Calreticulin Affects β-Catenin-associated Pathways*

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Calreticulin, a Ca\(^{2+}\) storage protein and chaperone in the endoplasmic reticulum, also modulates cell adhesiveness. Overexpression of calreticulin correlates with (i) increased cell adhesiveness, (ii) increased expression of N-cadherin and vinculin, and (iii) decreased protein phosphorylation on tyrosine. Among proteins that are dephosphorylated in cells that overexpress calreticulin is β-catenin, a structural component of cadherin-dependent adhesion complexes, a member of the armadillo family of proteins and a part of the Wnt signaling pathway. We postulate that the changes in cell adhesiveness may be due to calreticulin-mediated effects on a signaling pathway from the endoplasmic reticulum, which impinges on the Wnt signaling pathway via the cadherin/catenin protein system and involves changes in the activity of protein-tyrosine kinases and/or phosphatases.

The endoplasmic reticulum (ER)\(^1\) plays a critical role in the synthesis and chaperoning of membrane-associated proteins and secreted proteins (1). It is also an important site for the storage and release of Ca\(^{2+}\) (2). In fact, the ER is the major signal transducing organelle within the cell, continuously responding to extracellular stimuli by releasing Ca\(^{2+}\) (3, 4). The ER is also sensitive to intracellular signaling; in response to a variety of stimuli signals are transduced from the ER to the cytoplasm, the plasma membrane, and the nucleus, and they affect a variety of cellular activities including Ca\(^{2+}\) influx, protein folding, and synthesis, cholesterol synthesis, and transcription (5–8). Signaling from the extracellular space to the cell interior has long been the subject of intense research. In contrast, little is known about intracellular signaling from the ER. The lumen of the ER is a unique environment, which contains a high concentration of Ca\(^{2+}\)-binding chaperones (2). Calreticulin, a major Ca\(^{2+}\)-binding protein resident in the ER, is ubiquitous in eukaryotic cells (9). It functions as a molecular chaperone (10, 11) and also participates in ER-dependent Ca\(^{2+}\) homeostasis (2, 9). Calreticulin also affects processes external to the ER, such as steroid-sensitive gene expression and cell adhesiveness (12). Although the Ca\(^{2+}\) storage and chaperone functions of calreticulin are consistent with its localization to the lumen of the ER and with its structure, it is not obvious how it can affect cell adhesiveness at the level of the plasma membrane. To investigate the mechanisms behind calreticulin-dependent modulation of cell adhesiveness, we used well characterized mouse L fibroblasts differentially expressing calreticulin (13–15). Here we show that stable overexpression of full-length, ER-targeted calreticulin correlates with an increased adhesiveness in transformed fibroblasts, such that their cohesion resembles that of epithelial cells in culture. We also show that changes in the expression of calreticulin affect the tyrosine phosphorylation of cellular proteins, including β-catenin. β-Catenin is a structural component of cadherin-mediated adhesion complexes and is also a part of the Wnt signaling pathway (16). We found that calreticulin, from the lumen of the ER, influences tyrosine phosphorylation of β-catenin, resulting in the modulation of cell adhesiveness. We suggest that calreticulin may play a role in a signaling pathway from the ER (8), involving protein-tyrosine kinases and/or phosphatases.

EXPERIMENTAL PROCEDURES

Stably transfected lines of mouse L fibroblasts were generated as previously described (13). Briefly, to construct the calreticulin expression vector, the DraI/Smal restriction DNA fragment (nucleotides 20–1653) of pcDx-calreticulin (GenBank™ accession number J05138) was first inserted into the Smal-digested pSVL vector to generate pSchr-1. Next, the full-length calreticulin cDNA was excised from the pSchr-1 and cloned into the XbaI site of the pRe/CMV vector. Two constructs were generated and designated pRcR-DT-1 and pRcR-DT-2, which contain calreticulin cDNA in the sense and antisense orientations, respectively (13). These vectors were used to stably transfect L fibroblasts.

