Specific Substitutions at Amino Acid 256 of the Sarcoplasmic/Endoplasmic Reticulum Ca\textsuperscript{2+} Transport ATPase Mediate Resistance to Thapsigargin in Thapsigargin-resistant Hamster Cells\textsuperscript{*}

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High levels of resistance to thapsigargin (TG), a specific inhibitor of intracellular Ca\textsuperscript{2+} transport ATPases (SERCAs), can be developed in culture by stepwise exposure of mammalian cells to increasing concentrations of TG. We have identified, in two independently selected TG-resistant hamster cell lines of different lineages, mutant forms of SERCA. In the TG-resistant Chinese hamster lung fibroblast cell line DC-3F/TG, a T → C change at nucleotide 766 introduces a Phe\textsuperscript{256} → Leu alteration within the first cytosolic loop of the SERCA. In contrast, in the TG-resistant Syrian hamster smooth muscle cell line DDT/TG 4\muM, a T → C change at nucleotide 767 introduces a Phe\textsuperscript{256} → Ser mutation at that position. When these specific mutations are introduced into a wild-type full-length avian SERCA1 cDNA, transfection experiments reveal that Ca\textsuperscript{2+} transport function and ATP hydrolytic activity are not altered by such mutations. However, a 4–5-fold resistance to TG inhibition of ATPases (SERCAs)\textsuperscript{1} are intracellular Ca\textsuperscript{2+} pumps that play a central role in Ca\textsuperscript{2+} homeostasis. Several inhibitors of the SERCAs have been described, of which thapsigargin (TG) is the most potent and specific (1). By inhibiting SERCA function, TG depletes intracellular Ca\textsuperscript{2+} stores, resulting in inhibition of cell proliferation. However, high levels of resistance to TG inhibition of cell proliferation can be developed (2, 3). Several mechanisms that contribute to the increased production of SERCA protein become operative upon the selection of TG-resistant cells.\textsuperscript{2} In addition, resistance to TG can be associated with overexpression of the multidrug resistance transporter P-glycoprotein (Pgp) (2).\textsuperscript{3} Although increased expression of SERCA or Pgp may contribute to TG resistance, our previous studies suggest that a SERCA(s) that is directly resistant to TG inhibition can also be selected for during development of the TG-resistant phenotype (3). To determine whether altered forms of SERCA do in fact occur during TG selection and potentially contribute to TG resistance, we have begun to study the SERCAs from the TG-resistant cells.

Although TG affects SERCA function rather globally, the sites of interaction between TG and SERCA have not been clearly defined. Chimeric recombinations between SERCA1 and Na\textsuperscript{+},K\textsuperscript{+}-ATPase demonstrate that TG does not bind within the catalytic domain (i.e. the large cytosolic loop) of the ATPase (5). More recently, studies suggest that binding to TG may occur within the M3 transmembrane domain of SERCA (6). Hence, our initial efforts have been to analyze the 5' ends of SERCA, which encompass the M3 domain, from the TG-resistant cell lines. In this report, we demonstrate that substitutions at amino acid (aa) position 256 occur within the SERCAs obtained from two independently derived TG-resistant cell lines. The mutant ATPases contain either a Phe\textsuperscript{256} → Leu or a Phe\textsuperscript{256} → Ser mutation. Moreover, when either of these two specific mutations are introduced into wild-type (wt) SERCA1, both Ca\textsuperscript{2+} transport and ATP hydrolytic activities of the resulting ATPase become resistant to inhibition by TG. No mutations occur within the M3 domain (i.e. the putative TG binding site) of the TG-resistant cell derived SERCAs. This is the first demonstration of naturally occurring mutations within SERCA during development of the TG-resistant phenotype, and our results suggest that Phe\textsuperscript{256} which lies within the first cytosolic loop just upstream of the M3 domain, represents a "hot spot" for mutations upon TG selection, and is potentially involved in TG-SERCA interactions.

