Regulation of MDR1 Expression and Drug Resistance by a Positive Feedback Loop Involving Hyaluronan, Phosphoinositide 3-Kinase, and ErbB2* ♦

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Multidrug resistance is a potent barrier to effective, long term therapy in cancer patients. It is frequently attributed to enhanced expression of multidrug transporters or to the action of receptor kinases, such as ErbB2, and downstream anti-apoptotic signaling pathways, such as the phosphoinositide 3-kinase/Akt pathway. However, very few connections have been made between receptor kinases or anti-apoptotic pathways and multidrug transporter expression or function. Data presented herein show that constitutive interaction of the pericellular polysaccharide, hyaluronan, with its receptor, CD44, regulates assembly and activation of an ErbB2-containing signaling complex, which in turn stimulates phosphoinositide 3-kinase activity in multidrug-resistant MCF-7/Adr human breast carcinoma cells. Phosphoinositide 3-kinase activates Akt and downstream anti-apoptotic events, which contribute to drug resistance. However, hyaluronan and phosphoinositide 3-kinase stimulate expression of the multidrug transporter, MDR1 (P-glycoprotein), in an interdependent, but Akt-independent, manner. Furthermore, constitutively active phosphoinositide 3-kinase, but not Akt, stimulates hyaluronan production. These Akt-independent effects are dominant over the effects of Akt on doxorubicin resistance in MCF-7/Adr cells. Thus hyaluronan, phosphoinositide 3-kinase, and ErbB2 form a positive feedback loop that strongly amplifies MDR1 expression and regulates drug resistance in these cells. This pathway may also be important in progression of other malignant characteristics. These results illustrate the potential importance of hyaluronan as a therapeutic target in multidrug-resistant carcinomas.

Multidrug resistance in cancer can arise by several mechanisms but is frequently associated with enhanced drug efflux via broad specificity, ATP-dependent, ABC family transporters (herein termed multidrug transporters) (1). Recent work has also highlighted the importance of receptor kinases and cell survival/anti-apoptotic pathways in drug resistance (2–6), but very few connections have been made between the latter pathways and multidrug transporter expression or function. However, a recent study has shown that the PI3K/Akt pathway influences expression of the MRP1 transporter in prostate carcinoma cells (7). Hyaluronan, a very large polysaccharide composed of repeating disaccharides of glucuronic and N-acetylglucosamine, is a ubiquitous component of extracellular matrices. In many adult tissues the major function of hyaluronan is structural, but in developing and remodeling tissues hyaluronan plays a crucial, instructive, signal-transducing role (8–10). Hyaluronan is also a major component of the pericellular matrices within tumors and it influences the activity of tumor cell signaling pathways that are important in development of malignant cellular properties (11). However, these relationships are complex, since very high hyaluronan concentrations may inhibit rather than promote tumor progression (12), and turnover of hyaluronan may be crucial for the action of hyaluronan (13).

In particular, hyaluronan regulates the activity of ErbB family members, most likely via interaction with the hyaluronan receptor, CD44 (14–17). We have shown that hyaluronan constitutively regulates ErbB2 (18) and cell survival pathway activities, such as PTEN, PI3K, and the downstream effectors Akt, BAD, and FKHR (19), in colon and mammary carcinoma cells. Furthermore, increased hyaluronan production stimulates drug resistance in human breast carcinoma cells, whereas inhibition of constitutive hyaluronan-tumor cell interactions sensitizes multidrug-resistant cells to several chemotherapeutic drugs (20). In this study we have investigated possible interrelationships between hyaluronan, PI3K, ErbB2, and expression of MDR1 (P-glycoprotein) in MCF-7 and multidrug-resistant MCF-7/Adr human breast carcinoma cells. We have found that hyaluronan acts upstream and downstream of PI3K and that both hyaluronan and PI3K stimulate MDR1 expression and induce drug resistance in an interdependent, but Akt-independent, manner. We have also found that hyaluronan regulates activation of ErbB2, which in turn stimulates PI3K activity in these cells. This positive feedback loop is most likely critical in the regulation of multidrug resistance and other malignant characteristics of breast cancer cells.

