Intracellular Retention and Degradation of the Epidermal Growth Factor Receptor, Two Distinct Processes Mediated by Benzoquinone Ansamycins*

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Epidermal growth factor (EGF) stimulates the growth of various types of cells via its cell surface tyrosine kinase receptor. The EGF receptor (EGF-R) has an oncogenic potential when overexpressed in a wide range of tumor cells. Geldanamycin (GA) and herbimycin (HA), specific inhibitors of the cytosolic chaperone HSP 90 and its endoplasmic reticulum homologue GRP 94, were shown to accelerate degradation of the EGF-R and of its homologue p185erbB-2. Here we compared the effects of GA and HA on intracellular degradation and maturation of EGF-R. By using an inhibitor of pro teaseal degradation, we learned that GA, but not HA, blocks processing of newly synthesized EGF-R. The effects of GA and HA on receptor degradation are mediated by the cytosolic portion of EGF-R and could be conferred to the erythropoietin receptor (EPO-R), by employing the respective chimera. Neither HA nor GA affected stability of newly synthesized EGF-R lacking the cytosolic domain (Ex EGF-R), but GA caused intracellular retention of this mutant. Taken together, our results imply that GA has two distinct targets of action on the EGF-R, one for promoting its degradation and another for mediating its intracellular retention. Apparently, degradation of the EGF-R mediated by GA or HA requires the presence of the EGF-R cytosolic domain, whereas intracellular retention in the presence of GA is coupled to the extracellular domain of the EGF-R.

Herbimycin A (HA) and geldanamycin (GA) are benzoquinone ansamycins that specifically inhibit the cytosolic chaperone HSP 90 and its endoplasmic reticulum (ER) homologue GRP 94. These compounds have antiproliferative and antitumor effects as they bind to HSP 90, inhibit the HSP 90-mediated conformational maturation/refolding reaction, and thus promote degradation of HSP 90 substrates (reviewed in Ref. 1). The molecular basis underlying this inhibition is still under intensive investigation (2). GA was shown to accelerate the degradation of cell surface proteins including receptor Tyr kinases (3) and the cystic fibrosis transmembrane conductance regulator (CFTR) protein (4). Enhanced degradation in the presence of GA was also observed for non-membrane proteins including Ser/Thr kinases (5), Tyr kinases (6), and mutated p53 (7).

The epidermal growth factor receptor (EGF-R) and its homologue p185erbB-2 are well studied receptor Tyr kinases (8), which are degraded in the presence of GA and HA (9–11), presumably via the proteasome (3, 12). The cytosolic domain of the EGF-R contains Tyr kinase activity and may also contain determinants that are required for GA- and HA-mediated degradation. This is suggested by evidence that a soluble EGF-R, an EGF-R derivative containing only the extracellular domain of the receptor (11, 13), and p185erbB-2 lacking either the entire cytosolic domain or the kinase domain (10) were not degraded in the presence of HA (11, 13) or GA (10, 13). However, it is not clear whether Tyr kinase activity of the receptor or the presence of the entire kinase domain is required to confer GA-mediated degradation.

In the course of biosynthesis, the EGF-R is efficiently processed and is metabolically stable; thus, most of the receptor molecules are present on the cell surface at steady state (14–16). An EGF-R mutant that lacks the cytosolic domain and contains the extracellular and the transmembrane domains (Ex EGF-R (17)) is also metabolically stable, although its trafficking to the plasma membrane is slower than that of wild type (wt) EGF-R (18, 19). Ex EGF-R is therefore an appropriate experimental model system to address the net contribution of the EGF-R extracellular domain on receptor metabolism in the presence of GA and HA. The erythropoietin receptor (EPO-R) is a member of the cytokine receptor superfamily (20, 21). Unlike receptor Tyr kinases, the EPO-R has no kinase activity in its cytosolic domain and is phosphorylated by Janus kinase 2 following ligand binding (22). It was previously demonstrated that the EPO-R localizes intracellularly, mainly in the ER (23–27), and it has been postulated that its retention in the ER may be the result of impaired folding of the extracellular domain of the receptor (24).

In the present study we compared the effects of GA and HA on maturation and degradation of the EGF-R. We show that...
excreted by exogenous EGF-R (18, 19) is retained intracellularly but is not degraded in the presence of GA. In the presence of GA, however, exogenous EGF-R was efficiently transported to the cell surface, but, as with GA, its rate of degradation was not affected. No effect of GA was observed on degradation of newly synthesized EGF-R. Utilizing a chimera composed of the extracellular domain and the transmembrane domain (28), we demonstrate that the EGF-R cytosolic domain is sufficient to confer GA-mediated degradation. In addition, we show that GA-mediated degradation of the EGF-R does not require Tyr kinase activity of the receptor.