EXPERIMENTAL PROCEDURES

**Cell Lines**—The Chinese hamster lung fibroblast cell line DC-3F has been described previously (7). The TG-resistant cell line DC-3F/TG was derived from DC-3F cells by stepwise selection in TG, with the final

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\textsuperscript{2} The abbreviations used are: SERCA, sarcoplasmic/endoplasmic reticulum Ca\textsuperscript{2+} transport ATPase; TG, thapsigargin; Pgp, P-glycoprotein; PCR, polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; nt, nucleotide(s); bp, base pair(s); aa, amino acid(s); wt, wild-type; ASO, allele-specific oligonucleotide; MTX, methotrexate; DHFR, dihydrofolate reductase.

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The resulting pCDL-SR cDNA, and sequenced to ensure fidelity of the PCR amplification step. With appropriately designed primers, 720-bp PCR to introduce mutations at amino acid 256 in the avian fast muscle combination with primer 2.3, to RT-PCR amplify SERCA2 from DDT 1-2.4, corresponding to nt 936–953) (Table I) were used to RT-PCR 2.3, corresponding to nt 104–121) and 3 determined using the dideoxy chain termination method. Based on SERCA cDNA probe (11). The sequences of five positive clones were ligated were selected, and their sequence obtained by dideoxy sequenc-1g using T7 and T3 primers.

Cloning and Sequencing of cDNA Fragments—Based on the published rat SERCA2a cDNA sequence (9), 5’ and 3’ degenerate primers, designated 2.1 and 2.2, respectively, were designed between nucleotide (nt) 1–15 and nt 976–992, respectively (Table I). Total RNA isolated from DC-3F cells was reverse-transcribed (RT) using oligo(dT)12-15 primer, the double-stranded cDNA amplified by polymerase chain reaction (PCR) using primers 2.1 and 2.2, and the resulting 992-bp PCR product cloned into pBluescript SK-. Nicotellusose replica filters were screened as per Grunstein’s method (10) using radiolabeled avian SERCA cDNA probe (11). The sequences of five positive clones were determined using the dideoxy chain termination method. Based on these sequence data, hamster-specific 5’ forward primer (designated 2.3, corresponding to nt 104–121) and 3’ reverse primer (designated 2.4, corresponding to nt 936–953) (Table I) were used to RT-PCR amplify 580-bp 5’ end SERCA2 cDNA fragments from DC-3F/TG, DDT1-MF2, and DDT/TG 4 μm cells. The PCR products were separately ligated into pBluescript SK-, several independent clones from each ligation were selected, and their sequence obtained by dideoxy sequenc-1xing using T7 and T3 primers.

Allele-specific Oligonucleotide (ASO)-based PCR—Two antisense primers (designated 2.16 and 2.17) that are complementary to the DC-3F SERCA2a cDNA sequence between nt 766 and 782 were designed. Primers 2.16 and 2.17 are identical except at nt position 766 (Table I). Primer 2.16 is complementary to wt SERCA2 cDNA encoding Phe256, while primer 2.17 is complementary to mutant SERCA2 cDNA encoding Leu256. Each antisense primer (2.16 or 2.17) was used in combination with the sense primer 2.3 (Table I) to amplify SERCA2 cDNA using RT-PCR from DC-3F or DC-3F/TG cells (12). The PCR parameters consisted of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, extension at 72 °C for 1.5 min for 40 cycles. Two other 17-mer primers (2.27 and 2.28) (Table I) that are complementary to the DDT1-MF2, DDT/TG 4 μm SERCA2 cDNA sequence between nt 767 and 783, were used in combination with primer 2.3, to RT-PCR amplify SERCA2 from DDT1-MF2 and DDT/TG 4 μm cells under the above conditions. Primer 2.27 is complementary to wt SERCA2 cDNA encoding Phe256, and primer 2.28 is complementary to mutant SERCA2 cDNA encoding Ser256.

In Vitro Mutagenesis by PCR—Overlap extension PCR (13) was used to introduce mutations at amino acid 256 in the avian fast muscle SERCA1 cDNA (11). With appropriately designed primers, 720-bp PCR products, containing the desired mutations within the body of the amplified products as well as appropriate restriction site overhangs, were obtained using SERCA1 cDNA template. The PCR products were exchanged with the corresponding fragments from the wt SERCA1 cDNA, and sequenced to ensure fidelity of the PCR amplification step. The resulting plasmids were cotransfected into COS1 cells together with the wt SERCA1 cDNAs, were transfected as described previously (5, 14). As described by Zhang et al. (15), a c-myc tag at the 3′ terminus allowed assessment of SERCA1 expression after transfection.

