Caspase-dependent Cleavage of ErbB-2 by Geldanamycin and Staurosporin*

Received for publication, February 13, 2001, and in revised form, June 6, 2001
Published, JBC Papers in Press, June 11, 2001, DOI 10.1074/jbc.M101394200

Oleg Tikhomirov and Graham Carpenter‡

From the Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146

The geldanamycin-induced degradation of ErbB-2 produces a 23-kDa carboxyl-terminal fragment, which has been isolated and subjected to amino-terminal microsequencing. The obtained sequence indicates that the amino terminus of this fragment corresponds to Gly-1126 of ErbB-2. Analysis of the residues immediately before Gly-1126 suggests that cleavage may involve caspase activity. Site-directed mutagenesis of Asp-1125 in ErbB-2 prevents geldanamycin-provoked formation of the 23-kDa fragment, consistent with the requirement of this residue for caspase-dependent cleavage in known substrates. Also, the addition of the pan-caspase inhibitor Z-VAD-FMK blocks formation of the 23-kDa ErbB-2 fragment in cells exposed to geldanamycin. Interestingly, staurosporin and curcumin are also shown to provoke the degradation of ErbB-2 with formation of the 23-kDa carboxyl-terminal fragment. The generation of this fragment by staurosporin or curcumin is likewise blocked by caspase inhibition. Caspase inhibition does not prevent accelerated degradation of the 185-kDa native ErbB-2 in geldanamycin-treated cells but does significantly prevent staurosporin-stimulated metabolic loss of ErbB-2.

ErbB-2, a Type I transmembrane receptor tyrosine kinase, functions as a co-receptor by dimerizing with ligand-occupied members of the ErbB family, the EGF receptor, ErbB-3, or ErbB-4 (1–3). This heterodimerization event is considered to alter the signaling capacity and cellular responses provoked by homodimers of occupied ErbB receptors. No ligand has been identified that directly interacts with the ectodomain of ErbB-2.

ErbB-2 was originally identified as the transforming oncogene neu, which contains a point mutation in the transmembrane domain that is responsible for its oncogenic potential (4). Overexpression of ErbB-2 also produces a transformed phenotype in experimental systems (5–7). Importantly, ErbB-2 is frequently overexpressed in carcinomas, particularly mammary and ovarian carcinomas, and is associated with a poor prognosis (8–10). ErbB-2 antibodies (11, 12) and agents such as interferon (13) or tyrosine kinase inhibitors (14) decrease the growth of ErbB-2-expressing tumor cells and also reduce the cellular level of ErbB-2 (15). In many of these instances the decreased growth provoked by the loss of ErbB-2 is due to increased apoptosis. In contrast, the overexpression of ErbB-2 can prevent the induction of apoptosis (15). Hence, the growth-controlling activity of ErbB-2 is related to structural changes or alterations in its level of expression.

The benzoquinoid anasamycin antibiotic geldanamycin was first isolated and described as an inhibitor of tyrosine kinase activity (16). Subsequently, geldanamycin was shown to possess tumoricidal activity toward cell lines that overexpress ErbB-2 (17). Currently geldanamycin derivatives designed to reduce toxicity are in clinical trials for certain cancer patients (18). The addition of geldanamycin to cells results in an increased rate of degradation of several protein kinases, including ErbB-2 (17), Src (19), Raf (26), and focal adhesion kinase (20) as well as other growth-regulating proteins such as p53 (21). The mechanism by which geldanamycin provokes the degradation of these and other proteins is not clear but is thought to involve the Hsp90 family of chaperones, which are the major intracellular proteins that bind geldanamycin (22, 23). However, the manner in which Hsp90 or other geldanamycin-binding proteins influences the physiology of ErbB-2 is not known.

