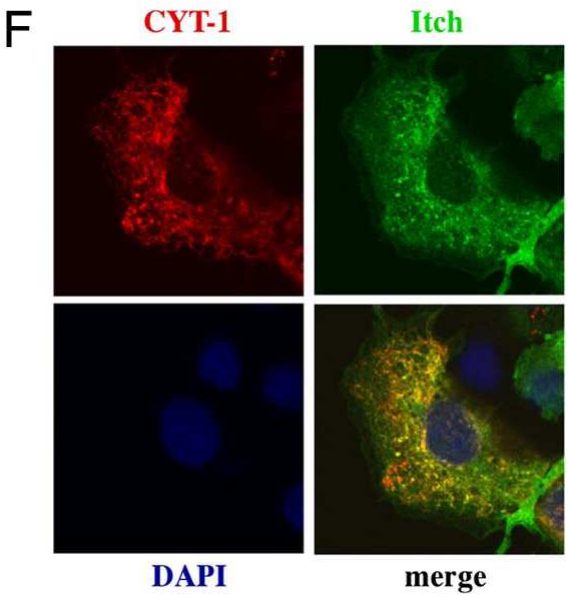
Fig. 3. Physical interaction and colocalization of ErbB4 with Itch E3 ubiquitin ligase.
(A) COS-7 cells were transfected with constructs encoding ErbB4 JM-a CYT-1 or ErbB4 JM-a CYT-2 and Myc-tagged Itch or Itch C830A. Lysates were immunoprecipitated with anti-ErbB4 antibody (HFR-1) followed by Western blotting with anti-Myc antibody. Membrane was reblotted with anti-ErbB4 antibody (sc-283). Expression of Itch was analyzed by Western blotting with anti-Myc antibody.
(B) COS-7 cells were transfected with constructs encoding HA-tagged ErbB4 JM-a CYT-1 or ErbB4 JM-a CYT-2 and Flag-tagged Itch. Lysates were immunoprecipitated with anti-Flag antibody followed by Western blotting with anti-ErbB4 antibody (Abcam). Membrane was reblotted with anti-Flag antibody. Expression of ErbB4 was analyzed by Western blotting with anti-ErbB4 antibody (Abcam).



(*C) COS-7 cells were transfected with constructs encoding ErbB4 JM-a CYT-1, ErbB4* JM-a CYT-1 Y1056F, ErbB4 JM-a CYT-1 P1054A, or ErbB4 JM-a CYT-2 with or without construct for Myc-tagged Itch. Lysates were immunoprecipitated with anti-ErbB4 antibody (HFR-1) followed by Western blotting with anti-Myc antibody. Membrane was reblotted with anti-ErbB4 antibody (sc-283). Expression of Itch was analyzed by Western blotting with anti-Myc antibody.



(D) Schematic structure of Itch including an N-terminal C2 domain for membrane anchoring, four WWdomains, and a C-terminal catalytic HECT domain. 4xWW indicates a protein region spanning all fourWWdomains. (E) GST fusion proteins including one of theWWdomains of Itch (WW1, WW2, WW3, or WW4) or all four of them in one fusion protein (4xWW) were incubated with lysates of COS-7 cells transiently expressing HA-tagged ErbB4 JM-a CYT-1. Material precipitating with glutathione Sepharose beads was analyzed by Western blotting with anti-HA antibody. Membranes were reblotted with anti-GST antibody.



(F) COS-7 cells simultaneously expressing HA-tagged ErbB4 JM-b CYT-1 and Myc-tagged Itch were stained with anti-HA (red) and anti-Myc (green) antibodies. The nuclei were stained with DAPI (blue).

