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Curcumin Treatment Abrogates Endoplasmic Reticulum Retention and Aggregation-Induced Apoptosis Associated with Neuropathy-Causing Myelin Protein Zero–Truncating Mutants

Mehrdad Khajavi,[1] Ken Inoue,[5] Wojciech Wiszniewski,[1] Tomoko Ohyama,[1] G. Jackson Snipes,[2] and James R. Lupski[1,3,4]

Departments of [1]Molecular and Human Genetics, [2]Pathology, and [3]Pediatrics, Baylor College of Medicine, and [4]Texas Children's Hospital, Houston; and [5]Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo

Mutations in *MPZ*, the gene encoding myelin protein zero (MPZ), the major protein constituent of peripheral myelin, can cause the adult-onset, inherited neuropathy Charcot-Marie-Tooth disease, as well as the more severe, childhood-onset Dejerine-Sottas neuropathy and congenital hypomyelinating neuropathy. Most *MPZ*-truncating mutations associated with severe forms of peripheral neuropathy result in premature termination codons within the terminal or penultimate exons that are not subject to nonsense-mediated decay and are stably translated into mutant proteins with potential dominant-negative activity. However, some truncating mutations at the 3′ end of *MPZ* escape the nonsense-mediated decay pathway and cause a mild peripheral neuropathy phenotype. We examined the functional properties of MPZ-truncating proteins that escaped nonsense-mediated decay, and we found that frameshift mutations associated with severe disease cause an intracellular accumulation of mutant proteins, primarily within the endoplasmic reticulum (ER), which induces apoptosis. Curcumin, a chemical compound derived from the curry spice tumeric, releases the ER-retained MPZ mutants into the cytoplasm accompanied by a lower number of apoptotic cells. Our findings suggest that curcumin treatment is sufficient to relieve the toxic effect of mutant aggregation-induced apoptosis and may potentially have a therapeutic role in treating selected forms of inherited peripheral neuropathies.

Introduction

Mutations in the gene encoding myelin protein zero (MPZ) cause dominantly inherited peripheral neuropathies that range in severity from adult-onset Charcot-Marie-Tooth disease (CMT) type 1B (CMT1B [MIM 118200]) to childhood-onset Dejerine-Sottas neuropathy (DSN [MIM 145900]) or congenital hypomyelinating neuropathy (CHN [MIM 605253]) (Lupski and Garcia 2001). CMT1B, a demyelinating neuropathy that results in progressive distal muscle atrophy, is characterized by a symmetrically slowed motor nerve conduction velocity (Shy et al. 2005). DSN is a more severe form of CMT that has an earlier onset of clinical symptoms, evidenced by the delayed achievement of motor-skill milestones. Clinical findings include hypertrophied nerves, increased cerebrospinal fluid protein, and, compared with CMT, more-significant slowing of nerve conduction velocity, more-pronounced demyelination, and neuropathology significant for more-numerous onion bulbs (Hayasaka et al. 1993; Rautenstrauss et al. 1994). The related CHN presents at birth and is distinguished from DSN by its congenital manifestations and, in some cases, the absence of myelin (Harati and Butler 1985).

We previously documented that the nonsense-mediated degradation of mRNA carrying premature termination codons in upstream exons is a mechanism for MPZ haploinsufficiency alleles (Inoue et al. 2004). The nonsense-mediated decay pathway is an mRNA surveillance system that specifically recognizes and degrades erroneous mRNA that harbors premature termination codons often resulting from abnormal splicing or frameshifts (Sun and Maquat 2000). Failure to eliminate mRNA with premature termination codons may result in the translation of aberrant proteins that can be toxic to cells through dominant-negative or gain-of-function effects (Wong and Filbin 1996; Frischmeyer and Dietz 1999; Mendell and Dietz 2001; Holbrook et al. 2004; Inoue et al. 2004). Essentially all nonsense and frameshift mutations that are associated with a relatively mild peripheral neuropathy phenotype have their mRNA degraded because of premature stop codons that

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Address for correspondence and reprints: Dr. James R. Lupski, Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Room 604B, Houston, TX 77030. E-mail: jlupski@bcm.tmc.edu

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are detected by nonsense-mediated decay. Therefore, haploinsufficiency is the disease mechanism (Inoue et al. 2004). Most late-occurring premature termination codons that result in mRNA that escapes nonsense-mediated decay encode apparent dominant-negative or gain-of-function proteins that convey a more severe neuropathy phenotype. However, a subset of truncating mutations at the 3′ end of *MPZ* escapes the nonsense-mediated decay surveillance pathway and causes a mild CMT phenotype (fig. 1*A*) (Inoue et al. 2004; Shy et al. 2004; Inherited Peripheral Neuropathies Mutation Database). Thus, the question remains: How can the same types of mutation—frameshift mutations that escape nonsense-mediated decay—result in distinct clinical phenotypes?