Geldanamycin binds to Hsp90 and inhibits its ATPase activity, which is required for its chaperone function (24, 25). Although association of Hsp90 with Src (19) and Raf (26) have been documented, only GRP94 was originally reported to be associated with ErbB-2 (27). The idea that GRP94 mediates geldanamycin-induced degradation of ErbB-2 was not widely accepted, however, due to its subcellular localization (31) and the fact that geldanamycin-sensitivity requires the tyrosine kinase domain of ErbB-2 (17, 29), which is never present within the lumen of the endoplasmic reticulum. However, recently it has been reported that Hsp90 does in fact, associate with ErbB-2 in a manner that is disrupted by the presence of geldanamycin (30). Hence, it is possible that Hsp90 is associated with the tyrosine kinase domain of ErbB-2 and may regulate the conformation or the enzymatic function of ErbB-2. Geldanamycin then represents a tool with which to perturb that association.

A previous study of ErbB-2 degradation stimulated by geldanamycin demonstrated the appearance of two fragments (29). One large fragment of 135 kDa included the ectodomain, the transmembrane domain, and part of the cytoplasmic domain. The other fragment of 23 kDa was derived from the carboxyl-terminal portion of the ErbB-2 cytoplasmic domain. These studies also indicated that the 135-kDa fragment was degraded by lysosomes, whereas degradation of the 23-kDa fragment depended on proteosome function. In the experiments reported in this manuscript, the exact nature of the 23-kDa fragment is described as is the nature of the enzyme that produces this fragment from ErbB-2.

* This work was supported by Department of the Army Breast Cancer Program Grant DAMD17-00-1-0483. 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.: 615-322-6678; Fax: 615-322-2931; E-mail: grahamcarpenter@mcmail.vanderbilt.edu.

This paper is available online at http://www.jbc.org
ErbB-2 Degradation

EXPERIMENTAL PROCEDURES

Materials—Geldanamycin, curcumin, staurosporin, and enhanced chemiluminescence (ECL) reagents were purchased from Sigma. Polyvinylidene difluoride membrane was from Bio-Rad. N-Acetyl-l-leucinyl-l-leucinyl-l-norleucinal (ALLN), 2 caspase inhibitor (2-VAD-FMK), and proteasome inhibitor I were from Calbiochem. Monoclonal antibody against the ErbB-2 extracellular domain (Ab5) was from Transduction Laboratories, whereas monoclonal antibody against the ErbB-2 carboxy-terminal domain (Ab3) was purchased from Calbiochem. The pcDNA3.1 vector was obtained from Invitrogen. Goat anti-mouse antibody cross-linked with horseradish peroxidase was from Zymed Laboratories Inc., and Lipofectamine was purchased from Life Technologies, Inc.

Cell Culture and Transfection—SKBr3 human breast cancer cells were grown to near confluence in 5% CO2 at 37 °C in McCoy's medium with 10% fetal bovine serum and then washed and treated with the indicated compounds in Dulbecco's modified Eagle's medium. Cos7 cells were grown overnight in Dulbecco's modified Eagle's medium with 10% fetal bovine serum to about 80% confluence and then transfected with Lipofectamine according to the manufacturer's recommendations (3 μg of DNA of pcDNA3.1 vector was used per 60-mm culture dish).

Construction of ErbB-2 Mutants—ErbB-2 mutants were prepared by site-directed mutagenesis using a megaprimer approach. To facilitate manipulations with the ErbB-2 intracellular region, a Sot restriction site downstream of the transmembrane region was generated by polymerase chain reaction (silent mutagenesis) using the following primers: primer 1, CTG CCT GCC GAG ACT GCT ACC (3'); primer 2, AAG TAC ATG CAT CGA CTT CGC (5'); primer 3, CTG CAG CAG CGT CAT CGT GTA (3'); primer 4, AGA CCC AAC ATG GCT GCC ACC ATG CAT GCG TGG CGC (5'). The resultant two polymerase chain reaction products (primers 1 and 2; primers 3 and 4) were cut with SalI and XbaI vector through NheI and XbaI sites (5-piece ligation).