For transfections, all plasmids were purified using Mega-plasmid preparation and Qiagen columns as recommended by the manufacturer. Mouse L fibroblasts were transfected with 20 mg of pRcR-DT-1, pRcR-DT-2, or pRe/CMV plasmid, by electroporation (1500 V/cm, 25 millifarad). The cells were then selected for resistance to genetin (200 mg/ml) for 14 days. The clones obtained were then screened for expression of calreticulin. Two cell lines expressed elevated (~2.0-fold, referred to as calreticulin overexpressors) and reduced (0.5-fold, referred to as calreticulin underexpressers) levels of calreticulin as determined by Western blot analyses (see Fig. IA). These two cell lines, together with a mock-transfected L fibroblast cell line (transfected with pRe/CMV vector and referred to as control) were recloned by a limiting dilution method and used in the present report. The cells were grown in high glucose Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (Life Technologies, Inc.) and genetin (Sigma)

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as previously described (15).

For aggregation assays, 10⁶ cells were plated in tissue culture dishes (100-mm diameter) and grown for 72 h. To prepare single cell suspensions, the cells were detached from the plates by treatment with EDTA-free trypsin, followed by scrubbing with a rubber policeman and pipetting several times. Trypsin was inactivated by adding fetal bovine serum, and the cells were then centrifuged, resuspended in medium, and plated in bacteriological dishes. The dishes were shaken for 48 h on an orbital shaker in a cell culture incubator (5% CO₂, 37 °C) at a constant speed of 50 cycles/min. The cell aggregates were then sized and counted.

For motility assays, cells were plated at a density of ~250,000 cells/60-mm cell culture dish. Time lapse recording, cell tracking, average cell velocity calculations, and morphometry were done as described previously (15). Wound closure rates were calculated as the time required for cells to cover a 3-mm-wide wound in cell monolayer.

For the induction of calreticulin with thapsigargin (Sigma), cells were plated at a density of 2 × 10⁵/3.5-cm dish and allowed to attach overnight. After changing the medium, the cells were incubated with thapsigargin (100 nM or 1 μM) for 16 h.

Semi-quantitative reverse transcription-PCR was carried out as follows. The cDNA was synthesized with a first strand cDNA synthesis kit (Amersham Pharmacia Biotech), using duplicate samples of 200 ng of total RNA and random hexamers. The first strand reactions were diluted to a volume of 200 μl with nuclease-free water (Promega). 3 μl of the cDNA solution was used in the subsequent PCR. Each PCR reaction was performed in 50 μl of mix Tris, pH 8.3, with 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM dNTPs, 1 μCi of [α-³²P]dCTP (DuPont Canada Inc.), and 250 ng of the appropriate primer, both forward and reverse. The following N-cadherin-specific primers were used: N-cadherin forward, 5'-CAAGAGCTTGTCAGAATCAGG-3' (nucleotides 1209–1227); hypoxanthine phosphoribosyltransferase forward, 5'-CCTGCTGGAT- TACATTAAAGGCATTG-3' (nucleotides 16–340); and hypoxanthine phosphoribosyltransferase reverse, 5'-GTTCAGGGCATTCGCAAAC-3' (nucleotides 643–667). The PCR was carried out by 30 cycles of preheating at 99 °C for 30 s, annealing at 55–60 °C for 45 s, and extending at 72 °C for 1 min. The PCR products were resolved by polyacrylamide gel electrophoresis (6% acrylamide). The gels were dried and exposed to a storage phosphor screen (Molecular Dynamics).

The antibodies used for immunodetection were as follows. A well-characterized goat polyclonal anti-calreticulin antibody (18) was used to detect calreticulin and a rabbit polyclonal antibody against C-terminal amino acids of chick N-cadherin (“pan-cadherin” from Sigma) was used to detect N-cadherin (19). Anti-α1, and anti-β1, integrin was from Santa Cruz (Santa Cruz, Calif.) and anti-α3, and anti-β3, integrins were from Dr. M. Ginsberg (Scripps Research Institute), and anti-α1- and anti-β1, integrins were from Dr. B. Chan (University of Western Ontario). Mouse monoclonal antibodies against phosphophoryn (clone PY20, Santa Cruz), α-catenin and pp125 focal adhesion kinase (Transduction Laboratories), β-catenin (Sigma), vinculin (ICN ImmunoBiologicals, Montreal, Canada), talin (Sigma), and rabbit polyclonal antibodies against α-smooth were used to detect their respective proteins. The rabbit anti-calnexin antibodies were purchased from StressGen. All secondary antibodies were from Jackson Immunoresearch Laboratories (West Grove, PA). For Western blotting, cells were homogenized in ice-cold lysis buffer (50 mM Tris-HCl, 120 mM NaCl, 0.5% Nonidet P-40, pH 8.0), boiled for 5 min, subjected to SDS-polyacrylamide gel electrophoresis, and then transferred to nitrocellulose membranes. Immunoprecipitations were performed on cellular lysates before SDS-polyacrylamide gel electrophoresis, as described by Ozawa and Kemler (20). Nitrocellulose membranes were incubated with primary antibodies for 1 h at room temperature followed by incubation with appropriate horseradish peroxidase-conjugated secondary antibodies (Jackson Immunoresearch). Immunoreactive bands were detected using enhanced chemiluminescence (Amersham Pharmacia Biotech). The nitrocellulose membranes were stripped by a 30-min incubation in a solution of 100 mM 2-mercaptoethanol, 2% SDS, and 62.5 mM Tris-HCl, pH 6.7, at 50 °C. The protein bands in each blot were scanned using a densitometer, and the areas under the curves were calculated using Gel Scan XL software. The mean and standard error of samples for each protein from each cell line was calculated, and the levels from each sample were compared by analysis of variance.

