Changes in Sodium Pump Expression Dictate the Effects of Ouabain on Cell Growth*

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Here we show that ouabain-induced cell growth regulation is intrinsically coupled to changes in the cellular amount of Na/K-ATPase via the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway. Ouabain increases the endocytosis and degradation of Na/K-ATPase in LLC-PK1, human breast (BT20), and prostate (DU145) cancer cells. However, ouabain stimulates the PI3K/Akt/mTOR pathway and consequently up-regulates the expression of Na/K-ATPase in LLC-PK1 but not BT20 and DU145 cells. This up-regulation is sufficient to replete the plasma membrane pool of Na/K-ATPase and to stimulate cell proliferation in LLC-PK1 cells. On the other hand, ouabain causes a gradual depletion of Na/K-ATPase and an increased expression of cell cycle inhibitor p21cip and slow the proliferation of LLC-PK1 cells. Consequently, we observe that small interfering RNA-mediated knockdown of Na/K-ATPase is sufficient to induce the expression of p21cip and slow the proliferation of LLC-PK1 cells. Moreover, this knockdown converts the growth stimulatory effect of ouabain to growth inhibition in LLC-PK1 cells. Mechanistically, both Src and caveolin-1 are required for ouabain-induced activation of Akt and up-regulation of Na/K-ATPase. Furthermore, inhibition of the PI3K/Akt/mTOR pathway by rapamycin completely blocks ouabain-induced expression of Na/K-ATPase and converts ouabain-induced growth stimulation to growth inhibition in LLC-PK1 cells. Taken together, we conclude that changes in the expression of Na/K-ATPase dictate the growth regulatory effects of ouabain on cells.

The Na/K-ATPase, a member of P-type ATPase family, was discovered as an energy transducing ion pump. It transports Na+ and K+ across the cell membrane and maintains ion homeostasis in animal cells (1, 2). Recent studies indicate that the Na/K-ATPase is also an important receptor that can transduce ligand binding into the activation of protein kinase cascades (3). Specifically, the Na/K-ATPase interacts with Src, which provides at least two important cellular regulations (4, 5). First, association with Na/K-ATPase keeps Src in an inactive state. Thus, the Na/K-ATPase serves as a native negative Src regulator (4). Second, this interaction forms a functional receptor complex for cardiotonic steroids (CTS) (3), a group of well characterized ligands of the Na/K-ATPase. Cardiotonic steroids include cardenolides (e.g. ouabain) and bufadienolides (e.g. marinobufagenin) (6). Although CTS are known cardiac drugs, some of them have now been identified as endogenous steroid hormones (6–8). Binding of CTS to the receptor complex activates the Na/K-ATPase-associated Src. Subsequently, the activated Src transactivates other tyrosine kinases, and together they recruit and further phosphorylate multiple membrane and soluble proteins, which results in the activation of protein kinase cascades and the generation of second messengers (3, 4, 6). Ultimately, this chain of signaling events would alter cellular functions and cell growth in a cell-specific manner (5, 9–12). For instance, we and others have demonstrated that ouabain-induced activation of ERK and PI3K/Akt/mTOR pathways are responsible for cell growth stimulation in transformed cell lines, in primary cultures, as well as in vivo (13–18).

It has also been recognized for a long time that CTS inhibit cell growth in many cancer cells (19–24). Of particular significance are studies that indicate the beneficial effects of CTS therapy in women with breast cancer (25–29). Consistently, recent in vitro and in vivo studies have identified several new CTS compounds that exhibit anti-cancer activities (30–32). Oleandrin, for example, is in clinical trials in the United States as an anti-cancer remedy for human cancers (31, 33). Although ouabain inhibits the pumping function of the Na/K-ATPase, it is important to note that the growth inhibitory effect of ouabain can occur at doses that neither cause significant changes in intracellular Na+ and K+ nor affect cell viability. Rather, much like its effect on cell growth stimulation, ouabain induces cell growth inhibition through the activation of protein kinases and the generation of second messengers (19–23, 34). For example, a recent report showed that these nontoxic concentrations of ouabain stimulated Src, resulting in activation of the epidermal growth factor receptor/ERK pathway and induction of the expression of cell cycle inhibitor p21cip and cell growth arrest (34). Thus, it becomes important to understand the molecular mechanisms that govern different fates of cells in response to CTS stimulation.