Mutations in the ErbB-2 carboxyl terminus were introduced in the first round of polymerase chain reaction with primer 1 as the 3' primer and the following 5' primers: primer 5, CTG CCC TCT GAG ACT GCT GCC TAC GTT (D1125-A); primer 6, TCT GAG ACT GAA GCC TAC GTT GC (G1126-A); primer 7, TCT GAG ACT GCC TAC GTT GC (D1125-A); one strand of these products was used as the 3' primer in the second round of polymerase chain reaction with primer 2 as the 5' primer. The final products were ligated through SoI and XbaI restriction sites into the pcDNA3.1 vector. All mutations were confirmed by sequencing.

Immunoblotting—At the end of each experiment the cells were solubilized by scraping into cold lysis buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM Na3VO4). The lysates were then clarified by centrifugation (14,000 g, 10 min), and equal aliquots (40 μg) were subjected to SDS-PAGE electrophoresis. Proteins were then transferred to a nitrocellulose membrane for Western blotting. The membrane was blocked by incubation with 5% bovine serum albumin in phosphate buffer saline for 1 h at room temperature. The membrane was then incubated for 1 h with the indicated primary antibody in TBSTw buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% Tween 20, 0.2% nonfat milk), washed 3 times in the same buffer, and incubated for 1 h with horseradish peroxidase-conjugated secondary antibody. The membrane was then washed five times with TBSTw buffer and visualized by ECL.

Amino-terminal Microsequencing—SKBr3 cells were grown on 20 150-mm tissue culture dishes to 80% confluence (~10 × 106 cells/plate) and washed with Dulbecco's modified Eagle's medium. The cells were then preincubated for 1 h with ALLN (250 μM) and treated with geldanamycin (3 μM) for 5 h. Subsequently, the cells were scraped with a rubber policeman into cold lysis buffer and centrifuged (20,000 g, 30 min). Twelve high molecular weight proteins, including native ErbB-2, were removed by ultrafiltration using a Centriplus membrane with a cut-off of 100 kDa (Millipore). The filtrate (~30 ml) was incubated with 40 μg of antibody against ErbB-2 carboxy terminus and protein G-Sepharose for 2 h and then washed five times with lysis buffer. Immunoreactive material was separated by 13% SDS-PAGE, transferred to the polyvinylidene difluoride membrane, and stained with Coomassie Blue. The 23-kDa band was cut out of the membrane and subjected to

7 cycles of Edman degradation using an Applied Biosystems Procise sequencer.

RESULTS

Sequencing of the 23-kDa Fragment of ErbB-2—Previously, we have shown that when the mammary carcinoma cell line SKBr3 is incubated with geldanamycin, a 23-kDa carboxy-terminal fragment of ErbB-2 is produced (29). This fragment is rapidly degraded by proteosome activity unless a proteosome inhibitor is added. The data in Fig. 1 show that a similar 23-kDa fragment accumulates when transfected Cos7 transiently expressing human ErbB-2 are treated with geldanamycin and ALLN, a proteosome inhibitor.

To isolate this 23-kDa ErbB-2 fragment for sequence analysis, we employed transfected Cos7 cells treated with geldanamycin and ALLN for 6 h. ErbB-2 was then immunoprecipitated from cell lysates with an antibody to the carboxyl terminus of ErbB-2. The resulting immunoprecipitate was subjected to SDS-PAGE, the separated proteins were transferred to a polyvinylidene difluoride membrane, and the membrane was stained with Coomassie. The stained protein band at 23 kDa representing the carboxy-terminal ErbB-2 fragment was clearly visible. The 23-kDa peptide was eluted from the membrane and subjected to automated Edman amino-terminal microsequencing, which yielded a sequence of GYVAFLT through the first seven cycles. There was no evidence of heterogeneity in this sequence, which if present might indicate proteolytic processing of the amino terminus of the fragment. The recovered sequence corresponds exactly to residues 1126–1132 in the deduced cDNA sequence of human ErbB-2 (32).