Transfection, Microsome Preparation, and Immunodetection—Trans- sient transfection into monkey kidney COS1 cells was carried out using the DEAE-dextran method as detailed previously (5, 14). For each experiment, 20 plates (150 × 25 mm of logarithmically growing COS1 cells were used. Preparation of microsomes from the transfected cell line DDT 1-MF2 (wild-type) 3543 was as described by Zhang et al. (15). The monoclonal antibody 9E10 to the c-myc epitope (16) was used to detect SERCA1 expression in the transfected COS1 cells via Western blotting.

Functional Assays—Ca2+ transport activity of the microsomal fractions was determined as described previously (3). ATPase activity was measured by the amount of P i released upon the addition of ATP to the microsomal fraction (15). Both Ca2+ transport and ATPase activities were normalized by the amount of expressed SERCA1 protein in the microsomal fractions obtained by Western blotting.

RESULTS

Identification of Amino Acid Substitutions at aa 256 of SERCA in the TG-resistant Cell Lines—Although cDNAs encoding the different isoforms of SERCA have been cloned from many species, the hamster SERCAs have yet to be cloned. We therefore used 5’ and 3’ degenerate oligonucleotide primers, corresponding to the published rat SERCA2a cDNA sequence, to clone a ~1-kilobase pair 5’ end SERCA2 cDNA from Chinese hamster DC-3F cells. Six different clones, obtained from two independent RT-PCR reactions, have been sequenced (data not shown). Based on these sequence data, hamster-specific 5’ and 3’ oligonucleotide primers were used to clone 850-bp 5’ end cDNA fragments of SERCA2 from the wt DDT1-MF2 cells, as well as the TG-resistant DC-3F/TG and DDT/TG 4 μm cells. To obtain sequence information of the translation start site region of hamster SERCA2, two additional primers were used to clone a 440-bp overlapping cDNA fragment encompassing the SERCA translation start site from the hamster cells. In the sequenced 5’ end of the molecule, the hamster and rat SERCA2 are identical at the protein level, although several nt changes between the two are noted. Nucleotide changes in SERCA are also noted to occur among different strains within the same species (i.e. between DC-3F and DDT1-MF2 SERCA).

Sequencing several independent clones from DC-3F/TG cells (which were obtained via separate RT-PCR reactions) reveals that the sequences of the clones are identical to that of wt DC-3F cells except at nt 766 (Table II). A T → C change at nt 766 occurs in five out of the eight clones, which predicts for a Phe256 → Leu change at the protein level. Since the clones were obtained from separate RT-PCR reactions, it is unlikely that this change represents a PCR artifact (see also below). That some clones have the wt phenotype and others the mutant phenotype suggests that the DC-3F/TG cell lines might represent a mixed population of cells, with some cells expressing the wt and others the mutant SERCA. The possibility also exists that the resistant cells represent a homogenous clone population, with the SERCA allele being heterogeneous with respect to nt 766 (i.e. aa 256). This is suggested by the fact that in clonally derived DC-3F/TG cell lines, both wt and mutant SERCA are expressed (see below).