Prior studies have demonstrated that CTS induce endocytosis of the Na/K-ATPase and regulate its cellular expression via receptor-mediated signal transduction (35, 36). Because the

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Na/K-ATPase has both pumping and signaling functions, it is conceivable that changes in the amount of cellular Na/K-ATPase could have significant consequences on cell growth. Therefore, we have conducted the following experiments to reveal the role of cellular Na/K-ATPase in ouabain-induced cell growth regulation.

EXPERIMENTAL PROCEDURES

Materials—Cell culture media, fetal bovine serum, and trypsin were purchased from Invitrogen; Optitran nitrocellulose membrane was from Schleicher & Schuell; the ECL Supersignal kit was purchased from Pierce; and the monoclonal anti-α1 antibody (α6F) was obtained from the Developmental Studies Hybridoma Bank at the University of Iowa. The anti-α2 antibody was from Millipore (Billerica, MA), and the anti-α3 was from Affinity Bioreagents (Golden, CO). The anti-p21 

Cell Culture—The pig kidney epithelia cells (LLC-PK1 cells), human breast cancer cells (BT-20 cells and MDA-MB-435s), and human prostate cancer cells (DU145 cells) were obtained from American Type Culture Collection (Manassas, VA). [3H]Ouabain was from PerkinElmer Life Sciences.

Cell Culture—The pig kidney epithelia cells (LLC-PK1 cells), human breast cancer cells (BT-20 cells and MDA-MB-435s), and human prostate cancer cells (DU145 cells) were obtained from American Type Culture Collection and maintained in Dulbecco’s modified Eagle’s medium or RPMI 1640 medium (for DU145 cells) in the presence of 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin in a 5% CO2 humidified incubator (37°C). The Na/K-ATPase knockdown (C2-9) cells were derived from LLC-PK1 cells and cultured in Dulbecco’s modified Eagle’s medium as described (38). To eliminate the confounding effect of growth factors in the serum, the cells were serum-starved for 24 h. Afterward, the cells were washed with serum-free medium and subjected to [3H]Ouabain binding assay as described (40). Briefly, the cells were incubated in K+-free Krebs solution (137 mM NaCl, 2.8 mM CaCl2, 0.6 mM NaH2PO4, 1.2 mM MgSO4, 10 mM dextrose, 15 mM Tris, pH 7.4) for 15 min and then exposed to 100 nM [3H]Ouabain for 30 min at 37 °C in the presence of 20 μM monensin. At the end of incubation, the cells were washed three times with ice-cold K+-free Krebs solution, solubilized in 0.1 M NaOH-0.2% SDS, and counted in a scintillation counter for [3H]Ouabain. Nonpecific binding was measured in the presence of 5 μM unlabeled ouabain and subtracted from total binding. Parallel plates were used for counting cell numbers. [3H]Ouabain binding data were calculated as binding sites/cell.

Confocal Imaging and Immunocytochemistry—LLC-PK1, BT20, and DU145 cells were serum-starved for 24 h and treated with ouabain (10 nm) for 6 h on coverslips. The cells were fixed with ice-cold methanol for 15 min and blocked with Signal Enhancer from Invitrogen. A monoclonal anti-Na/K-ATPase α1 antibody was then added and incubated overnight at 4°C. After three washes, a secondary Alexa 488-conjugated anti-mouse antibody was added and incubated for 2 h at room temperature. The coverslip was washed, mounted, and imaged using a Leica confocal microscope as described (5).