Caspase Cleavage of ErbB-2—Although it is possible that post-cleavage amino-terminal processing has taken place to yield the isolated 23-kDa fragment, we considered whether sequence information would predict a known proteolytic cleavage consensus site between residues 1125 and 1126 in ErbB-2. This analysis did show that there is a significant similarity between the ErbB-2 sequence immediately upstream from Gly-1126, which is FSETD, and caspase consensus cleavage sites that have been defined using a positional scanning combinatorial substrate library (33–35) and analysis of known caspase cleavage sites in proteins (36, 37). These caspase cleavage sequences are characterized by an essential Asp residue at the P1 position and frequently a Glu residue at the P3 position which correspond, respectively, to Asp-1125 and Glu-1123 in the ErbB-2 sequence.

To test the possibility that this putative caspase cleavage sequence in ErbB-2 was necessary to generate the 23-kDa

\[ \text{ALLN} \quad - \\ \text{GA} \quad - \\ \text{W.B.: anti-ErbB2(CT)} \]

\[ \text{FIG. 1. Geldanamycin-induced formation of the 23-kDa ErbB-2 fragment.} \]

The cells were then treated with ALLN (250 μM for 1 h, and geldanamycin (GA, 3 μM) was added for an additional 6 h. The cells were then lysed, and proteins in an aliquot of the lysate (40 μg) were separated by SDS-PAGE. A Western blot (W.B.) was performed using antibody against the ErbB-2 carboxy-terminal domain (C7), and bound antibody was detected by ECL.

\[ \text{GA} \quad - \\ \text{ALLN} \quad - \\ \text{W.B.: anti-ErbB2(CT)} \]

\[ \text{FIG. 1. Geldanamycin-induced formation of the 23-kDa ErbB-2 fragment.} \]
ErbB-2 Degradation

ErbB-2 fragment, site-directed mutagenesis was employed. The Asp at residue 1125 of ErbB-2 was mutated to either Ala or Glu, and Gly-1126 was changed to Ala. In regard to the conservative D1125E mutation, published structural data show that the aspartyl carboxylate side chain at the P1 position in a caspase substrate fits into a highly restrictive “socket” of enzyme that does not accommodate the side chain of a glutamate residue (36). The wild-type and mutant ErbB-2 constructs were transiently expressed in Cos7 cells and exposed to geldanamycin without or with ALLN. The results in Fig. 2 show that mutagenesis of Asp-1125 to either Glu or Ala prevented production of the 23-kDa fragment from ErbB-2. Interestingly, in the case of these two mutations a new fragment of 30 kDa was generated from the carboxyl terminus of ErbB-2, and the depletion of the cellular level of native ErbB-2 by geldanamycin was not affected. In the case of the G1126A mutation, there was no effect on geldanamycin-induced ErbB-2 degradation or formation of the 23-kDa fragment. That mutagenesis of Gly-1126 did not abrogate production of the 23-kDa fragment is not surprising in view of the fact that caspase cleavage sites, although preferring Gly at the P1 position, exhibit considerable flexibility at this position (35).

To test the possibility that caspase activity is required for production of this 23-kDa fragment from ErbB-2, the pan-caspase inhibitor Z-VAD-FMK (38, 39) was tested. As can be seen in the data presented in Fig. 3, Z-VAD-FMK completely prevented the formation of the 23-kDa ErbB-2 fragment induced by geldanamycin. In the D1125E ErbB-2 mutant geldanamycin induces a 30-kDa fragment instead of the 23-kDa fragment. To determine whether the formation of this 30-kDa fragment might also be caspase-dependent, we incubated cells expressing the D1125E mutant with geldanamycin in the presence and absence of Z-VAD-FMK. The data shown in Fig. 4 indicate that the amount of the 30-kDa fragment is significantly reduced when these cells are exposed to geldanamycin in the presence of the caspase inhibitor.