We noted an apparent correlation between the type of frameshift mutations occurring after the transmembrane domain and the associated phenotype. Mutations associated with CMT retain their transmembrane domain and have a net addition of a +2 frameshift mutation (a 2-bp insertion or a 1-bp deletion), mainly in downstream exons (fig. 1*A*). We hypothesized that frameshift mutations associated with severe disease may lead to a mutant protein with a gain-of-function that causes a more deleterious effect by being misprocessed in the cell. To examine this hypothesis, we selected four mutations for functional analyses. These all represent frameshift alleles that, as we documented elsewhere (Inoue et al. 2004), escape nonsense-mediated decay and consist of two mutations from each of two categories: severe DSN- or CHN-associated mutations (*MPZ* 506delT and 550del3insG) and relatively mild CMT-associated mutations (*MPZ* 554delG and 676insCA) (fig. 1*B*). Notably, both 550del3insG and 554delG were located after the transmembrane domain but differed in frame and associated phenotype. We introduced such disease-associated mutations (fig. 1*B*) in an expression vector and used both a transiently transfected HeLa cell line and human embryonic kidney cells (HEK293) to study the effects of wild-type *MPZ* cDNA and each disease-causing mutation on its intracellular processing. The severe alleles that cause DSN and CHN appear to be retained in the endoplasmic reticulum (ER) and induce increased apoptotic cell death that can be partially mitigated by pretreatment with curcumin.

Material and Methods

Recombinant Constructs

Full-length human *MPZ* cDNA (IMAGE: 3926008) was obtained (OpenBiosystems) and was subcloned into pcDNA3.1 (Invitrogen) to generate pcDNAMPZ. Mutations were generated in each construct with use of the QuikChange site-directed mutagenesis kit (Stratagene). Clones were verified by direct double-strand DNA sequencing with use of the DyePrimer chemistry and ABI377 sequencer (Applied Biosystems).

Tissue Culture and Transfection

HeLa cells and HEK293 cells were grown in Dulbecco's modified Eagle medium (BioWhittaker), supplemented with 10% fetal bovine serum, and were transfected with use of FuGENE 6 transfection reagents (Roche Applied Science). Cells were incubated for 24 h at 37°C in a humidified incubator containing 10% CO_2.

Immunostaining

Cells were fixed with 2% paraformaldehyde in PBS at room temperature for 10 min. Cells were then washed and permeabilized with 0.1% Triton X-100 in PBS on ice for 2 min. Cells were rinsed twice with PBS and were blocked with 5% normal goat serum for 1 h at 37°C. Fixed cells were incubated with primary antibodies diluted in PBS with 5% normal goat serum at appropriate concentrations for 1 h at 37°C. Antibodies used in this study include the polyclonals directed against MPZ protein (Trapp et al. 1979) and mouse monoclonal protein disulfide isomerase (PDI; 1:1,000 [Affinity Bioreagents]). This incubation was followed by two washes in PBS and another incubation with Alexa Fluor goat anti-mouse or anti-rabbit antibody (1:1,000 [Molecular Probes]) for 1 h at 37°C. For visualizing the nuclei, SlowFade Light Antifade Kit with 4′, 6-diamidino-2-phenylindole (DAPI) (Molecular Probes) was used in accordance with the manufacturer's instructions. Fluorescently labeled cells were visualized by standard fluorescence microscopy.

Apoptosis Assay

TUNEL staining was performed with use of an in situ cell death detection kit, Flourescein (Roche Applied Science). Cells were grown in four chamber slides and were fixed with 4% paraformaldehyde in PBS at room temperature for 10 min. Cells were then washed and permeablized with 0.1% Triton X-100 in PBS on ice for 2 min. TUNEL staining was performed in accordance with conditions recommended by the supplier (1 h at 37°C). The average numbers of TUNEL-positive and DAPI-positive cells were calculated, and the standard deviation of the ratio was determined for each slide. Student's *t* tests comparing wild-type and MPZ mutants were performed. Statistical significance was defined as $P < .05$.