Quantitative RT-PCR—The cells were seeded in 6-cm dishes and serum-starved for 24 h after the confluence reached 90%. The cells were then treated with 10 nm ouabain for 24 h. Total RNA was extracted using TRIzol and subjected to quantitative RT-PCR analysis. The assays were performed on the AB 7500 real time PCR system with the following protocol: 1 cycle (6 min at 95 °C), 40 cycles (30 s at 95 °C, 30 s at 52 °C, 30 s at 72 °C, and 1 min at 85 °C), followed by a dissociation stage to ensure a single product. The reactions are in a total volume of 25 μl, including 1.25 μl of Sybr Green (Invitrogen), 0.125 μl of DNA polymerase (Denivel), 2.5 μl of dNTP (Invitrogen), 10 ng of cDNA. Primer pairs were used at 5 μM concentration, and the sequences are: human GAPDH, 5′-GGGAGGTTGAGGTCGGAGT and 3′-TCCACTCTTACACAGTAAAAGCG; human Na/K-ATPase α1, 5′-TGCTGATGCGTTGTTGAGCG and 3′-TGCCCCTTTAAATAGGAAGT; human Na/K-ATPase α1, 5′-TGGCTGTGCTGAGATGGTGGG and 3′-AGATGATGACCTTTTGGGCTC (42); and pig Na/K-ATPase α1, 5′-ATGCTGGTCTGTTCATCTTG and 3′-AATGGCTTGGCTTGTGATGATG. The relative Na/K-ATPase α1 gene-expression was determined using the comparative Ct method.
expression was calculated by comparative threshold (\(C_T\)) method (\(\Delta\Delta C_T\)). To normalize the difference amounts of cDNA, GAPDH was used as an internal control.

Statistics—The data are presented as the means ± S.E. and compared using Student’s \(t\) test. Significance was accepted at \(p < 0.05\).

RESULTS

Ouabain Regulates Cell Growth and Na/K-ATPase Expression in a Cell-specific Manner—It is known that ouabain can either stimulate or inhibit cell proliferation in a cell-specific manner (16, 20, 34, 43–46). To understand the molecular mechanism of these opposing effects, we first compared the effects of ouabain on cell growth in two human epithelia-derived cancer cell lines (BT20 and DU145) and a pig kidney epithelial cell line (LLC-PK1). As shown in Fig. 1A, MTT assays indicated that ouabain at 1–50 nM stimulated cell proliferation in LLC-PK1 cells. These findings are consistent with the observed effects of ouabain on other renal epithelial cells (47, 48). In contrast, we failed to detect any stimulatory effect of ouabain on either breast cancer BT20 or prostate cancer DU145 cells. Moreover, 10–50 nM ouabain caused a significant inhibition of cell proliferation in these cells (Fig. 1A). To further confirm these observations, we counted cell numbers in the presence or absence of ouabain as a function of time. As depicted in Fig. 1B, ouabain increased the number of LLC-PK1 cells in a time-dependent manner, confirming that ouabain stimulates the proliferation of these cells. On the contrary, the same treatment significantly inhibits the proliferation of BT20 cells and DU145 cells. These findings are in accordance with what has been reported in the literature (22, 34).

Because ouabain inhibited cell proliferation by stimulating the expression of the cell cycle inhibitor \(p21\) in human breast cancer MDA-MB-435s cells (34), we measured the effects of ouabain on \(p21\) expression. As depicted in Fig. 1C, whereas 10 nM ouabain stimulated the expression of the cell cycle inhibitor \(p21\) in BT20 and DU145 cells, it failed to do so in LLC-PK1 cells.