EXPERIMENTAL PROCEDURES

Materials—Hyaluronan oligomers used in this study are a highly purified, mixed fraction of average molecular weight ~2.5 × 10^5 and are composed of 3–10 disaccharide units (19, 21). Plasmids for constitutively PI3K and Akt, and dominant-negative Akt, were obtained from Upstate Biotechnology, Lake Placid, NY. The dominant-negative PI3K plasmid was provided by Dr. L. Cantley, Harvard Medical School, in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Hyaluronan, PI3K, ErbB2, and MDR1 in Drug Resistance

FIG. 1. Regulation of doxorubicin resistance by hyaluronan. A, multidrug-resistant MCF-7/Adr human breast carcinoma cells were infected with recombinant adenoviruses driving expression of β-galactosidase (as control), soluble CD44, or the link module of brevican (BLM), then incubated for 72 h with various concentrations of doxorubicin. Cell survival was assayed and compared with cells that were incubated with doxorubicin but not treated with adenovirus (untreated). B, MCF-7/Adr cells were transfected with CD44 siRNA or control RNA, then incubated with various concentrations of doxorubicin. Cell survival was assayed and compared with cells that were incubated with doxorubicin without other reagents (untreated) or with 100 μg/ml hyaluronan oligomers (α-HA). C, drug-sensitive, parental MCF-7 cells were treated with recombinant adenoviruses driving expression of β-galactosidase (as control) or HAS2; the latter produce elevated levels of hyaluronan. HAS2-transfected cells were treated with and without 100 μg/ml hyaluronan oligomers. The cells were incubated with various concentrations of doxorubicin, then cell survival was assayed and compared with cells that were incubated with doxorubicin but not treated with other reagents (untreated). Results in A–C are given for three experiments as means ± S.E.

Boston, MA. Antibodies against total ErbB2 (extracellular domain), phosphorylated ErbB2, p85 subunit of PI3K, and ezrin were from Upstate Biotechnology Inc.; antibodies against p110α subunit of PI3K, Akt, and phosphorylated Akt were from Santa Cruz Biotechnology Inc.; antibodies against MDR1 was from Signet Laboratories (Bedford, MA). Antibody against cdc7 was a gift from Dr. N. Grammatikakis (22). The secondary antibodies used were ECL anti-rabbit IgG (peroxidase-linked species-specific Fab’/2 fragment from donkey) and ECL anti-mouse IgG (peroxidase-linked species-specific Fab’/2 fragment from sheep) from Amersham Biosciences. Enhanced chemiluminescence reagents (Western Lightning Chemiluminescence Reagent Plus) were from PerkinElmer Life Sciences Inc. AG825 and doxorubicin were from Calbiochem. Unless specified, all other reagents were the highest grade from Sigma.

Cell Lines and Transfectants—MCF-7/Adr human mammary carcinoma cells were obtained from Dr. K. Cowan, University of Nebraska. Transient transfections were carried out with Lipofectamine 2000 according to manufacturer’s instructions (Invitrogen). Stable transfectants were prepared using linearized plasmids with Lipofectamine 2000 and selected using G418 (22). MCF-7 cells were purchased from ATCC (Manassas, VA).

Cell lines were routinely maintained in complete medium, i.e. RPMI plus Glutamax I medium (Invitrogen), containing 4.5 g/ml glucose and 10% fetal bovine serum, 100 units of penicillin/ml, 100 μg of streptomycin/ml, and an additional 1 mm glutamine (Invitrogen). The cell lines were maintained at 37 °C in 5% CO2 and passaged every 3–4 days.

Recombinant Adenoviruses—Recombinant adenoviruses, driving expression of β-galactosidase, Has2, soluble CD44, or the link module of brevican were prepared as described previously (24–26). The adenoviruses for dominant-negative and constitutively active Akt were obtained from Dr. K. Walsh, Boston University (27). Cells were plated in 6-well plates and allowed to grow under routine conditions until ~70% confluent. The growth medium was then replaced with serum-free medium. The cells were infected with the adenovirus constructs at 200–300 multiplicity of infection per cell by incubation for 90 min with intermittent gentle rocking. The virus-containing medium was removed and replaced with 3 ml per well of complete serum-containing medium. The cultures were then incubated at 37 °C overnight and transferred to 150-mm dishes before use.

RNA Silencing—siRNA for human CD44 was designed as described previously (18). The nonspecific control IX RNA was purchased from Dharmaco (Lafayette, CO). Cells were transfected with the siRNA in 2 ml of complete medium in 6-well plates with cells at 70–90% confluence. Transfections were performed with 200 pmol of siRNA, using Oligofectamine (Invitrogen), according to manufacturer’s instructions. The cells were then incubated at 37 °C in 5% CO2 for 24 h and replated in 150-mm dishes before use.