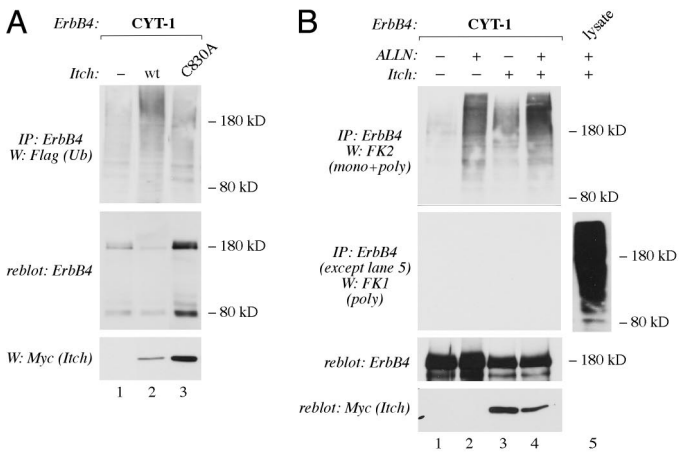
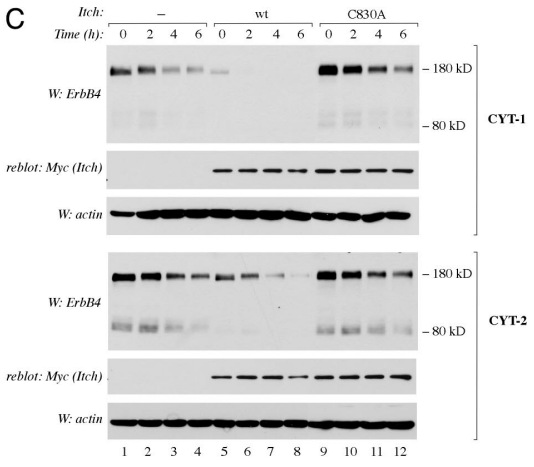
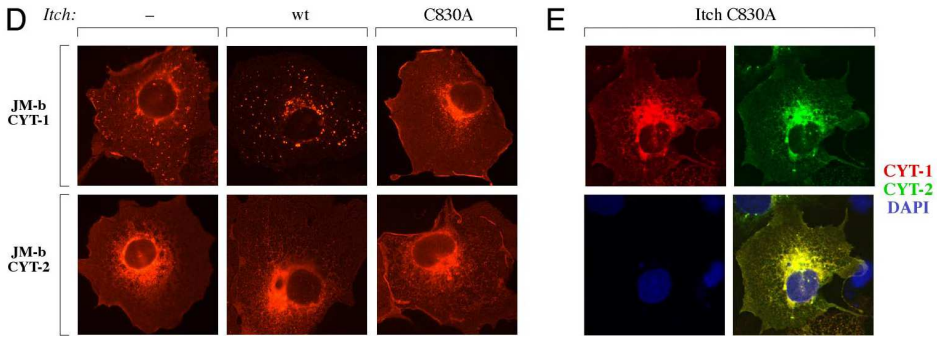


Fig. 4. Function of Itch in ErbB4 ubiquitination, endocytosis, and degradation. (*A) COS-7 cells expressing ErbB4 JM-a CYT-1 and Flag-tagged ubiquitin in the* presence or absence of Myc-tagged Itch or Itch C830A were lysed, and the lysates were immunoprecipitated with anti-ErbB4 antibody (HFR-1) followed by Western blotting with anti-Flag antibody. Membrane was reblotted with anti-ErbB4 antibody (sc-283). Expression of Itch was analyzed by Western with anti-Myc antibody. (*B) COS-7 cells expressing ErbB4 JM-a CYT-1 and Flag-tagged ubiquitin in the presence or absence of Myc-tagged Itch were treated for 2 h with or* without 150 M ALLN. Lysates were immunoprecipitated with anti-ErbB4 antibody (HFR-1) followed by Western blotting with anti-FK1 or anti-FK2 antibody. Membrane was reblotted with anti-ErbB4 antibody (sc-283). Expression of Itch was analyzed by Western blotting with anti-Myc antibody. Total lysate from cells expressing Itch-Myc and treated with ALLN was also analyzed by Western blotting with FK1 antibody without prior immunoprecipitation (lane 5).