**EXPERIMENTAL PROCEDURES**

**Materials**—All materials were obtained from sources previously listed (19, 25). Geldanamycin (GA) and herbimycin (HA) were obtained from Life Technologies, Inc. Cbz-leucinyl-leucinyl-leucinal vinyl sulfone (Z-L3VS) was kindly provided by Dr. C. Carlin (Case Western Reserve University, Cleveland). pCDNA3 EGF-R and its derivative encoding a kinase-defective receptor (K721A) have been described (31).

**Cell Culture and Transfection**—COS 7 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. Cells were cultured to 60% confluence and were transiently transfected using the DEAE-dextran/chloroquine method (32) with 5 μg of plasmid containing the appropriate cDNA.

CHO cell lines stably expressing wt EGF-R or K721A EGF-R were maintained in Dulbecco’s modified Eagle’s medium/Ham’s F-12 supplemented with 10% fetal calf serum. Brefeldin A (BFA; Epicenter Technologies) was added to the cells at a final concentration of 5 μM to the cells during starvation, and the inhibitors were present during the pulse and throughout the chase periods. A, non-treated cells. B, GA-treated cells. C, HA-treated cells. Detergent extracts of the cells were immunoprecipitated with anti-EGF-R antibodies. Immunoadsorbed material was divided into 2 aliquots that were incubated at 37 °C with no addition (−) or with Endo H (+). Samples were resolved by 7.5% SDS-PAGE and subjected to autoradiography. The solid and empty arrows indicate the Endo H-sensitive and Endo H-resistant forms of exogenous EGF-R, respectively. Arrowhead points at partially processed forms of exogenous EGF-R in the presence of GA. Metabolism of the endogenously expressed wt EGF-R is depicted for comparison.

**RESULTS**

**Metabolic Processing of EGF-R in the Presence of GA**—It has previously been reported that GA affects both maturation and degradation of EGF-R and p185 erbB-2 (9, 10, 13) and that the cytosolic domain of p185 erbB-2 is required for receptor degradation. We addressed the question whether retarded maturation and enhanced degradation are two independent processes mediated by GA. To this end we compared the metabolic profile of an EGF-R mutant devoid of its cytosolic domain (EGF-R(17)) to that of full-length EGF-R, in the presence of GA and HA, specific inhibitors of HSP 90 and GRP 94.

To enable comparison of EGF-R and exogenous EGF-R metabolism in the same cell, we transiently expressed EGF-R in COS 7 cells that also express an endogenous EGF-R. COS 7 cells were transiently transfected with exogenous EGF-R, and after 48 h the cells were metabolically labeled in medium containing [35S]cysteine-methionine and chased for periods up to 4 h (Fig. 1). In control non-treated cells newly synthesized EGF-R was stable throughout the chase periods and acquired resistance to Endo H (Fig. 1A), whereas in the presence of either GA or HA it was rapidly degraded (Fig. 1, B and C). In comparison, newly synthesized Ex EGF-R was metabolically stable, and typically 40–50% of the receptor molecules acquired resistance to Endo H after a 4-h period of chase in the absence of inhibitors (Fig. 1A (19)). It should be noted that Endo H-resistant Ex-EGF-R
runs as a more smeared band (probably due to a range of glycosylation products) as compared with Endo H-sensitive Ex EGF-R. Unlike EGF-R, in the presence of either GA or HA newly synthesized Ex EGF-R was metabolically stable throughout the chase periods (Fig. 1, B and C, respectively), suggesting a role for the EGF-R cytosolic domain for degradation in the presence of either GA or HA. After 4 h of chase in the presence of GA, the Ex EGF-R remained largely unprocessed and did not display Endo H-resistant forms (Fig. 1B), whereas processing of newly synthesized Ex EGF-R was practically identical in the presence of HA and in control cells (Fig. 1, C and A, respectively). These results show that GA but not HA inhibited maturation of Ex EGF-R, suggesting that the extracellular domain of the receptor mediates this effect of GA.