Assays for Cell Survival/Doxorubicin Sensitivity, Inhibition of ErbB2 Activity, PI3K Activity, and Hyaluronan Concentration—For analysis of sensitivity to doxorubicin treatment, cells were plated in 24-well plates (Corning) in complete serum-containing medium. After overnight incubation, various concentrations of doxorubicin (10 nM to 100 μM) plus other reagents as specified were added to the cultures, followed by further incubation for 72 h. The cultures were then washed three times with phosphate-buffered saline to remove dead cells, harvested by trypsinization, and cell numbers measured in a Coulter Counter.

RESULTS AND DISCUSSION

Hyaluronan Regulates Drug Resistance—In a previous study we demonstrated that small hyaluronan oligosaccharides (oligomers) sensitize drug-resistant breast carcinoma cells to several different chemotherapeutic drugs (20). Our interpretation of these results was that the oligomers antagonize constitutive hyaluronan polymer-CD44 interaction by competitively replacing a multivalent, signal-transducing interaction with a monovalent, non-signaling interaction (11). However, other interpretations are possible, e.g. the oligomers might signal directly through another receptor (8, 29). Thus, to confirm that perturbation of constitutive hyaluronan interactions inhibits drug resistance, we used two other antagonists of hyaluronan-CD44 interactions, i.e. soluble CD44 and the hyaluronan-binding link module of brevican, both of which act as competitive decoys for binding endogenous hyaluronan (11, 25). We found that both reagents sensitize multidrug-resistant MCF-7/Adr breast carcinoma cells to doxorubicin treatment (Fig. 1A). To demonstrate that hyaluronan-CD44 interaction is specifically involved, we used an siRNA that blocks CD44 expression by 70–90% (18). This reagent was also found to sensitize MCF-7/Adr cells to doxorubicin to a similar degree to the hyaluronan oligomers (Fig. 1B).

In addition to using hyaluronan antagonists, we determined the effect of increased hyaluronan synthesis. We stimulated hyaluronan synthesis rather than adding exogenous hyaluronan, since the latter does not necessarily mimic the cellular localization and interactions available to endogenously produced hyaluronan (11, 30), and since, in a previous study, we showed that up-regulation of hyaluronan synthesis stimulates further...
ErbB2 phosphorylation in MCF-7 cells, whereas addition of exogenous hyaluronan does not (18). Thus, we infected the parental, drug-sensitive MCF-7 cells with a recombinant Has2 adenovirus that induces ~5-fold increase in hyaluronan production (26) and showed that this treatment causes increased resistance to doxorubicin in the MCF-7 cells. This effect was reversed by concomitant treatment with hyaluronan oligomers (Fig. 1C).

Thus we conclude that hyaluronan-CD44 interaction regulates doxorubicin resistance in these cells.

**Hyaluronan and PI3K Cooperatively Regulate Drug Resistance in an Akt-independent Manner**—Numerous studies have shown that PI3K activity increases drug resistance, and it has been widely assumed that this is due to the downstream anti-apoptotic effects of the PI3K/Akt cell survival pathway (3). We have shown that hyaluronan constitutively regulates the PI3K/Akt pathway (19, 20, 26), and so we sought evidence for the postulate that hyaluronan regulates drug resistance via its effects on the PI3K/Akt pathway. To do this we prepared MCF-7/Adr cells that express dominant-negative and constitutively active forms of PI3K and Akt. We manipulated PI3K activity levels by stable or transient transfection with the relevant constructs. We manipulated Akt activity by transient transfection or by infection with adenoviruses expressing the constructs. We showed that each of the dominant-negative constructs of PI3K or Akt inhibits Akt phosphorylation and that the constitutively active constructs of PI3K and Akt stimulate Akt phosphorylation (Fig. 2A and B). We also showed that the PI3K transfections had the expected effects on PI3K activity (Fig. 2C). Since the different methods of delivering the dominant-negative and constitutively active constructs gave similar results, we used stable transfection with the PI3K constructs and recombinant adenoviral delivery of the Akt constructs for most of the subsequent experiments.

We then examined the effects of these manipulations on doxorubicin sensitivity. We found that dominant-negative PI3K sensitizes the cells by at least 2 orders of magnitude, whereas the constitutively active PI3K increases resistance in these already resistant cells by another order of magnitude (Fig. 2D). However, the Akt constructs showed much smaller effects (Fig. 2E); we used both transient transfection and adenoviral delivery in several experiments, but the effects obtained were always less than 10-fold. Thus, even though the PI3K constructs and the Akt constructs have very similar effects on Akt phosphorylation (Fig. 2A and B), only the PI3K constructs elicit strong effects on drug resistance. Consequently, we conclude that a large part of the effect of PI3K on drug resistance is Akt-independent.