ErbB-2 Degradation by Staurosporin—Geldanamycin induces ErbB-2 degradation, in part through a caspase-dependent mechanism, and geldanamycin is known to induce apoptosis in some cell lines (40). Therefore, we tested whether other reported inducers of apoptosis, particularly in mammary carcinoma cell lines, also provoke ErbB-2 degradation. Two known stimulators of apoptosis, which are also known protein kinase inhibitors, did induce ErbB-2 degradation in a manner similar to geldanamycin.

The results with staurosporin are shown in Fig. 5. The data in the upper panel of Fig. 5A show that staurosporin decreases the level of native ErbB-2 with about the same time course as that previously reported for geldanamycin (17), whereas the results in the upper panel of Fig. 5B show that maximal degradation of ErbB-2 was achieved with a staurosporin concentration of −5 μM. Significantly, the ErbB-2 degradation elicited by staurosporin is accompanied by the production of a 23-kDa carboxyl-terminal ErbB-2 fragment, shown in the lower panels of Figs. 5, A and B, similar to that recorded for degradation induced by geldanamycin. The data in Fig. 6 demonstrate that formation of this 23-kDa fragment from ErbB-2 in the presence of staurosporin is prevented by the pan-caspase inhibitor Z-VAD-FMK. Comparison of geldanamycin and staurosporin-induced ErbB-2 degradation in the same cell line (SKBr3) can be made by comparing the data in Figs. 3 and 6, respectively. The results show that in the absence of ALLN, the 23-kDa fragment produced by staurosporin accumulates to a significantly greater level than the same fragment generated in geldanamycin-treated cells. Also, the presence of ALLN significantly increases the level of this fragment in geldanamycin-treated cells but actually decreases the fragment level in staurosporin-
treated cells. These results suggest significant, but unknown, distinctions in the mechanisms of action of geldanamycin and staurosporin.

Curcumin is another protein kinase inhibitor that has been reported to induce apoptosis in numerous cell lines and also to deplete cellular level of ErbB-2 (41–44). The data in Fig. 7 show that curcumin depletion of cellular ErbB-2 involves production of a 23-kDa ErbB-2 carboxyl-terminal fragment, which is stabilized in the presence of proteasome inhibitor I. Furthermore, the formation of this fragment in cells treated with curcumin is blocked in the presence of Z-VAD-FMK.

However, not all inducers of apoptosis provoked ErbB-2 degradation in SKBr3 cells. Etoposide (100 μM), actinomycin D (5 μg/ml), hydroxyurea (5 μM), camptothecin (100 μM), paclitaxel (1 μM) with or without the MEK (mitogen-activated protein kinase/extracellular signal-regulated kinase kinase) inhibitor PD98059 (50 μM) (45) and the potent kinase inhibitor H7 (100 μM) did not decrease ErbB-2 levels in SKBr3. In many cases, these compounds did induce dramatic morphological changes in the cells.

Both staurosporin and geldanamycin induce the degradation of ErbB-2 with formation of a 23-kDa fragment that is dependent on caspase activity. We have asked whether the inhibition of caspase activity interferes with the drug-induced degradation of the native 185-kDa ErbB-2 molecule. The data in Fig. 8 show that Z-VAD-FMK does not block geldanamycin-induced ErbB-2 degradation, but the inhibitor significantly attenuates degradation induced by staurosporin. Like geldanamycin-induced ErbB-2 degradation, curcumin-mediated degradation of this transmembrane protein was not prevented by Z-VAD-FMK (data not shown).