With respect to the DDT/TG 4 μm cell line, 12 independent SERCA clones, obtained from three separate RT-PCR reac-
demonstrates that the SERCA allele is heterozygous with respect to the positions 766 and 782 are depicted, along with the antisense primers, 16 and 17 in parentheses. Note the T→C change at position 766 between the wt and mutant sequence. B. PCR products obtained from reverse-transcribed total RNA are run on 2% agarose gels. DC-3F, lanes 1–3: lane 1, primer pair 2.3 and 2.4; lane 2, primer pair 2.3 and 2.16; lane 3, primer pair 2.3 and 2.17. DC-3F/TG, lanes 4–6: lane 4, primer pair 2.3 and 2.4; lane 5, primer pair 2.3 and 2.16; lane 6, primer pair 2.3 and 2.17. Note that the forward primer 2.3 (nt 104–121) plus the reverse primer 2.4 (nt 936–953) amplify an 850-bp fragment from either cell line, primer 2.3 plus reverse primer 2.16 (nt 766–782) amplify a 679-bp fragment from DC-3F cells, while both primer pairs 2.3 plus 2.16 and 2.3 plus 2.17 amplify a 679-bp fragment from DC-3F/TG cells. C, three independent clonal sublines were derived, by limiting dilution, from the DC-3F/TG mass population of cells depicted in lanes 4–6 of B. Lanes 1 and 2, subclone 1 (lane 1, primers 2.3 plus 2.16; lane 2, primers 2.3 plus 2.17); lanes 3 and 4, subclone 2 (lane 3, primers 2.3 plus 2.16; lane 4, primers 2.3 plus 2.17); lanes 5 and 6, subclone 3 (lane 5, primers 2.3 plus 2.16; lane 6, primers 2.3 plus 2.17); lanes 7 and 8, parental DC-3F cells (lane 7, primers 2.3 plus 2.16; lane 8, primers 2.3 plus 2.17). In all three subclones, SERCA is mutated at nt 766 (lanes 2, 4, and 6). The presence of wt SERCA (lanes 1, 3, and 5) in each subclone demonstrates that the SERCA allele is heterozygous with respect to the mutation.

The DC-3F/TG cell line shown in Fig. 1B represents a mass population. Three independent clonal sublines have been derived from the DC-3F/TG mass population by limiting dilution. Analysis of the clonal lines demonstrates that in each cell line both wt and mutant SERCA are expressed (Fig. 1B, lanes 3). In contrast, DC-3F/TG cells express both wt and mutant SERCA. Hence, either primer pair (i.e., 2.3 and 2.16 for wt, or 2.3 and 2.17 for mutant) should amplify a 679-bp fragment when RNA from DC-3F/TG cells is used as template (Fig. 1B, lanes 5 and 6).

The DC-3F/TG cell line shown in Fig. 1B represents a mass population. Three independent clonal sublines have been derived from the DC-3F/TG mass population by limiting dilution. Analysis of the clonal lines demonstrates that in each cell line both wt and mutant SERCA are expressed (Fig. 1C, lanes 1 and 2, clone 1; lanes 3 and 4, clone 2; lanes 5 and 6, clone 3; lanes 7 and 8, wt DC-3F). Since each clonal population was presumably derived from a single cell, the above data are consistent with the SERCA allele being heterozygous with respect to the Phe256→Leu mutation in the DC-3F/TG cells.

Fig. 2 shows the results of a typical ASO-based PCR assay performed on DDT/TG 4μM cells. In Fig. 2A are shown the two antisense primers 2.27 and 2.28, which are identical to each other except at nt 767. Under appropriate conditions of annealing, primer 2.27 hybridizes to the complementary DNA strand containing T at nt 767 (which predicts for Phe256), while primer 2.28 hybridizes to the complementary strand containing C at nt 767 (which predicts for Ser256). That the primer pair 2.3 and 2.27 amplifies a 680-bp PCR product from both DDT1-MF2 cells (Fig. 2B, lane 8) and DDT/TG 4μM cells (Fig. 2B, lane 5) demonstrates that the wt Phe256 SERCA exists in both drug-sensitive and drug-resistant cells. The primer pair 2.3 and 2.28 amplifies a 679-bp fragment from DDT/TG 4μM cells (Fig. 2B, lane 6) but not from wt DDT1-MF2 cells (Fig. 2B, lane 9). These data confirm and extend the initial sequencing data with respect to the heterozygous Phe256→Ser change in DDT/TG 4μM cells. The specificity of the above primers is demonstrated by the data in Fig. 2B, lanes 2 and 3. Lane 2 shows the presence of a 679-bp fragment when reverse-transcribed RNA from DDT/TG 4μM cells is amplified with the primer pair 2.3 plus 2.16. Primer 2.16 is identical to primer 2.27, except for the former
extends from nt 766 to 782, while the latter extends from 767 to 783. Importantly, the primer pair 2.3 plus 2.17 (which recognizes Leu256; Fig. 1) fails to amplify any PCR product from DDT/TG 4 μM cells (Fig. 2B, lane 3), thus confirming the ability of the above primers in recognizing only specific nt changes.