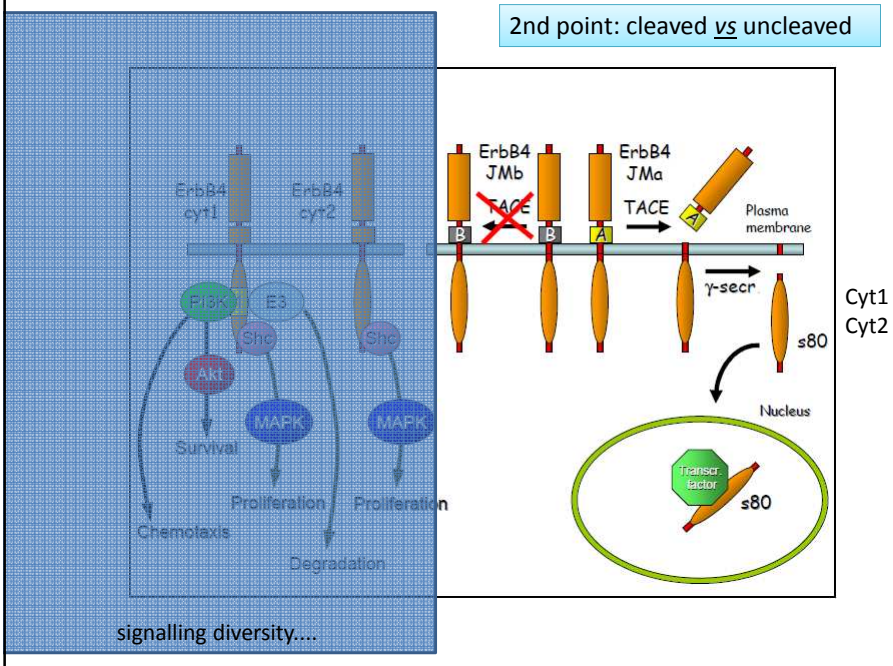


(*C) COS-7 cells* expressing ErbB4 JM-a CYT-1 or ErbB4 JM-a CYT-2 in the presence or absence of Myc-tagged Itch or Itch C830A were treated with 100 M cycloheximide for 0, , 4, or 6 h and lysed. ErbB4 expression was analyzed by Western blotting with anti-ErbB4 antibody (Abcam). Membranes were reblotted with anti-Myc antibody. Protein loading was controlled by blotting with anti-actin.



(*D) COS-7 cells expressing HA-tagged ErbB4 JM-b CYT-1 or HA-tagged ErbB4 JM-b CYT-2 in the* presence or absence of Myc-tagged Itch or Itch C830A were stained with anti-HA antibody (red) and photographed under a fluorescence microscope. Itch expression was confirmed by staining with anti-Myc antibody (data not shown). (*E) COS-7 cells expressing HA-tagged ErbB4 JM-a CYT-1, Myc-tagged ErbB4 JM-a* CYT-2, and Flag-tagged Itch C830A. Cells were stained with anti-HA (red), anti-Myc (green), and DAPI (nuclear stain; blue).

2nd point: cleaved *vs* uncleaved



signalling diversity....

γ-Secretase Cleavage and Nuclear Localization of ErbB-4 Receptor Tyrosine Kinase

Chang-Yuan Ni,[1] M. Paul Murphy,[3] Todd E. Golde,[3] Graham Carpenter[1,2]*

ErbB-4 is a transmembrane receptor tyrosine kinase that regulates cell proliferation and differentiation. After binding of its ligand heregulin (HRG) or activation of protein kinase C (PKC) by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), the ErbB-4 ectodomain is cleaved by a metalloprotease. We now report a subsequent cleavage by γ-secretase that releases the ErbB-4 intracellular domain from the membrane and facilitates its translocation to the nucleus. γ-Secretase cleavage was prevented by chemical inhibitors or a dominant negative presenilin. Inhibition of γ-secretase also prevented growth inhibition by HRG. γ-Secretase cleavage of ErbB-4 may represent another mechanism for receptor tyrosine kinase–mediated signaling.

Materiale per uso didattico

Ectodomain cleavage of ErbB-4, but not other ErbB receptors, can be stimulated by the PKC activator TPA *or by HRG (also known as neu* differentiation factor or neuregulin-NRG [ndr]), the growth factor that binds to ErbB-4. This cleavage requires the metalloprotease TACE (*4), a transmembrane* molecule with an ectodomain protease. Products of this cleavage event are an 80-kD fragment that contains the cytoplasmic and transmembrane domains and a few ectodomain residues, plus a 120-kD ectodomain fragment that is released into the extracellular medium (*2).*

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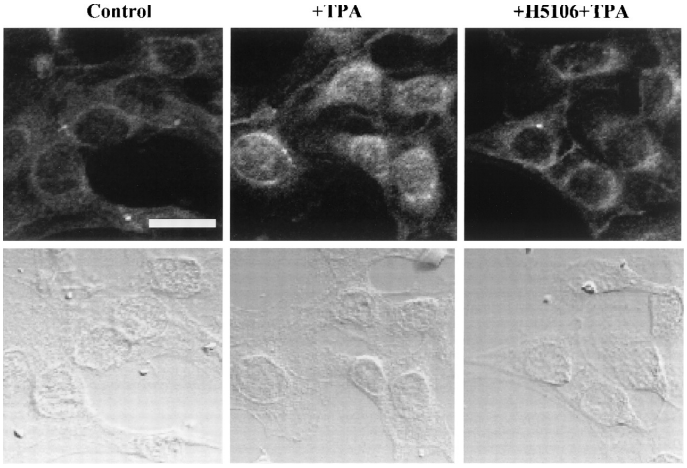

Fig. 3. Immunochemical localization of ErbB-4. Subconßuent T47-14 cells, grown on cover slips as described (*2), were treated with dimethyl sulfoxide (control cells) or TPA (100 ng/ml) for 90 min,* or were preincubated with H5106 (10 mM) for 30 min before addition of TPA for an additional 90 min. After Þxation with Ð20¡C methanol, the cells were rehydrated, blocked with goat serum, and immunostained with C-18 antibody to the ErbB-4 COOH-terminal domain as described (*14). The* primary antibody was detected with goat antibody to rabbit immuno-globulin G conjugated with Alexa Fluor 546 and examined by confocal microscopy. Differential interference contrast microscopy images are also presented (bottom panels). Scale bar, 25 mm.