Because the Na/K-ATPase is the receptor for ouabain, we next tested whether ouabain affects the expression of Na/K-ATPase. Western blot analyses indicated that all three cell lines expressed a ouabain-sensitive \(\alpha_1\) isoform of Na/K-ATPase. Under the same experimental conditions, the expression of \(\alpha_2\) or \(\alpha_3\) isoform was undetectable (data not shown). Interestingly, the effects of ouabain on \(\alpha_1\) expression were also cell-specific. Although ouabain caused induction of \(\alpha_1\) expression in LLC-PK1 cells after either 24 h or 72 h exposure, it reduced the cellular amount of \(\alpha_1\) in both BT20 and DU145 cells (Fig. 2A). Because ouabain also inhibited the proliferation of MDA-MB-435s cells (34), we measured the effects of ouabain on \(\alpha_1\) expression after these cells were exposed to 5 nM ouabain for 24 h. A significant reduction in \(\alpha_1\) expression was detected (82 ± 2% of control, \(n = 3\), \(p < 0.05\)). Thus, ouabain-induced changes in \(\alpha_1\) expression were clearly correlated with its effects on cell proliferation.
It is known that the β1 subunit plays an important role not only in the formation of a functional Na/K-ATPase but also in the formation of tight junctions and in the suppression of tumor cell growth (49–51). Thus, we assessed whether ouabain affects β1 expression in LLC-PK1 and cancer cell lines. The cells were treated with 10 nM ouabain for 24 h, and the amount of β1 was measured in the cell lysates by Western blot. As depicted in Fig. 2B, ouabain caused a modest increase in β1 expression in LLC-PK1 but not BT-20 cells.

To assess the functional consequence of these changes in α1 expression, we measured the number of active Na/K-ATPase in the plasma membrane. The cells were exposed to different concentrations of ouabain for 72 h and then subjected to [³H]ouabain binding. It is important to note that this assay measured the number of functional Na/K-ATPase that was not occupied by 5 or 10 nM ouabain after 72 h treatment. As depicted in Fig. 2C, ouabain up to 10 nM barely changed the number of active Na/K-ATPase in LLC-PK1 cells, indicating that the ouabain-induced up-regulation of β1 is just sufficient to replete the plasma membrane pool of active Na/K-ATPase. In contrast, ouabain caused a significant decrease in the number of active plasma membrane Na/K-ATPase in both BT20 and DU145 cells (Fig. 2C). Taken together, the data suggest that the decrease in the plasma membrane Na/K-ATPase may be involved in the ouabain-induced growth inhibition in cancer cells.

Reduction of the Na/K-ATPase Slows Cell Proliferation and Enables Ouabain to Inhibit Cell Proliferation in LLC-PK1 Cells—LLC-PK1 cells and the two cancer cell lines are derived from different organs and species. To further test the proposal that a decrease in cellular Na/K-ATPase is involved in ouabain-induced inhibition of cell proliferation, we measured the effects of a graded knockdown of the Na/K-ATPase on cell proliferation in LLC-PK1 cells. The generation of the knockdown cell lines has been previously reported (4, 52). A4–11 and PY-17 cells expressed about 40 and 10% of the Na/K-ATPase in comparison with the control vector-transfected LLC-PK1 (P-11) cells, respectively (4). As shown in Fig. 3, P-11 cells behaved similarly to the parent LLC-PK1 cells in response to ouabain stimulation. Ouabain, at concentrations from 1 to 10 nM, stimulated cell proliferation. In contrast, A4–11 and PY-17 cells behaved like BT20 and DU145 cells. No growth stimulation was detected. Instead, reduction of Na/K-ATPase expression by small interfering RNA-mediated mechanism sensitized these cells to ouabain-induced cell growth inhibition (Fig. 3). For example, 10 nM
ouabain caused more than a 80% reduction in cell proliferation in PY-17 cells in comparison with that in P-11 cells. Moreover, the growth inhibitory effects of ouabain were clearly correlated with the cellular amount of Na/K-ATPase.

To test whether this growth inhibitory effect of ouabain applies to other cardiotonic steroids, we measured the effects of digoxin, digitoxin, and marinobufagenin on cell proliferation in PY-17 cells. We observed that these compounds caused a growth inhibition similar to that of ouabain (data not shown).

To further examine the consequences of Na/K-ATPase reduction in cell growth regulation, we compared the cell proliferation curves of stable cell lines P-11 and PY-17 as well as AAC-19, a rat α1-rescued PY-17 cell line (4, 52). As shown in Fig. 4A, PY-17 cells proliferated much slower than P-11 cells, whereas reintroduction of rat Na/K-ATPase α1 restored cell growth in AAC-19 cells.