RESULTS

Western blotting (Fig. 1A) showed that calreticulin overexpressers expressed ~2.0-fold elevated levels of calreticulin, whereas calreticulin underexpressers expressed 0.5-fold reduced levels of calreticulin, compared with control, mock-transfected L fibroblasts. Functionally, in a single cell suspension under constant shear stress, cells overexpressing calreticulin aggregated more efficiently than either cells underexpressing underexpressers (under) or control cells (mock transfectants, control). Shown is the number of aggregates with a diameter of 50–110 μm formed from a single cell suspension (10⁶ cells) shaken for 48 h. Similar distributions were observed for aggregates sized 110–220 and 220–1100 μm (not shown). The data shown are the means ± S.E. C, in an in vitro scrape wound closure assay calreticulin overexpressers (over) form a cell sheet and maintain it throughout the experiment. Calreticulin underexpressers (under) cover the wound but maintain fairly substantial distance between adjacent cells. The images shown are taken after 2 and 17 h of recording.

Confocal microscopy was carried out as previously described (15) on a Bio-Rad MRC-600 confocal fluorescence microscope.

FIG. 1. A, Western blotting shows that the total amount of calreticulin is reduced in L fibroblasts underexpressing calreticulin (under) and elevated in L fibroblasts overexpressing calreticulin (over) in comparison with the control cell line (control). B, L fibroblasts overexpressing calreticulin (over) aggregate more efficiently than either calreticulin underexpressers (under) or control cells (mock transfectants, control). Shown is the number of aggregates with a diameter of 50–110 μm formed from a single cell suspension (10⁶ cells) shaken for 48 h. Similar distributions were observed for aggregates sized 110–220 and 220–1100 μm (not shown). The data shown are the means ± S.E. C, in an in vitro scrape wound closure assay calreticulin overexpressers (over) form a cell sheet and maintain it throughout the experiment. Calreticulin underexpressers (under) cover the wound but maintain fairly substantial distance between adjacent cells. The images shown are taken after 2 and 17 h of recording.
was detected between these cell lines. In an in vitro scrape wound closure assay, calreticulin overexpressers closed a 3-mm-wide wound in less than 72 h, which is 2.5 times faster than the underexpressers (170–190 h) and ~2 times faster than the control mock-transfected cells (120–160 h). Importantly, in this assay, calreticulin overexpressers migrated like an epithelial sheet, maintaining intercellular contacts and a well defined leading edge of cell monolayer, whereas calreticulin underexpressers dissociated early from the wound edge and migrated independently (Fig. 1C). In comparison, both wild type (parent) and control cells exhibited intermediate monolayer migration patterns. Thus, phenomenologically, changes in cell adhesiveness parallel alterations in the level of expression of calreticulin.

Reverse transcription-PCR established that it is N-cadherin that is likely the cell surface protein responsible for the increased intercellular adhesiveness of L fibroblasts overexpressing calreticulin. Semi-quantitative Western blot and reverse transcription-PCR analysis showed that amounts of the protein N-cadherin and its mRNA were elevated in calreticulin overexpressers, ~2-fold and ~12-fold, respectively (Fig. 2, A and B). The Western blot and RT-PCR data were further supported by immunofluorescence labeling of cells with anti-cadherin antibodies (Fig. 2, C–F). Calreticulin underexpressers did not attach to each other, had few cadherin-positive intercellular junctions, and showed a tendency to multilayer. In contrast, calreticulin overexpressers flattened out and, by developing abundant cadherin-rich intercellular junctions, formed a flat monolayer that closely resembled an epithelial cell sheet. The formation of a monolayer by calreticulin overexpressers is especially evident with optical sectioning along the optical axis of the microscope (XZ sectioning). Control cells formed scattered cadherin-positive intercellular contacts. Cadherin-positive intercellular contacts were also prominent in an optical cross-section of an aggregate of calreticulin overexpressers (Fig. 2F).