The Effect of GA on Cell Surface Expression of Ex EGF-R.—To determine whether GA treatment inhibited transport of Ex EGF-R or only affected its glycosylation pattern, cell surface expression of newly synthesized Ex EGF-R and EGF-R was examined in the presence of GA. COS 7 cells were transiently transfected with Ex EGF-R and were pulse-labeled in medium containing [35S]cysteine-methionine and chased in nonradioactive medium for 4 h. GA (1 μg/ml) or HA (1 μg/ml) was added to the cells during starvation, and the inhibitors were present during the pulse and throughout the chase periods. BFA (5 μg/ml) was added to the cells only during the chase period. After a 4-h chase period, the cells were subjected to cell surface biotinylation for 30 min. Samples were processed as follows prior to SDS-PAGE and autoradiography. A, cell surface biotinylated Ex EGF-R was immunoprecipitated with anti-EGF-R antibodies, followed by streptavidin-agarose. B, total EGF-R was immunoprecipitated using anti-EGF-R antibodies. C, biotinylated cell surface proteins were precipitated with streptavidin-agarose. Molecular weight markers are depicted on the right. The asterisk, circle, and triangle point at cell surface biotinylated proteins that were reduced by both HA and GA, reduced in size in the presence of GA, and remained unchanged by GA or HA treatment, respectively.

**Fig. 2.** GA inhibits trafficking of the Ex EGF-R to the cell membrane. COS 7 cells expressing Ex EGF-R were pulse-labeled in medium

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**Fig. 2.** GA inhibits trafficking of the Ex EGF-R to the cell membrane. COS 7 cells expressing Ex EGF-R were pulse-labeled in medium containing [35S]cysteine-methionine and chased in nonradioactive medium for 4 h. GA (1 μg/ml) or HA (1 μg/ml) was added to the cells during starvation, and the inhibitors were present during the pulse and throughout the chase periods. BFA (5 μg/ml) was added to the cells only during the chase period. After a 4-h chase period, the cells were subjected to cell surface biotinylation for 30 min. Samples were processed as follows prior to SDS-PAGE and autoradiography. A, cell surface biotinylated Ex EGF-R was immunoprecipitated with anti-EGF-R antibodies, followed by streptavidin-agarose. B, total EGF-R was immunoprecipitated using anti-EGF-R antibodies. C, biotinylated cell surface proteins were precipitated with streptavidin-agarose. Molecular weight markers are depicted on the right. The asterisk, circle, and triangle point at cell surface biotinylated proteins that were reduced by both HA and GA, reduced in size in the presence of GA, and remained unchanged by GA or HA treatment, respectively.
domain, rather to the presence of the EGF-R cytosolic domain, the metabolic profiles of sEPO-R in the absence or the presence of GA were examined (Fig. 4C). The degradation kinetics of sEPO-R was essentially similar in the presence and absence of GA, supporting the notion that the EGF-R cytosolic domain is sufficient to confer GA-mediated degradation to the EPO-R/EGF-R chimera.

To address whether kinase activity of the EGF-R is necessary for GA-mediated degradation of the receptor, we followed the metabolism of the EGF-R K721A mutant, which is devoid of Tyr kinase activity (Fig. 5). CHO cells stably expressing K721A EGF-R or wt EGF-R were pulse-labeled with [35S]cysteine-methionine and chased in growth medium for 1 and 2 h in the absence or presence of GA. The kinetics of K721A EGF-R and wt EGF-R degradation in GA-treated cells was virtually identical, indicating that Tyr kinase activity of the EGF-R is not required for GA-mediated degradation.

Both HA and GA are inhibitors of HSP 90 and GRP 94, the cytosolic and ER-lumen chaperones, respectively. Here we demonstrate that the minute molecular differences between these compounds (35, 36) are sufficient to elicit distinct biological effects on metabolism of the EGF-R. GA has two different effects on metabolism of the EGF-R. It enhanced degradation, and it inhibited maturation of the receptor. HA, however, only accelerated EGF-R degradation. Enhanced EGF-R degradation mediated by GA and HA is conferred by the cytosolic domain of the receptor, whereas GA-mediated intracellular retention requires its extracellular domain.