**Functional Analysis of the Phe256 → Leu and Phe256 → Ser Mutations**—To determine whether the observed mutations do in fact contribute to TG resistance, the appropriate substitutions within codon 256 have been introduced by PCR-based in vitro mutagenesis in a wild-type full-length avian SERCA1-encoding cDNA (11). The resulting SERCA-containing expression plasmids have been transfected into COS1 cells. Western blot analysis demonstrates that introduction of the above mutations at aa 256 do not affect the expression of the transfected mutant SERCAs, when compared with transfected wt SERCA, in COS1 cells (Fig. 3). Furthermore, measurement of Ca²⁺ transport and ATPase activities of the respective microsomal fractions demonstrate that neither the Phe256 → Leu nor the Phe256 → Ser change substantially alters either the Ca²⁺ transport function or the ATPase activity of the mutants when compared with wt SERCA (Table III).

Upon measuring Ca²⁺ transport activity as a function of TG concentration, each aa substitution renders COS1 microsomal fraction ⁴⁵Ca²⁺ uptake 4-fold resistant to inhibition by TG (Fig. 4). Moreover, each mutation results in a 5-fold increase in resistance to TG in terms of hydrolysis of ATP by the mutant SERCA (Fig. 5).

It is of note that, although the above mutations lead to essentially equivalent levels of resistance to TG with respect to both Ca²⁺ transport (Fig. 4) and ATP hydrolytic activities (Fig. 5), the absolute concentrations of TG required to inhibit Ca²⁺ transport function appear to be somewhat lower than that required to inhibit the ATPase activity of both wt and mutant SERCAs (compare Figs. 4 and 5). The apparent discrepancy in the absolute concentrations of TG between Figs. 4 and 5 is in part due to the very nature of the two assays, i.e. Ca²⁺ transport function is determined by the amount of ⁴⁵Ca²⁺ retained by microsomal fractions, while ATPase function is assessed spectrophotometrically. Nevertheless, these data clearly demonstrate that certain mutations at aa 256 can modify SERCA’s transport and catalytic properties with respect to the enzyme’s response to its specific inhibitor TG.

**DISCUSSION**

In this report, we identified mutant forms of SERCA that can confer resistance to TG inhibition. To our knowledge, this is the first demonstration of naturally occurring mutations within SERCA that occur upon long term exposure of mammalian cells to SERCA’s highly specific inhibitor TG. Although wt cells in culture are exquisitely sensitive to SERCA pump inhibition, high levels of resistance to TG can be developed by long term selection so that the selected cells continue to proliferate in TG (2, 3).² Previously, we reported several adaptive changes that the TG-selected cells employ to maintain growth in TG (2, 3).² Among these is overexpression of the multi-drug resistance transporter Pgp (2), as well as overproduction of SERCA protein by a multiplicity of mechanisms including gene amplification, transcriptional up-regulation, and post-transcriptional alterations.²

Previous in situ studies, as well as analysis of Ca²⁺ transport activity following isolation of microsomal fractions from TG-resistant cells, suggested qualitative changes that directly contribute to TG resistance might also occur within these ATPase (3). Our present studies clearly demonstrate that aa 256 is susceptible to specific mutations (Table II, Figs. 1 and 2). That

**Table III** 

| SERCA         | ATPase activity (mean ± S.E.) | Ca²⁺ uptake (mean ± S.E.) |
|---------------|------------------------------|---------------------------|
| Wild-type     | 93.16 ± 2.00⁶                 | 67.0 ± 8.69               |
| Leu²⁵⁶        | 93.14 ± 12.91                | 69.4 ± 14.2               |
| Ser²⁵⁶        | 113.16 ± 0.97                | 76.8 ± 16.4               |

² The data were obtained by two separate experiments and normalized with respect to the expression of SERCA in each microsomal preparation.