We then analyzed the effects of manipulating hyaluronan interactions on doxorubicin sensitivity in the PI3K and Akt transfectants. We found that the highly resistant, constitutively active PI3K transfectant was no longer susceptible to the usual dose of hyaluronan oligomers, i.e. 100 μg/ml (Fig. 3A). However, treatment with soluble CD44 or with CD44 siRNA still sensitized the cells to doxorubicin treatment (Fig. 3A). Since hyaluronan oligomers interact with CD44 with low affinity (31, 32), we used higher doses of oligomers and found that these doses sensitize the cells to doxorubicin (Fig. 3B).

Thus, we conclude that the increased doxorubicin resistance caused by constitutively active PI3K is dependent on endogenous hyaluronan-CD44 interaction. We also found that experimentally increasing hyaluronan production in the dominant-negative PI3K transfectant partially reverses its increased sensitivity to doxorubicin (Fig. 3C) and that hyaluronan oligomers sensitize both the constitutively active and dominant-negative Akt transfectants in similar fashion to non-transfected cells (Fig. 3D).

**Constitutively Active PI3K, but Not Akt, Stimulates Hyaluronan Production**—As shown in the previous section, larger amounts of hyaluronan oligomers are required to inhibit doxorubicin resistance in the constitutively active PI3K-MCF-7/Adr transfectant than in non-transfected or vector-transfected MCF-7/Adr cells. Thus we considered the possibility that PI3K

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**FIG. 2. Characterization of PI3K and Akt transfectants.** A, levels of phosphorylated Akt (pAkt) and total Akt in the PI3K transfectants. Lane 1, MCF-7/Adr cells; lane 2, dominant-negative transient transfectant; lane 3, constitutively active transient transfectant; lane 4, dominant-negative stable transfectant; lane 5, constitutively active stable transfectant. Vector transfectants show similar levels to untreated cells. B, levels of phosphorylated Akt (pAkt) and total Akt in the Akt transfectants. Lane 1, MCF-7/Adr cells; lane 2, dominant-negative adenovirus; lane 3, constitutively active adenovirus; lane 4, dominant-negative transient transfectant; lane 5, constitutively active transient transfectant. Vector transfectants show similar levels to untreated cells. C, PI3K activity in PI3K transfectants. Lane 1, vector-transfectant; lane 2, dominant-negative stable transfectant; lane 3, constitutively active stable transfectant. D, doxorubicin sensitivity of PI3K stable transfectants compared with untreated and vector-transfected cells. Each cell type was treated for 72 h with a range of doxorubicin concentrations, then assayed for cell survival. DN, dominant-negative; CA, constitutively active. E, doxorubicin sensitivity of recombinant Akt adenovirus-treated transfectants, compared with untreated and β-galactosidase adenovirus-treated cells (as control). Results in C–E are given for three experiments as means ± S.E.
adenoviruses driving expression of CD44 or oligomers or transiently transfected with CD44 siRNA or control RNA or treated with recombinant adenoviruses driving expression of soluble CD44 or β-galactosidase (as control). Cell survival was measured after incubation for 72 h with various concentrations of doxorubicin. B, MCF-7/Adr cells, stably transfected with constitutively active PI3K (CA-PI3 kinase), were treated with recombinant adenoviruses driving expression of β-galactosidase (as control) or HAS2. HAS2-transfected cells were treated with and without 100 μg/ml hyaluronan oligomers. The cells were incubated for 72 h with various concentrations of doxorubicin and compared with cells that were incubated with doxorubicin but not treated with other reagents (untreated). D, MCF-7/Adr cells, infected with recombinant adenoviruses driving expression of dominant-negative (DN) or constitutively active (CA) Akt, were treated with and without 100 μg/ml hyaluronan oligomers (o-HA) plus doxorubicin for 72 h. Results in A–D are given for three experiments as means ± S.E.

stimulates hyaluronan production, in which case higher levels of oligomer would be required to compete for interaction with endogenous CD44. We found that constitutively active PI3K greatly stimulates hyaluronan production, whereas constitutively active Akt does not; the dominant-negative PI3K and Akt both exhibit modest inhibitory effects (Fig. 4).