DISCUSSION

The induction of ErbB-2 degradation by geldanamycin is associated with proteolytic fragmentation of the receptor (29). This includes the formation of a 23-kDa fragment that is recognized by antibodies as an epitope, within residues 1242–1255, in the carboxyl-terminal domain of the ErbB-2. Sequencing of this fragment shows that its amino terminus corresponds to Gly-1126 in the human ErbB-2 sequence. If the 23-kDa...
ErbB-2 Degradation

fragment includes all residues from Gly-1126 to the ErbB-2 carboxyl terminus Val-1255, then it has a calculated molecular mass of 16,091. Hence its apparent $M_r$ value on SDS-PAGE is an overestimation of the actual mass due to anomalous migration in the gel. This fragment would contain most of the known ErbB-2 autophosphorylation sites that provide its coupling to signal transduction proteins (46, 47) as well as the carboxy-terminal PDZ domain recognition motif that is involved in targeting ErbB-2 to the basolateral surface of polarized cells (48).

It is possible that cleavage of ErbB-2 occurs between residues 1125 and 1126 to generate the 23-kDa fragment or that the primary cleavage event occurs upstream of these residues, and post-cleavage processing occurs at the amino terminus to produce the observed fragment. Several facts suggest that the former is more plausible. First, we did not detect heterogeneity in the amino-terminal amino acids recovered in microsequencing of this fragment. Second, the residues immediately upstream of Gly-1126 constitute a site favorable for caspase-dependent cleavage. This includes the essential Asp at residue 1125. Analysis of caspase cleavage sites shows that cleavage invariably occurs immediately after an Asp residue at the P1 position and frequently includes a Glu residue at the P3 position (34, 35), which in this instance corresponds to Glu-1123 in the ErbB-2 sequence. Our data show that mutagenesis of the essential Asp at position P1 (residue 1125) prevents geldanamycin-induced formation of the 23-kDa fragment. This includes the conservative D1125E mutation, which on the basis of the structure of the site of caspase active sites with model substrates, is predicted to inhibit peptide bond hydrolysis by these proteases (36). Last, the pan-caspase inhibitor Z-VAD-FMK blocks geldanamycin-induced formation of the 23-kDa peptide. Hence, we conclude that this fragment is formed by cleavage between residues 1125 and 1126 in ErbB-2.

It is interesting that when Asp-1125 is mutated in ErbB-2, geldanamycin treatment of cells gives rise to a new carboxy-terminal fragment of 30 kDa instead of the 23-kDa fragment. Formation of this fragment in the ErbB-2 mutant is partially sensitive to the caspase inhibitor. There are consensus caspase cleavage sites upstream of Asp-1125 that could generate the 30-kDa fragment. These include Asp-1087 and Asp-1016 and Asp-1019, which constitute a nested cleavage site. Cleavage at Asp-1087 would directly produce a carboxy-terminal ErbB-2 fragment of about 30 kDa, whereas cleavage at Asp-1016 or Asp-1019 would generate a theoretical 40-kDa fragment, which would require additional processing to produce the observed 30-kDa fragment.

It is important to note that when the caspase-dependent cleavages at the carboxy terminus are prevented by the addition of Z-VAD-FMK to geldanamycin-treated cells, the cellular content of the native ErbB-2 molecule is still decreased. This implies that cleavage at the ErbB-2 carboxyl terminus is not necessary to initiate the geldanamycin-dependent reduction in the ErbB-2 levels. However, Z-VAD-FMK does prevent staurosporin-dependent degradation of the native ErbB-2 molecule, suggesting that caspase activity is more significant in the degradation of ErbB-2 by staurosporin than by geldanamycin.