**Fig. 3.** Immunodetection of SERCA1 expression. The microsomal fractions derived from transfected COS1 cells were analyzed by Western blot as described under “Experimental Procedures.” The monoclonal antibody 9E10 was used to detect the c-myc tag at the C terminus of SERCA1. Lane 1, wt; lane 2, Leu²⁵⁶; lane 3, Ser²⁵⁶.

**Fig. 4.** Thapsigargin inhibition of ATP-dependent Ca²⁺ uptake activity by microsomal fractions obtained from SERCA1-transfected COS1 cells. Microsomes were isolated from wt (●), Leu²⁵⁶ (○), and Ser²⁵⁶ (□) transfected COS1 cells. ATP-dependent Ca²⁺ uptake was determined either in the absence or presence of TG by the accumulation of ⁴⁵Ca²⁺ in microsomal vesicles separated from the reaction mixture by filtration (0.45-μm Millipore filters), as described previously (3). Ca²⁺ uptake activity is expressed relative to the activity obtained in the absence of TG. Each point represents the average of two independent measurements, and standard errors (S.E.) are indicated as bars.

**Fig. 5.** Thapsigargin inhibition of Ca²⁺-dependent ATPase activity by microsomal fractions obtained from SERCA1-transfected COS1 cells. ATPase activities in the microsomal fractions obtained from wt (●), Leu²⁵⁶ (○), and Ser²⁵⁶ (□) transfected COS1 cells were measured by determination of P in the presence or absence of TG as described by Zhang et al. (14). The Ca²⁺-dependent ATPase activity was calculated by subtracting the Ca²⁺-independent ATPase activity from the total ATPase activities. ATPase activities are expressed relative to the activity obtained in the absence of TG. Each point represents the average of two independent measurements, and standard errors (S.E.) are indicated as bars.
cell lines of two different lineages, selected independently for TG resistance, give rise to two different mutations at the same location suggests that this aa is particularly susceptible to mutations. The data also suggest that aa 256 might be closely linked with TG-SERCA interactions.

Since the M3 domain is the putative TG binding site, our initial efforts have been to analyze regions of SERCA encompassing this domain. If any mutations occur within SERCA in the TG-selected cells, they would be expected to occur within the TG binding pocket. Such mutations could potentially alter SERCA’s affinity for TG, thus rendering the ATPase resistant to inhibition by TG. For instance, antifolates like methotrexate (MTX) inhibit dihydrofolate reductase (DHFR) (18). However, specific mutations in DHFR (e.g. Leu\(^{22}\) → Phe or Leu\(^{22}\) → Arg) within the binding pocket of MTX can alter MTX’s affinity for DHFR, resulting in a MTX-resistant phenotype (19, 20).

Interestingly, no mutations in the TG-resistant cell lines have been found within the M3 domain upon natural drug selection (data not shown). However, Phe\(^{256}\), which is only 4 aa upstream from where the M3 domain begins (4), is mutable under our conditions with drug selection. Although isoform-specific changes distinguish the different isoforms of SERCA within and across species, at the protein level and with respect to the overall topology, the various SERCAs have a high degree of homology to each other. The M3 domain is highly conserved, but not identical, between SERCA1 and SERCA2 (4). Interestingly, the 14 aa within the first cytosolic loop that are immediately upstream of the M3 domain (i.e. aa 247–260) are identical between SERCA1 and SERCA2 across diverse species (4), and are also similar to but not identical with the corresponding region in SERCA3 (4). Although our initial observations in terms of mutations at aa 256 are with hamster SERCA2, the fact that introduction of the observed mutations in a different isoform from a different species (i.e. avian SERCA1) renders the resulting mutant enzymes resistant to TG (Figs. 4 and 5) suggests that the aa 256 position is particularly important in TG-SERCA interactions. Interestingly, we have only observed heterozygous changes at aa 256 in the TG-resistant cells (Figs. 1 and 2). Although no mutations are observed within the actual M3 domain in our resistant cell lines, previous studies have suggested that this region is involved in TG-SERCA interactions (5, 6).