As stated above, we have previously shown that hyaluronan stimulates PI3K activity (20, 26). The results obtained here show that PI3K stimulates hyaluronan production, thus establishing a positive feedback loop that would augment the action of these agents.

**Hyaluronan and PI3K, but Not Akt, Regulate Expression of MDR1 (P-glycoprotein)—**Since Akt activity appears to play a minor role in doxorubicin resistance in the MCF-7/Adr cells, we explored the possibility that PI3K and hyaluronan, but not Akt, affect levels of multidrug transporter expression in these cells.

First we confirmed that MCF-7/Adr cells express higher levels of MDR1 than the parental MCF-7 cells (Fig. 5, lane 1 in A versus lane 1 in B). We then showed that treatment with hyaluronan oligomers reduces constitutive MDR1 expression in the MCF-7/Adr cells (Fig. 5A, lane 2) and that increased hyaluronan production increases MDR1 expression in the MCF-7 cells (Fig. 5B, lane 2); the latter effect is reversed by co-treatment with hyaluronan oligomers (Fig. 5B, lane 3). Similar effects on expression of another multidrug transporter, MRP2, were also observed, i.e. increased hyaluronan induced expression of MRP2 in MCF-7 cells, and treatment with hyaluronan oligomers suppressed expression of MRP2 in MCF-7/Adr cells (data not shown).

We also found that constitutively active PI3K increases MDR1 expression (Fig. 5A, lane 3) and that soluble CD44 and CD44 siRNA reverse this increase (Fig. 5A, lanes 5 and 6). The usual dose of hyaluronan oligomers, i.e. 100 μg/ml, was only partially effective (Fig. 5A, lane 4, and Fig. 5C, lane 2), but higher doses strongly reverse the effect of constitutively active PI3K (Fig. 5C, lanes 4 and 5). Dominant-negative PI3K inhibits MDR1 expression in the MCF-7/Adr cells (Fig. 5, lane 4 in B versus lane 1 in A), but this inhibition is reversed by increased hyaluronan production (Fig. 5B, lane 5). Although manipulation of PI3K and hyaluronan-CD44 interaction has these large effects on MDR1 expression, manipulation of Akt activity has little effect (Fig. 5D).

The results described here closely match the effects of hyaluronan, PI3K, and Akt on sensitivity to doxorubicin (Fig. 2, D and E, and Fig. 3). Thus we conclude that the Akt-independent effects of PI3K on doxorubicin sensitivity are most likely due to its effects on multidrug transporter expression and that hyaluronan-CD44 interaction is required for the effects of PI3K.

**Hyaluronan Constitutively Activates ErbB2 in MCF-7/Adr Cells—**ErbB2 has been implicated in induction of drug resist-

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**Fig. 3.** Effects of manipulating hyaluronan interactions on doxorubicin sensitivity of PI3K and Akt transfectants. A, MCF-7/Adr cells, stably transfected with constitutively active PI3K (CA-PI3 kinase), were treated with (o-HA) or without (untreated) 100 μg/ml hyaluronan oligomers or transiently transfected with CD44 siRNA or control RNA or treated with recombinant adenoviruses driving expression of soluble CD44 or β-galactosidase (as control). Cell survival was measured after incubation for 72 h with various concentrations of doxorubicin. B, MCF-7/Adr cells, stably transfected with constitutively active PI3K, were treated with 0–1000 nM doxorubicin for 72 h.

**Fig. 4.** Stimulation of hyaluronan production by constitutively active PI3K. The amounts of hyaluronan produced by vector-transfectant (control) and by dominant-negative (DN) and constitutively active (CA) PI3K and Akt transfectants were measured in culture media after 24-h incubation. Results are given for three experiments as means ± S.E.
ance in several tumor types, at least in part through activation of the PI3K/Akt pathway (5). Hyaluronan activates ErbB2 (14, 18) and the PI3K/Akt pathway (19, 20, 33) in various transformed and carcinoma cells. Since, as found herein, hyaluronan and PI3K cooperatively regulate drug resistance in MCF-7/Adr cells, and since MCF-7/Adr cells express high levels of activated ErbB2, we determined the extent to which ErbB2 is responsible for PI3K activity in these cells. We did this by measuring Akt phosphorylation at various concentrations of the ErbB2 inhibitor, AG825 (0–2 μM), on expression of phosphorylated ErbB2 (pErbB2), phosphorylated Akt (pAkt), and total Akt. A, immunoblots. B, graphic representation of the immunoblots after densitometry.