The EGF-R cytosolic domain is sufficient to confer degradation in the presence of GA, and this is supported by the rapid degradation of EPO-R/EGF-R chimera induced by this compound (Fig. 4). The specificity of GA was supported by the lack of its effect on degradation of full-length EPO-R and sEPO-R.
metabolism. Utilizing this EPO-R/EGF-R chimera provided further support for the notion that GA independently affects receptor maturation and degradation. Although present at low levels, cell surface EPO-R and EPO-R/EGF-R chimera are detected upon binding to radioactively labeled EPO (28). In the presence of GA, the expression levels of both cell surface EPO-R and EPO-R/EGF-R chimera were reduced by 50% (data not shown). This decrease in cell surface receptors may be due to accelerated degradation or inhibition of transport. The fact that the degradation of the chimeric receptor molecule was sensitive to GA, but that of the wild type EPO-R was not, indicates that degradation and retention are distinct processes mediated by GA. We speculate that retarded maturation of EGF-R in the presence of GA is mediated predominantly by inhibition of GRP 94 localized in the lumen of the ER and that enhanced degradation in the presence of HA and GA are mediated via inhibition of the cytosolic chaperone HSP 90. This possibility is currently under investigation.

It was previously reported that the cytosolic domains of EGF-R (13) and p185<sup>ERB-B2</sup> (10) are required for degradation stimulated by GA and that the kinase domain of p185<sup>ERB-B2</sup> is involved in this process (10). The data hereby presented demonstrate that a kinase-defective EGF-R is degraded similarly to WT EGF-R in GA-treated cells, ruling out the contribution of kinase activity per se to this process. This finding alludes to the existence of other, yet unidentified, sequence or structural motifs in the cytosolic domain of the EGF-R directing its degradation in the presence of GA.

Figs. 1 and 2 demonstrate the differential effects of GA and HA on turnover and processing of Ex EGF-R, an EGF-R mutant that lacks the cytosolic domain. Cell surface biotinylation experiments indicate that GA treatment significantly reduced expression levels of newly synthesized Ex EGF-R on the cell membrane, whereas the total levels of Ex EGF-R remained unchanged. Slightly lower levels of newly synthesized Ex EGF-R at the cell surface were observed in the presence of 1 µg/ml HA, compared with control non-treated cells. It should be noted that no further reduction was observed at higher concentrations (up to 10 µg/ml) of HA (data not shown). It was previously reported that a soluble EGF-R molecule was secreted in the presence of GA, although to a somewhat lower extent (13). It is conceivable that GA differentially affects membrane versus soluble secreted proteins. This might explain the differences in the level of inhibition of transport by GA observed for the soluble and membrane-bound forms of EGF-R. Furthermore, analysis of the general profile of newly synthesized biotinylated cell surface proteins indicates that GA affects only a subgroup of these proteins, whereas the others remain unaffected. Noteworthy is the fact that in the presence of BFA, newly synthesized cell surface proteins were barely detected. This confirms that indeed no cell surface labeling of EGF-R is detected when ER to Golgi transport is blocked. Hence, it seems that GA-mediated block of protein transport applies to proteins in addition to the EGF-R, yet it does not apply to all cell surface proteins.

The different activities of HA and GA were also demonstrated in experiments utilizing the combination of these inhibitors with the proteasomal inhibitor Z-L<sub>V</sub>S<sub>V</sub>. In the presence of Z-L<sub>V</sub>S<sub>V</sub>, HA treatment yielded fully processed EGF-R, whereas GA treatment under these conditions resulted in EGF-R forms impaired in their glycosylation. These data lend support to the conclusion that intracellular retention of the EGF-R mediated by GA is a separate effect from its effect on enhanced degradation of the receptor. A similar effect of GA in the presence of proteasomal inhibitors was also assessed for the CFTR (4), although the effects of HA in the presence of proteasomal inhibitors on CFTR degradation was not measured. Based on our results, a possibility exists that unlike GA, HA may not prevent maturation of the CFTR. The mechanism by which GA causes intracellular retention of the EGF-R and possibly other membrane proteins remains to be elucidated.