With Western blotting we detected no changes in the abundance of actin, α, and β integrins (i.e. fibronectin receptor), αv and β3 integrins (i.e. vitronectin receptor), pp125 focal adhesion kinase, talin, α-actinin, and α- and β-catenins (Fig. 3B). It should be noted that, in our L fibroblast cell lines, the expression level of α, β integrin was very low compared with that of αv, β3 integrin. In keeping with earlier findings (15, 21), the amounts of vinculin and its mRNA were elevated in calreticulin overexpressers and reduced in calreticulin underexpressers (Fig. 3A). The induction of vinculin in the calreticulin overexpressers was accompanied by its translocation to junctional areas when the cells packed to form cell sheets (not shown).

Finally, using Western blotting we detected no changes in the expression of SERCA2, the inositol 1,4,5-triphosphate receptor (not shown), immunoglobulin-binding protein, calnexin, ERP57, ERP72, GRP 94, and protein-disulfide isomerase (PDI).
Calreticulin and Cell-Cell Adhesion

Fig. 4. A, Western blotting shows that the total amount of tyrosine phosphorylated proteins is elevated in cells underexpressing calreticulin (under) and reduced in cells overexpressing calreticulin (over) in comparison to the control cell line (control). The positions of molecular marker proteins are indicated. B, incubation of L fibroblasts with 100 nM thapsigargin (TG) for 16 h is accompanied by an increase in calreticulin abundance. After stripping the nitrocellulose membrane, Western blotting with an anti-phosphotyrosine antibody reveals a decrease in the total amount of tyrosine phosphorylated proteins in thapsigargin-treated cells. C, Western blotting with an anti-phosphotyrosine antibody shows that phosphotyrosine content of β-catenin immunoprecipitated (IP) from cells overexpressing (over) calreticulin is dramatically reduced in comparison to either calreticulin underexpressers (under) or control cells (control). Western blotting with an anti-β-catenin antibody shows that the abundance of β-catenin does not change in these cell lines. D, Western blotting with antibodies to β-catenin after immunoprecipitation with an anti-phosphotyrosine antibody shows a decrease in amount of β-catenin in immunoprecipitates from extracts of thapsigargin-treated (1 μM) cells.

In adherens-type junctional complexes cadherins link to the actin cytoskeleton via proteins of the catenin family (27–29). It appears that both the localization and function of β-catenin depend on its degree of tyrosine phosphorylation (30, 31), although the mechanisms are still unclear (32). To determine whether the tyrosine phosphorylation of β-catenin is affected by overexpression of calreticulin, we immunoprecipitated β-catenin from cell extracts and assessed its phosphotyrosine content by Western blotting with anti-phosphotyrosine antibodies. In calreticulin underexpressers, the phosphotyrosine content of β-catenin was readily detected, whereas in calreticulin overexpressers the phosphotyrosine content of β-catenin was below detection level (Fig. 4C). To address the question of whether induction of calreticulin by the ER stress will also affect the phosphotyrosine content of β-catenin, we immunoprecipitated phosphotyrosine-containing proteins from extracts of thapsigargin-treated cells. Western blotting with antibodies to β-catenin (Fig. 4D) shows a decrease in amount of PY-20-precipitable β-catenin from thapsigargin-treated cell extracts. Importantly, using immunofluorescence microscopy (Fig. 5), we found that β-catenin, albeit barely detectable in all the cell lines, could be found in patches localized to areas of cell-cell contact in cells overexpressing calreticulin but not in the underexpressers and control cells.