**Fig. 5. Effects of hyaluronan, PI3K, and Akt on MDR1 expression.** A, effects of hyaluronan antagonists on expression of MDR1 in MCF-7/Adr cells stably transfected with vector (Adr) or constitutively active PI3K (CA-PI3k). Lanes 1 and 2, Western blots of lysates of MCF-7/Adr cells treated without (lane 1) and with 100 μg/ml hyaluronan oligomers (lane 2); lanes 3–6, CA-PI3k cells treated without (lane 3) or with 100 μg/ml hyaluronan oligomers (lane 4), soluble CD44 recombinant adenovirus (lane 5), or CD44 siRNA (lane 6). β-Tubulin (β-tub) was used as a loading control. B, effects of increased hyaluronan expression on MCF-7 cells or on MCF-7/Adr cells stably transfected with dominant-negative PI3K (DN-PI3k). Lanes 1–3, MCF-7 cells treated without (lane 1) or with HAS2 recombinant adenovirus in the absence (lane 2) or presence of 100 μg/ml hyaluronan oligomers (lane 3); lanes 4–6, dominant-negative PI3K (DN-PI3k) cells treated without (lane 4) or with HAS2 recombinant adenovirus in the absence (lane 5) or presence of 100 μg/ml hyaluronan oligomers (lane 6). C, constitutively active PI3K (CA-PI3k) cells treated without (lane 1) or with 100 μg/ml (lane 2), 200 μg/ml (lane 3), 500 μg/ml (lane 4), or 1000 μg/ml (lane 5) hyaluronan oligomers. D, MCF-7/Adr cells treated with recombinant adenoviruses driving expression of β-galactosidase (Adr lane 1), constitutively active Akt (CA-Akt; lanes 2 and 3), or dominant-negative Akt (DN-Akt; lanes 4 and 5).

We have recently found that hyaluronan constitutively regulates assembly of a lipid raft-associated complex, containing activated ErbB2, CD44, PI3K, ezrin, and the chaperones, Hsp90 and cdc37, in HCT116 human colon and TA3/St murine mammary carcinoma cells (18). Thus we determined whether
endogenous hyaluronan induces this complex in MCF-7/Adr cells by measuring the effects of hyaluronan antagonists on its assembly. We found that such a complex could be immunoprecipitated from MCF-7/Adr cell lysates with antibody against ErbB2 or the p85 subunit of PI3K and that hyaluronan antagonists inhibits its assembly (Fig. 7B).

We conclude from these results that regulation of PI3K activity by hyaluronan in MCF-7/Adr cells is at least in part mediated by constitutive, hyaluronan-induced activation of ErbB2.

Conclusions—The major findings of this and our previous studies of breast epithelial and carcinoma cells (18–20,26) are: (a) that hyaluronan acts both upstream and downstream of PI3K, (b) that hyaluronan and PI3K stimulate expression of MDR1 in an interdependent, but Akt-independent, manner, and (c) that hyaluronan regulates activation of ErbB2, which in turn stimulates PI3K activity. It is possible that a similar phenomenon occurs with other receptor kinases, such as c-MET (34) and TGF-β receptor 1 (35). Downstream anti-apoptotic events in the PI3K/Akt pathway clearly play a role in drug resistance (3), but our data indicate that, at least in MCF-7/Adr cells, a more important effect of PI3K may be Akt-independent stimulation of multidrug transporter expression. In the case of MCF-7/Adr cells, both MDR1 and MRP2 are induced by PI3K. A recent study showed that PI3K was also responsible for stimulation of MRP1 expression in prostate carcinoma cells, but this study did not address whether this effect was Akt-dependent or -independent (7). At this time it is not clear whether PI3K stimulates multidrug transporter expression directly. We observed that constitutively active PI3K requires endogenous hyaluronan for its effect on transporter expression. This dependence may be due to the positive feedback amplification of PI3K activity by hyaluronan, but this would only be the case if this amplification outweighed the contribution of the constitutively active PI3K introduced into the cells. Thus it seems more likely that hyaluronan stimulates transporter expression via another pathway and that PI3K contributes by amplifying hyaluronan expression. Recent studies suggest that hyaluronan may interact directly with drug transporters as well as influence their expression (36, 37). Our current studies are directed toward determining the pathways whereby hyaluronan and PI3K collaborate in the induction of multidrug resistance and other malignant properties. These results highlight the potential importance of hyaluronan as a therapeutic target in multidrug-resistant carcinomas.

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