The current proposed mechanism for geldanamycin-induced ErbB-2 degradation involves the drug-dependent dissociation of Hsp90 from the kinase domain of ErbB-2 (27, 28, 30). Since geldanamycin is known to induce apoptosis and some but not all cell types (40, 49, 53) and, as mentioned above, caspase activity seems involved in ErbB-2 degradation induced by this drug, we tested other known inducers of apoptosis for their capacity to provoke ErbB-2 degradation and formation of the 23-kDa fragment. Of the various inducers of apoptosis tested, two compounds (staurosporin and curcumin) were found to promote formation of a 23-kDa fragment. Others report that curcumin inhibits the tyrosine kinase activity of ErbB-2 and depletes the cellular content of native ErbB-2 (41). Curcumin also induces an apoptotic response in mammary carcinoma cells (50). Curcumin has been reported to dissociate the endoplasmic reticulum heat shock protein GRP94 from ErbB-2 (41). However, it is not known whether curcumin actually binds to GRP94, and its effect on Hsp90 has not been reported.

Less is known regarding the effects of staurosporin on ErbB-2. Staurosporin, a broad spectrum protein kinase inhibitor, has not been reported to interact with heat shock proteins and is well described as an inducer of apoptosis (51, 52). Staurosporin, like geldanamycin and curcumin, is a weak inhibitor of receptor tyrosine kinases (51) but has not been previously shown to alter the cellular level of ErbB-2. The similarities in ErbB-2 metabolism between cells treated with geldanamycin or staurosporin suggest that the observed degradation of ErbB-2 by these compounds does not necessarily have to proceed through dissociation of heat shock proteins from ErbB-2. It may be that inhibition of kinase activities along with promotion of caspase activities is sufficient for ErbB-2 degradation. The identity of the target kinases, however, is unclear. It has been reported that the metabolic stability of a kinase-negative ErbB-2 mutant is decreased by geldanamycin (30), which suggests that perhaps non-tyrosine kinases are the target of these drugs.