Since the relative resistance to TG by the documented mutations at aa 256 is only 4–5-fold (Fig. 4), we cannot rule out the possibility that additional qualitative changes within other regions of SERCA could also occur, and potentially contribute to the overall TG resistance phenotype. In summary, our studies show that the SERCAs exhibit a variety of responses, including point mutations, that enable cells to survive in the presence of their highly selective and potent inhibitor TG. Thus, the SERCAs follow the same paradigm exhibited by enzymes like DHFR that are subjected to selective pressures by their specific inhibitors such as the antifolates.

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Additions and Corrections

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Expression cloning of a novel suppressor of the Lec15 and Lec35 glycosylation mutations of Chinese hamster ovary cells.

Felecia E. Ware and Mark A. Lehrman

This paper reported the cloning of a cDNA for a new gene, which we termed SL15. We reported that SL15 cDNA corrected two separate glycosylation-defective cell lines known as Lec15 and Lec35. In experiments to be reported in the future we have found that the Lec35 cells used in that study have a defect in the SL15 gene. This provides a clear explanation for correction of Lec35 cells by SL15 cDNA, a result that has now been obtained by three separate individuals in this laboratory as well as by another laboratory (T. Kinoshita, personal communication). However, similar attempts to repeat the correction of Lec15 cells by SL15 cDNA have consistently failed. Based upon further analysis of cell samples from that study, we now conclude that the Lec15 population was contaminated with Lec35 cells. Lec15 cells described in Figs. 2, 3, and 4 as being transfected with SL15 cDNA and having normal glycosylation phenotypes have now been shown to be Lec15-Lec35 hybrids, most likely formed during electroporation. It is likely that SL15 cDNA was carried by contaminating Lec35 cells during expression cloning and that the contaminating Lec35 cells accounted for the greater number of colonies that survived lectin selection after transfection with SL15 cDNA as compared with vector controls.

Since there is no evidence that SL15 cDNA can correct the Lec15 phenotype certain aspects of our paper must be rescinded, specifically Fig. 2, panel C; Fig. 3, all data with T-ptLec15 microsomes; Fig. 4, lane 3; and all sections of the text indicating that SL15 can correct Lec15 cells. However, we remain confident that the correction of Lec35 cells by SL15 cDNA is a valid result. We sincerely apologize to the readers of this journal for any confusion or inconvenience this may have caused.

Vol. 273 (1998) 3542–3546

Specific substitutions at amino acid 256 of the sarcoplasmic/endoplasmic reticulum Ca2+ transport ATPase mediate resistance to thapsigargin in thapsigargin-resistant hamster cells.

Myounghee Yu, Lilin Zhong, Arun K. Rishi, Mohammed Khadeer, Giuseppe Inesi, and Arif Hussain

Dr. Zhong's name was misspelled. It is correct in the author line above.

Vol. 272 (1997) 7595–7601

Identification of the 170-kDa melanoma membrane-bound gelatinase (seprase) as a serine integral membrane protease.

Mayra L. Piñeiro-Sánchez, Leslie A. Goldstein, Johannes Dodt, Linda Howard, Yuyun Yeh, Huan Tran, W. Scott Argraves, and Wen-Tien Chen

Page 7595: Drs. Tran and Argraves were omitted. The corrected author line is shown above.

Pages 7597 and 7600: Due to an error in assembling Figs. 1F and 7C, the two D28 blot lanes in Fig. 1F were mounted upside down and Fig. 7C was mistaken as LOX melanoma seprase but it was actually derived from placental seprase data. The former had been shown previously (Monsky, W. L., Lin, C.-Y., Aoyama, A., Kelly, T., Mueller, S. C., Akiyama, S. K., and Chen, W.-T. (1994) Cancer Res. 54, 5702–5710), and the latter should be referenced with similar figures derived from two LOX seprase experiments done on July 9, 1992 and January 15, 1993. In all of these changes, there is no new scientific information added or changed, and there is no change in the conclusions of the paper.

We suggest that subscribers photocopy these corrections and insert the photocopies at the appropriate places where the article to be corrected originally appeared. Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.