Because ouabain stimulated the expression of p21cip1 and inhibited cell proliferation in human cancer cells (Fig. 1) (34), we tested whether knockdown of cellular Na/K-ATPase affects the expression of this cell cycle inhibitor. As expected, the expression of p21cip1 was quite low in the control P-11 cells (Fig. 4B). Knockdown of the Na/K-ATPase caused a significant induction of this cell cycle inhibitor in PY-17 cells. Moreover, rescuing PY-17 cells by knocking in rat α1 was sufficient to reduce the expression of p21cip1 in AAC-19 cells. Consistently, we also detected more G0/G1 cells in PY-17 cells than that in both P-11 and AAC-19 cells (Fig. 4C).

Ouabain Has No Effect on α1 mRNA—To probe the molecular mechanism of ouabain-induced cell-specific regulation of α1, we first measured α1 mRNA using a quantitative RT-PCR after the cells were treated with 10 nm ouabain. As depicted in Fig. 5, ouabain had no effect on the levels of α1 mRNA in LLC-PK1, BT20, and DU145 cells. Thus, the ouabain-induced changes in the α1 protein are likely via translational/post-translational mechanisms.

Ouabain Stimulates Endocytosis and Degradation of Na/K-ATPase—We showed that ouabain stimulated Na/K-ATPase endocytosis in LLC-PK1 cells (35). If ouabain also stimulates the endocytosis in BT20 and DU145 cells, this could result in an increased degradation of Na/K-ATPase and a decrease in the α1 protein in these cells. To test this postulation, the cells were exposed to 10 nm ouabain and then immunostained for α1. As expected, most of α1 resided in the plasma membrane in BT20 and DU145 cells as in LLC-PK1 cells (Fig. 6A). After 6 h of ouabain exposure, many α1-positive vesicles were accumulated in BT20 and DU145 cells.

To determine whether ouabain-induced endocytosis also leads to increased degradation of Na/K-ATPase, LLC-PK1 cells and BT-20 cells were pretreated with cycloheximide, a protein synthesis inhibitor, for 1 h, and then exposed to 10 nm ouabain for 6, 16, and 24 h. As depicted in Fig. 6 (B and C), ouabain treatment produced more degradation of the α1 subunit in both BT-20 and LLC-PK1 cells.

Ouabain Activates Akt in LLC-PK1 but Not BT-20 and DU145 Cells—We and others have demonstrated that ouabain stimulates the PI3K/Akt/mTOR pathway. It is known that activation of mTOR could increase the translation of many mRNAs. Because ouabain did not increase α1 mRNA in LLC-PK1 cells, we tested whether ouabain-induced up-regulation of α1 is sensitive to inhibitors of PI3K and mTOR. As depicted in Fig. 7A, pretreatment of cells with either PI3K inhibitor LY294002 or mTOR inhibitor rapamycin completely abolished ouabain-induced up-regulation of α1 subunit. Interestingly, both LY294002 and rapamycin reduced the basal expression of α1. Moreover, in the presence of rapamycin, ouabain treatment actually resulted in a further decrease in the expression of α1. This is not surprising, because rapamycin could theoretically...
block ouabain-induced α1 expression but not ouabain-induced α1 endocytosis and degradation.

To test whether activation of the Akt/mTOR pathway is involved in ouabain-induced cell-specific regulation of α1 expression, we subsequently measured the effect of ouabain on Akt in three different cell lines. As shown in Fig. 7 (B and C), whereas ouabain stimulated Akt phosphorylation in LLC-PK1 cells, it failed to do so in both BT20 and DU145 cells. To be sure that the Akt pathway is intact in cancer cells, we exposed DU145 cells to IGF and measured for Akt activation. As shown in Fig. 7C, IGF caused a remarkable stimulation of Akt in DU145 cells. Consistently, when the same experiments were repeated in PY-17 cells, ouabain showed no effect on Akt phosphorylation (data not shown).