As previously reported (15), subconfluent calreticulin overexpressers were more spread than the other cell types (Fig. 5). Next, we asked whether a change in the level of tyrosine phosphorylation of cellular proteins could itself affect the degree of cell spreading. Fig. 6 shows immunolocalization of phosphotyrosine in of calreticulin underexpressers (A, B, and C), overexpressers (A′, B′, and C′), and control cells (A″, B″, and C″). Row A shows untreated cultures, row B shows cultures treated with pervanadate (vanadate/H2O2) to inhibit tyrosine phosphatases, and row C shows cultures treated with herbimycin A to inhibit tyrosine kinases. The phosphotyrosine signal in the underexpressers was mostly cytoplasmic and strong (Fig. 6A), whereas in the overexpressers it was confined to focal adhesions and weak (Fig. 6A′). Herbimycin A treatment lowered the cytosolic level of phosphotyrosine in all cell lines and caused all cells to spread more. Conversely, pervanadate treatment elevated the cytosolic level of phosphotyrosine in all cell lines and caused the cells to retract thus making control cells (Fig. 6B″) to look like the untreated calreticulin underexpressers shown in (Fig. 6A). Treatment of cells with Tyrphostin A25, an inhibitor more specific for receptor-type protein-tyrosine phosphatase, resulted in a significant decrease in the phosphotyrosine signal in all cell lines. Western blotting with anti-phosphotyrosine antibodies revealed a decrease in the amount of phosphorylated proteins in cells treated with herbimycin A, consistent with the decreased phosphotyrosine signal seen in the immunofluorescence experiments.
kinases (33), was less effective in induction of cell spreading (not shown). Morphometric analysis of the degree of spreading of treated cells is shown in Fig. 6D.

DISCUSSION

In this study we have shown that increased expression of calreticulin, a Ca$^{2+}$-binding chaperone resident in the lumen of the ER, correlates with an increase in cell adhesiveness. When overexpressing calreticulin, fibroblasts (i) aggregate effectively at a shear stress, which prevents aggregation in other cell lines, and (ii) when released from contact inhibition display a pattern of mobility resembling that of epithelia. Further, cells overexpressing calreticulin have significantly decreased tyrosine phosphorylation of cellular proteins including $\beta$-catenin, a component of cadherin-based junctional complexes. Our results indicate that calreticulin can affect cell adhesiveness via alterations in tyrosine phosphorylation. Because calreticulin is resident in the lumen of the ER, its modulation of cell adhesion must involve signaling from the ER. This signaling could occur via multiple pathways, including activation of gene expression (15, 21, 34) and alteration of tyrosine phosphorylation (Ref. 21 and this work).

Protein phosphorylation on tyrosine plays a significant role in cell adhesion (35, 36). Therefore, calreticulin-dependent alteration of tyrosine phosphorylation may represent a novel pathway via which the ER signals to the plasma membrane to modulate cell adhesiveness. In this study, we have shown that the reduced phosphorylation of tyrosine in cells that overexpress calreticulin is not an artifact of transfection, because a similar reduction is seen in untransfected parental cells (Fig. 1E) and in NIH3T3 cells (not shown) when calreticulin expres-
sion is induced by exposure to thapsigargin (22). This conclusion is further supported by the observation that, in a Tet-on inducible HeLa cell line (in which a calreticulin gene is controlled by a promoter dependent on doxycycline-controlled transactivator), increased expression of calreticulin caused a similar decrease in tyrosine phosphorylation of proteins (not shown). Also, we have recently shown that retinal pigmented epithelial cells, which overexpressed calreticulin, had lower levels of tyrosine phosphorylation, compared with calreticulin underexpressing cells (21). Although the mechanisms are still unclear, protein phosphorylation on appears to regulate the stability and, possibly, function, of cell adhesions (31, 32). It must be stressed, however, that the effects of tyrosine phosphorylation on junctional function are very dynamic, may be transient, and are often cell type-dependent (37–40). The majority of work regarding effects of tyrosine phosphorylation utilized cells commencing spreading or recovering from starvation. A few reports that used well spread nonstarved cells focused on stability and phosphorylation status of focal adhesions and suggest that inhibitors of tyrosine kinases and phosphatases affect most effectively adhesions in statu nascendi, whereas mature adhesions are either less sensitive or insensitive to those inhibitors (41–44). Here we show that well spread, nonstarved L fibroblasts treated with inhibitors of tyrosine kinases and phosphatases undergo dramatic changes in shape and degree of spreading. Interestingly, these changes further enhance the differences that exist between the cell lines differentially expressing calreticulin such that cells that overexpress calreticulin are well spread and have less phosphotyrosine spread even more after inhibition of tyrosine kinases and vice versa.