REFERENCES

1. Karunagaran, D., Tzahar, E., Beerli, R. R., Chen, X., Graus-Porta, D., Ratzkin, B., Seger, R., Hynes, N., and Yarden, Y. (1996) EMBO J. 15, 254–264.
2. Riese, D. J., III, and Stern, D. F. (1998) Bioessays 20, 41–48.
3. Alroy, I., and Yarden, Y. (1997) FEBS Lett. 410, 83–86.
4. Segato, O., King, C. R., Pierce, J. H., Di Fiore, P. P., and Aaronsen, S. A. (1988) Mol. Cell. Biol. 8, 5570–5574.
5. Di Marco, E., Pierce, J. H., Knaus, C. L., and Di Fiore, P. P. (1996) Mol. Cell. Biol. 11, 3191–3202.
6. Di Fiore, P. P., Pierce, J. H., Kraus, M. H., Segato, O., King, C. R., and Aaronsen, S. A. (1987) Science 237, 178–182.
7. Hudes, R. M., Schlessinger, J., and Ullrich, A. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 7159–7163.
8. Revillion, F., Bonnetterre, J., and Peyrat, J. P. (1998) Eur. J. Cancer 34, 791–808.
9. Klapper, L. N., Kirschbaum, M. H., Sela, M., and Yarden, Y. (2000) Adv. Cancer Res. 77, 25–79.
10. Slamon, D. J., Clark, G. M., Wong, S. G., Keith, D. E., Levin W. J., Ullrich, A., and Herbst, R. S. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 7190–7194.
11. Zhou, B., Hu, M., Miller, S. A., Yu, Z., Xia, W., Lin, S., and Hung, M.-C. (2000) J. Biol. Chem. 275, 30849–30855.
30. Xu, W., Minnaugh, E. G, Rosser, M. F., Niechitta, C., Marcu, M., Yarden, Y., and Neckers, L. (2001) J. Biol. Chem. 276, 3702–3708
31. Nicchita, C. (1998) Curr. Opin. Immunol. 10, 103–109
32. Coussen, L., Yang-Feng, T. L., Liao, Y.-C., Chen, E., Gray, A., McGrath, J., Seeburg, P. H., Libermann, T. A., Schlessinger, J., Francke, U., Levinson, A., and Ullrich, A. (1985) Science 230, 1132–1139
33. Margolin, N., Raybuck, S. A., Wilson, K. P., Chen, W., Fox, T., Gu, Y., and Livingston, D. J. (1997) J. Biol. Chem. 272, 7223–7228
34. Thornberry, N. A., Rano, T. A., Peterson, E. P., Rasper, D. M., Timkey, T., Garcia-Calvo, M., Hutzager, V. M., Nordstrom, P. A., Roy, S., Vaillancourt, J. P., Chapman, K. T., and Nicholson, D. (1997) J. Biol. Chem. 272, 17907–17911
35. Talanian, R. V., Quinlan, C., Trautz, S., Hackett, M. C., Mankovich, J., Banach, D., Ghayur, T., Brady, K. D., and Wong, W. W. (1997) J. Biol. Chem. 272, 9677–9682
36. Nicholsen, D. W. (1999) Cell Death Differ. 6, 1028–1042
37. Cohen, G. M. (1997) Biochem. J. 326, 1–16
38. McColl, K. S., He, H., Zhong, H., Whitacre, C. M., Berger, N. A., and Distelhorst, C. W. (1998) Mol. Cell. Endocrinol. 139, 229–238
39. Pronk, G. J., Ramer, K., Amiri, P., and Williams, L. T. (1996) Science 271, 808–810
40. Khan, S. M., Oliver, R. H., Dauffenbach, L. M., and Yeh, J. J. (2000) Fertil. Steril. 74, 359–365
41. Hong, R.-L., Spohn, W. H., and Hung, M.-C. (1999) Clin. Cancer Res. 5, 1884–1891
42. Khar, A., Mubarak, A. A., Pardhasaradhi, B. V., Begum, Z., and Anjum, R. (1999) FEBS Lett. 445, 165–168
43. Chen, H., Zhang, Z., Zhang, Y., and Zhou, D. (1999) Anticancer Res. 19, 3675–3680
44. Kuo, M.-I., Huang, T.-S., and Lin, J.-K. (1996) Biochim. Biophys. Acta 1317, 95–100
45. McKeigan, J. P., Collins, T. S., and Ting, J. P.-Y. (2000) J. Biol. Chem. 275, 38953–38956
46. Margolis, B. L., Lax, I., Kris, R., Dombalagian, M., Honnegger, A. M., Howk, R., Givol, D., Ullrich, A., and Schlessinger, J. (1989) J. Biol. Chem. 264, 10667–10671
47. Hazan, R., Margolis, B., Dombalagian, M., Ulirich, A., Zilberstein, A., and Schlessinger, J. (1990) Cell Growth Differ. 1, 3–7
48. Berg, J., Marchetto, S., Le Bivic, A., Ollendorff, V., Jaulin-Bastard, F., Saito, H., Fournier, E., Adelaide, J., Margolis, B., and Birnbaum, D. (2000) Nat. Cell Biol. 2, 407–414
49. Davis, M., and Carbott, D. (1999) Toxicol. Appl. Pharmacol. 161, 59–74
50. Ramachandran, C., and You, W. (1999) Breast Cancer Res. 54, 269–278
51. Andersson, M., Sjustrand, J., Petersen, A., Honarvar, A. R., and Karlsson, J. (2000) Invest. Ophthalmol. Vis. Sci. 41, 2623–2632
52. Rokhlin, O. W., Glover, R. A., and Cohen, M. R. (1998) Cancer Res. 58, 5870–5875
53. Nimmanapalli, R., O’Bryan, E., and Bhalla, K. (2001) Cancer Res. 61, 1799–1804
Caspase-dependent Cleavage of ErbB-2 by Geldanamycin and Staurosporin
Oleg Tikhomirov and Graham Carpenter

J. Biol. Chem. 2001, 276:33675-33680.
doi: 10.1074/jbc.M101394200 originally published online June 11, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M101394200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 53 references, 29 of which can be accessed free at
http://www.jbc.org/content/276/36/33675.full.html#ref-list-1