**Rapamycin Sensitizes LLC-PK1 Cells to Ouabain-induced Cell Growth Inhibition**—The above data indicate that activation of PI3K/Akt/mTOR is required for ouabain to increase the expression of α1. Because repletion of α1 is essential for ouabain-induced cell growth stimulation, we reasoned that inhibition of mTOR by rapamycin would convert ouabain-induced cell growth into growth inhibition in LLC-PK1 cells. Indeed, as shown in Fig. 7D, the addition of rapamycin was sufficient to block ouabain-induced cell proliferation in LLC-PK1 cells. Moreover, it also sensitized the cell to ouabain-induced cell growth inhibition. For example, 10 nM ouabain was sufficient to cause significant inhibition of cell proliferation in LLC-PK1 cells pretreated with the inhibitor.

**Involvement of Src and Caveolin-1**—
We have shown that the Na/K-ATPase signals through caveolae by forming a receptor complex with Src. To further understand the molecular mechanism of ouabain-induced up-regulation of α1, we first addressed the role of Src. SYF cells (in which the Src family kinases Src, Yes, and Fin were knocked out) were exposed to ouabain for 24 h. Because these cells were from mice and express a ouabain-resistant Na/K-ATPase, 0.5 and 1.0 μM ouabain were used in the experiments. As depicted in Fig. 8A, knock-out of Src completely blocked ouabain-induced α1 expression. Consistently, when SYF cells were rescued by c-Src, ouabain was able to cause a 3-fold induction of α1 expression.
To test the involvement of caveolin-1 and caveolae, we utilized caveolin-1-specific small interfering RNA and established a caveolin-1 knockdown LLC-PK1 cell line (C2-9 cells) that expressed about 20% of caveolin-1 in comparison with the parent LLC-PK1 cells (36). As shown in Fig. 8B, knockdown of caveolin-1 completely abolished ouabain-induced increases in α1 expression. Consistently, ouabain failed to stimulate Akt in these cells (Fig. 8C). Moreover, knockdown of caveolin-1 not only abolished ouabain-induced cell proliferation but also sensitized the cells to ouabain-induced cell growth inhibition (Fig. 8D).

DISCUSSION

We have made the following major observations in this report. First, the stimulatory or inhibitory effect of ouabain on cell proliferation depends on whether ouabain can activate the Akt/mTOR pathway and replete cellular Na/K-ATPase against ouabain-induced endocytosis and subsequent degradation of the enzyme. Second, inhibition of the Akt/mTOR pathway by rapamycin increases ouabain-induced depletion of Na/K-ATPase and sensitizes cells to ouabain-induced growth inhibition. Finally, reduction of cellular Na/K-ATPase by means other than ouabain can also stimulate the expression of cell cycle inhibitor p21cip, resulting in cell growth inhibition.

Although ouabain exhibited both stimulatory and inhibitory effects on cell proliferation in a cell-specific manner, the activation of proximal signaling events (e.g., Src and ERK) has been recorded in both types of cells (34). Thus, the ouabain-activated downstream events must significantly diverge in cells whose growth response to ouabain went in opposite directions. In accordance, we observed that ouabain stimulated the Akt/mTOR pathway, resulting in translational up-regulation of Na/K-ATPase and stimulation of cell proliferation in LLC-PK1 cells. In contrast, ouabain failed to activate the Akt/mTOR pathway and replete the Na/K-ATPase in human cancer cells such as BT20 and DU145 cells as well as in the Na/K-ATPase knockdown cells (Fig. 7). Interestingly, ouabain-induced activation of the PI3K/Akt/mTOR pathway has been reported in several cell lines and primary cultures (13, 15, 35). Consistently, this activation appears to be required for ouabain to stimulate growth in these cells (13).