The reduced phosphorylation of tyrosine of cellular proteins in general, and β-catenin in particular, observed in cells that overexpress calreticulin can be also achieved by induction of the ER stress. The calreticulin-dependent effect, however, is unlikely to be a part of a simple stress response, because the 2-fold overexpression of calreticulin is not sufficient to cause ER overload and is not accompanied by other changes considered typical of a stressed ER (5–8). The transcription factor NF-κB, which is activated by release of Ca\(^{2+}\) from the stressed ER (45), is not affected by changes in expression of calreticulin (not shown). These observations suggest that calreticulin-dependent modulation of cell adhesiveness involves pathways related by other than the ER signaling pathways that have already been described (5–8). Calreticulin is a relatively unique ER luminal protein. It not only binds Ca\(^{2+}\) but also interacts with other chaperones in a Ca\(^{2+}\)-dependent way, likely regulated by Ca\(^{2+}\) binding to calreticulin (46–48). It is also as the only known soluble chaperone with a lectin-like activity (9, 10). Collectively, these unique properties of calreticulin support our hypothesis that changes in tyrosine phosphorylation possibly represent a new, calreticulin-dependent pathway for ER signaling.

Recent evidence indicates that calreticulin may affect gene expression (15, 21, 22, 25, 26). In keeping with this suggestion, we have shown that cells that overexpress calreticulin also have elevated expression of N-cadherin and vinculin and their respective mRNAs (15, 21). However, we did not detect changes in the expression of several other adhesion-related proteins, including actin, talin, α- and β-catenin, α\(_5\)β\(_1\) and α\(_6\)β\(_3\) integrins, and pp125 focal adhesion kinase (this report and Refs. 15 and 21). Although the involvement of other, as yet unidentified, proteins cannot be excluded, the observed changes in expression of N-cadherin and vinculin are sufficient to account for the changes in adhesion that we observed in these fibroblasts (49, 50). Overall, our results indicate that calreticulin, from within the lumen of the ER, affects transcriptional control of selected genes involved in cell adhesion (N-cadherin and vinculin).

The most important finding of this study is that β-catenin is underphosphorylated in cells that overexpress calreticulin. The level of tyrosine phosphorylation of β-catenin affects its ability to interact with other members of the cadherin-based junctional complexes (20, 51–54). For example, when dephosphorylated on tyrosine, β-catenin is stabilized within junctional complexes where it may participate in linking junctional components to the cytoskeleton (55, 56). In contrast, when phosphorylated on tyrosine, β-catenin displaces to the cytoplasm, where it may bind lymphocyte enhancer factor/T cell factor transcription factors and translocate to the nucleus (29, 57, 58). The affinity of β-catenin for E-cadherin is reduced over 5-fold after β-catenin has been phosphorylated on tyrosine-645 (59). It has been shown, however, that v-Src regulates E-cadherin-mediated adhesion in the absence of β-catenin (60). On the other hand, other kinases and/or phosphatases (52–54, 61, 62) may use β-catenin as a substrate. The precise role of tyrosine phosphorylation in general and that of β-catenin in particular in stability and function of cell junctions have been unclear and controversial (see Refs. 31, 32, and 63 for reviews). In the case of calreticulin overexpressers, they exhibit increased adhesiveness, and they also show reduced tyrosine phosphorylation of β-catenin, which is properly localized to cell-cell junctions. Importantly, the level of expression of β-catenin is not altered in calreticulinoverexpressers. Our results indicate that calreticulin-dependent effects on cell adhesion may not occur via transcriptional regulation of β-catenin but via changes in tyrosine kinase/phosphatase pathway(s). Calreticulin may affect the phosphorylation status of β-catenin by either inhibition of specific phosphotyrosine kinase(s) or activation of phosphotyrosine phosphatase(s). The change in phosphorylation of β-catenin is likely to affect the proportions of complexed and free protein, and hence it is likely to impinge further down in the Wnt signaling pathway (29, 58). Preliminary experiments indicate that calreticulin overexpressers have up-regulated levels of mRNA encoding protein phosphatase 2C.2 This finding suggests that expression of protein phosphatase 2C may be regulated in these cells and may be responsible for calreticulin-dependent ER signaling as this enzyme participates in Wnt signaling (64). Changes in the expression of calreticulin appear to affects tyrosine-dependent phosphorylation of cytoplasmic, adhesion related molecules. Consequently, we infer that signaling enzymes involved must be distinct from serine/threonine such as IRE and/or PERK. It is likely that calreticulin, because of its unique properties, participates in activation of as yet undescribed ER-dependent signaling pathway or a new branch of a described pathway, the elucidation of which awaits further experimentation.

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