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REFERENCES

1. Pratt, W. B. (1998) Proc. Soc. Exp. Biol. Med. 217, 420–434
2. Buchner, J. (1999) Trends Biochem. Sci. 24, 136–141
3. Schulte, T., Blagosklonny, M. V., Romanova, L., Mushinski, J. F., Monia, B. P., Johnston, J. F., Nguyen, P., Trepel, J., and Neckers, L. M. (1996) Mol. Cell. Biol. 16, 5839–5845
4. Uehara, Y., Murakami, Y., Sugimoto, Y., and Mizuno, S. (1989) Cancer Res. 49, 760–785
5. Blagosklonny, M. V., Toretsky, J., and Neckers, L. (1995) Oncogene 11, 933–939
6. Wella, A. (1999) Int. J. Biochem. Cell Biol. 31, 657–643
7. Chavany, C., Mimnaugh, E., Miller, P., Bitten, K., Nguyen, P., Trepel, J., Whitesell, L., Schnur, R., Moyer, J., and Neckers, L. (1996) J. Biol. Chem. 271, 4974–4977
8. Miller, P., DiOrio, C., Meyer, M., Schnur, R. C., Bruskin, A., Cullen, W., and Moyer, J. D. (1994) Cancer Res. 54, 2724–2730
9. Murakami, Y., Mizuno, S., and Uehara, Y. (1994) Biochem. J. 301, 63–68
10. Mimnaugh, E. G., Chavany, C., and Neckers, L. (1996) J. Biol. Chem. 271, 22796–22801
11. Sagagami, M., Morrison, P., and Welch, W. J. (1999) Cell Stress Chaperones 4, 19–28
12. Felder, S., Miller, K., Mocheg, U., Ullrich, A., Schlessinger, J., and Hopkins, C. R. (1990) Cell 61, 623–634
13. Carlin, C. R., and Knowles, B. B. (1984) J. Biol. Chem. 259, 7902–7908
14. Kurten, R. C., Cadena, D. L., and Gill, G. N. (1996) Science 272, 1008–1010
15. Hober, M., and Carlin, C. (1995) J. Cell. Physiol. 162, 434–446
16. Hober, M. R., EI, S. J., Medof, M. E., and Carlin, C. R. (1997) J. Biol. Chem. 272, 32901–32909
17. Supino-Rosin, L., Yoshimura, A., Altaraz, H., and Neumann, D. (1999) Eur. J. Biochem. 363, 410–419
18. Yoshimura, A., and Misawa, H. (1998) Curr. Opin. Hematol. 5, 171–176
19. Yamasaki, M., Nagata, M., Ohara, T., and Ueda, Y. (1989) Cell 67, 223–2236
20. Witthuhn, B. A., Quelle, F. W., Silvennoinen, O., Yi, T., Tang, B., Miura, O., and Lodish, H. F. (1993) Cell 74, 227–236
21. Sawyer, S. T., and Hankins, W. D. (1995) Proc. Natl. Acad. Sci. U. S. A. 90, 6849–6853
22. Hilton, D. J., Watowich, S. S., Murray, P. J., and Lodish, H. F. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 190–194
23. Neumann, D., Wittem, L., Watowich, S. S., and Lodish, H. F. (1995) J. Biol. Chem. 268, 13639–13649
24. Levin, I., Cohen, J., Supino-Rosin, L., Yoshimura, A., Watowich, S. S., and Neumann, D. (1998) FEBS Lett. 427, 164–170
25. Cohen, J., Altaraz, H., Zick, Y., Klangmuller, U., and Neumann, D. (1997)
28. Ohashi, H., Maruyama, K., Liu, Y. C., and Yoshimura, A. (1994) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{91}, 158–162
29. Kris, R. M., Lax, I., Gullick, W., Waterfield, M. D., Ullrich, A., Fridkin, M., and Schlessinger, J. (1985) \textit{Cell} \textbf{40}, 619–625
30. Neumann, D., Yuk, M. H., Lodish, H. F., and Lederkremer, G. Z. (1996) \textit{Biochem. J.} \textbf{313}, 391–399
31. Levkowitz, G., Waterman, H., Zamir, E., Kam, Z., Oved, S., Langdon, W. Y., Beguinot, L., Geiger, B., and Yarden, Y. (1998) \textit{Genes Dev.} \textbf{12}, 3663–3674
32. Aruffo, A., and Seed, B. (1987) \textit{Proc. Natl. Acad. Sci. U. S. A.} \textbf{84}, 8573–8577
33. Lippincott-Schwartz, J., Yuan, L., Tipper, C., Amherdt, M., Orci, L., and Klausner, R. D. (1991) \textit{Cell} \textbf{67}, 601–616
34. Dunphy, W. G., Brads, R., and Rothman, J. E. (1985) \textit{Cell} \textbf{40}, 463–472
35. Iwai, Y., Nakagawa, A., Sadakane, N., Omura, S., Oiwa, H., Matsumoto, S., Takahashi, M., Ikai, T., and Ochiai, Y. (1980) \textit{J. Antibiot. (Tokyo)} \textbf{33}, 1114–1119
36. Sasaki, K., Rinehart, K. L., Jr., Slomp, G., Grostic, M. F., and Olson, E. C. (1970) \textit{J. Am. Chem. Soc.} \textbf{92}, 7591–7593