It is important to note that our experiments were conducted in serum-free cultures. Because serum is required for promoting and maintaining normal cell growth, the observed ouabain effects on cell signaling and growth might be due to serum starvation-induced changes in cell cycle. Thus, future studies will be required to resolve whether ouabain affects cell signaling and growth differently when cells are in normal logarithmic growth with serum.

In many types of cells, binding of ouabain and other cardioactive steroids to the Na/K-ATPase is known to induce endocytosis and degradation of the enzyme (35, 53–55). Functionally, ouabain-induced endocytosis, even by low concentrations of ouabain, could lead to a significant reduction of the Na/K-ATPase over time if it is not compensated by up-regulation of Na/K-ATPase expression and cell growth regulation (34). Consistently, we found that blocking ouabain-induced
repletion of the Na/K-ATPase by rapamycin sensitized these cells to ouabain-induced cell growth inhibition. Moreover, we observed that knockdown of the Na/K-ATPase by small interfering RNA was equally effective in stimulating the expression of p21cip1 and inhibiting cell proliferation in LLC-PK1 cells. Taken together, it is clear that the amount of cellular Na/K-ATPase is important for cell growth control.

Needless to say, the molecular mechanism that prevents ouabain from stimulating the PI3K/Akt/mTOR pathway in BT20 and DU145 cells remains to be revealed. Nevertheless, it is important to discuss the potential role of caveolin-1, the Na/K-ATPase, and caveolae in this regulation. It is known that caveolae compartmentalize signaling events (56). Specifically, disruption of caveolae by depletion of either caveolin-1 or cholesterol can significantly alter insulin/IGF-induced activation of PI3K/Akt/mTOR pathways (57, 58). Interestingly, expression of caveolin-1 and/or Na/K-ATPase is significantly reduced in many cancer cell lines. Specifically, the expression of caveolin-1 is almost absent in BT20 cells, whereas expression of the Na/K-ATPase is significantly reduced in DU145 cells.

Because both caveolin-1 and Na/K-ATPase are important for the formation of caveolae (59), it is possible that reduction of either caveolin-1 or the Na/K-ATPase may contribute to the uncoupling of ouabain binding to the activation of PI3K/Akt/mTOR pathway in these cells. Consistently, as shown in Fig. 8, we found that knockdown of caveolin-1 indeed abolished ouabain-induced activation of Akt. Similarly, ouabain also failed to stimulate Akt in the Na/K-ATPase knockdown PY-17 cells.

A large increase in intracellular Na+ is known to stimulate transcription of α1 mRNA (60–62). This occurs when cells are exposed to low extracellular K+ or ouabain concentrations that produce more than 50% inhibition of the Na/K-ATPase (60–62). Apparently, this is unlikely the molecular mechanism by which 1–10 nM ouabain increased expression of the Na/K-ATPase in LLC-PK1 cells. Because both caveolin-1 and Na/K-ATPase are important for the formation of caveolae (59), it is possible that reduction of either caveolin-1 or the Na/K-ATPase may contribute to the uncoupling of ouabain binding to the activation of PI3K/Akt/mTOR pathway in these cells. Consistently, as shown in Fig. 8, we found that knockdown of caveolin-1 indeed abolished ouabain-induced activation of Akt. Similarly, ouabain also failed to stimulate Akt in the Na/K-ATPase knockdown PY-17 cells.

Epidemiological studies in the late 1970s and early 1980s indicated the potential benefits of using digitalis drugs in patients with breast cancer (26, 27). Phase I and II clinical trials are underway in the United States and in Europe to test several new digitalis compounds as anti-cancer drugs. Taking the liberty of extrapolating in vitro to in vivo conditions, our new findings suggest that expression of the Na/K-ATPase may be used to assess the effectiveness of digitalis therapy on certain cancers or individual patients. Moreover, they suggest the potential use of rapamycin in the digitalis therapy of cancer. To this end, it is of interest to note that rapamycin is currently in clinical trials as an anti-cancer agent. It remains to be tested...
whether rapamycin-induced down-regulation of the Na/K-ATPase contributes to its effectiveness as an anti-cancer drug.

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