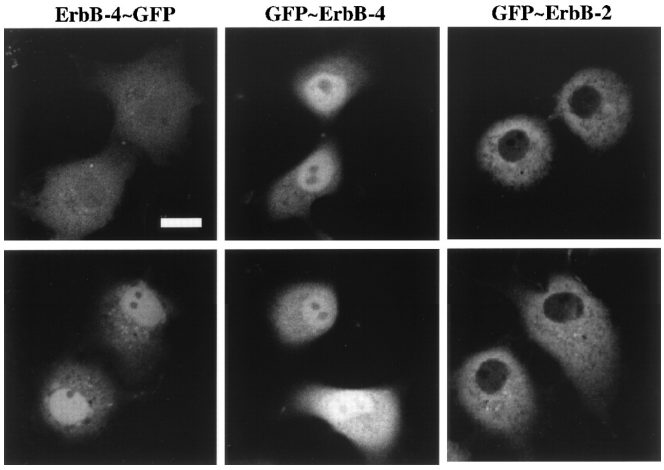

Fig. 4. Localization of GFP-ErbB fusion proteins. The columns labeled ErbB-4ÐGFP and GFPÐErbB-4 show GFP fusions at the COOH- or NH2-terminus, respectively, of the ErbB-4 cytoplasmic domain (residues 676 to 1308); the right column shows GFP fusion at the NH2-terminus of ErbB-2 (residues 676 to 1255). Fusions were constructed and expressed in COS-7 cells as described (*17). After 48* hours, the cells were examined by confocal microscopy. The cells in the bottom panels were incubated for 1 hour with LMB (100 ng/ml) before microscopy. Scale bar, 25 mm.

Several studies have confirmed this original finding.

Furthermore, ERB4 ICD was found to act as a coactivator of STAT5a and of the estrogen receptor

Additionally, ERBB4 ICD is foudn in a complex with the nuclear coreressor NCoR and the shuttling protein Tab2 in neural precursors.

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J Mammary Gland Biol Neoplasia (2008) 13:247–258

Table 1 4ICD interacting proteins.

Interacting protein	Function	References
BCL-2	Suppressor of apoptosis	[11]
ERα	Nuclear receptor; transcription factor	[21]
ETO2	Transcriptional repressor	[55]
MDM2	Negative regulator of p53; ubiquitin ligase	[57]
PI3K (p85)	Membrane signaling complex	[66]
PSD95	Synapse associated scaffolding complex	[67]
STAT5A	Transcription factor; mammary differentiation factor	[30]
TAB2	Signaling protein	[56]
WWOX	Tumor suppressor gene; dehydrogenase/ reductase	[68]
YAP	Transcription factor	[39]

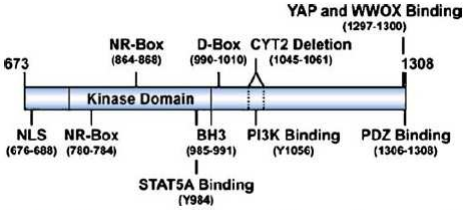

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Figure 2 4ICD functional domains and interacting proteins. Details are described in text.

Presenilin-Dependent ErbB4 Nuclear Signaling Regulates the Timing of Astrogenesis in the Developing Brain

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DOI 10.1016/j.cell.2006.07.037

SUMMARY

Embryonic multipotent neural precursors are exposed to extracellular signals instructing them to adopt different fates, neuronal or glial. However, the mechanisms by which precursors integrate these signals to make timely fate choices remained undefined. Here we show that direct nuclear signaling by a receptor tyrosine kinase inhibits the responses of precursors to astrocyte differentiation factors while maintaining their neurogenic potential. Upon neuregulin-induced activation and presenilin-dependent cleavage of ErbB4, the receptor's intracellular domain forms a complex with TAB2 and the corepressor N-CoR. This complex undergoes nuclear translocation and binds promoters of astrocytic genes, repressing their expression. Consistent with this observation, astrogenesis occurs precociously in *ErbB4* knockout mice. Our studies define how presenilin-dependent nuclear signaling by a receptor tyrosine kinase directly regulates gene transcription and cell fate. This pathway could be of importance for neural stem cell biology and for understanding the pathogenesis of Alzheimer's disease.

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Figure 7. Mechanism for the Regulation of NP Fate by ErbB4 Nuclear Signaling.

ErbB4 activation by NRG1 promotes receptor phosphorylation and its association with TAB2 and N-CoR. Ligand-induced ErbB4 cleavage by TACE and presenilin allows for the nuclear translocation of the E4ICD/TAB2/N-CoR complex. This complex binds to specific promoters (GFAP and S100b), leading to their transcriptional repression and preventing their activation by factors that induce astrocyte differentiation, such as CNTF.