Flow-Cytometric Analysis

Cells were transiently transfected for 48 h and then harvested. Annexin V–fluorescein isothiocyanate (FITC) and propidium iodide (PI) staining (BD Biosciences Pharmingen) was performed by the incubation of cells (1×10^6 cells/ml) in the dark for 15 min at room temperature in a binding buffer (10 mM HEPES, 140 mM

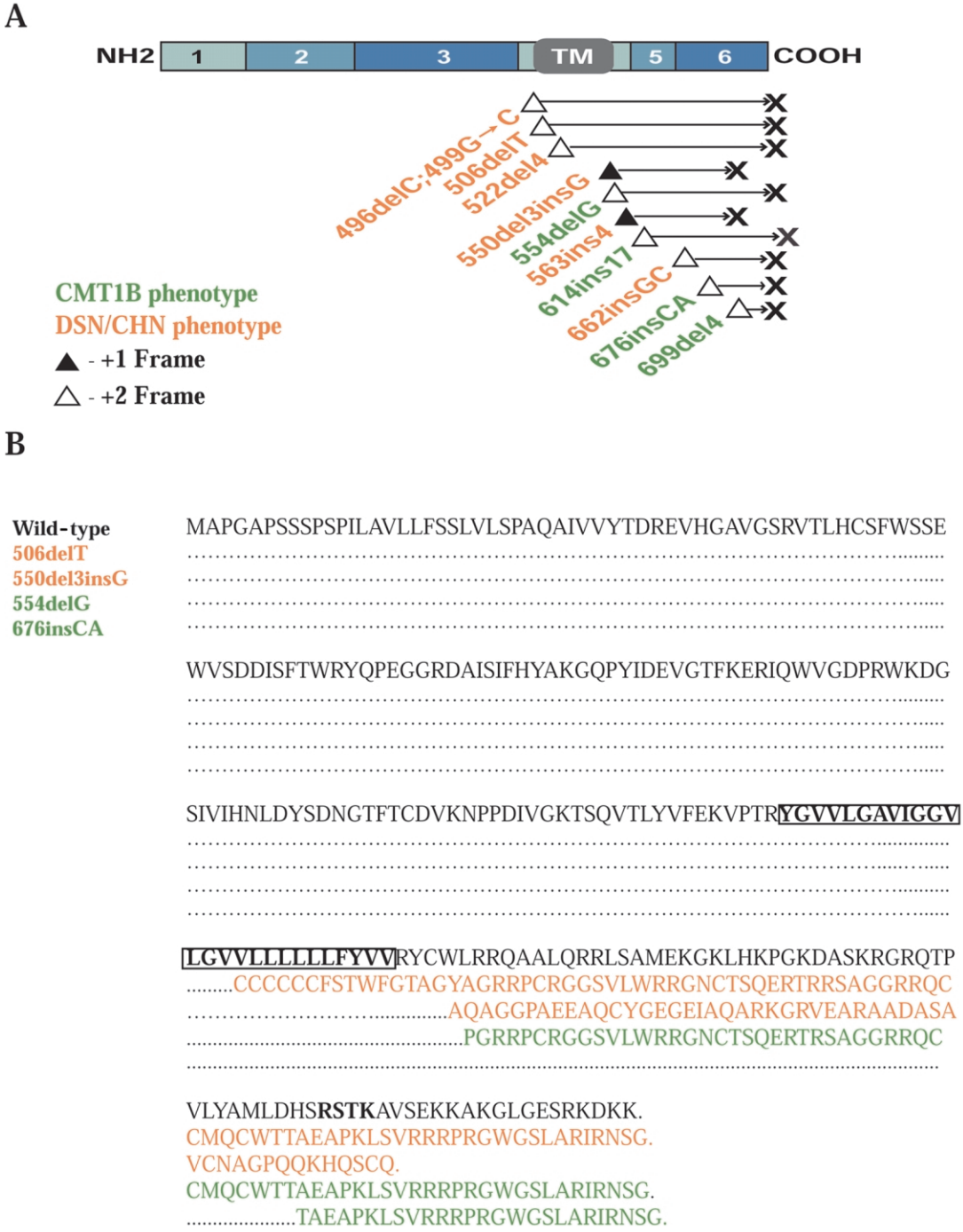


Figure 1 Genotype-phenotype correlation of *MPZ*. *A*, Published *MPZ*-truncating mutations that escape nonsense-mediated decay and are associated with inherited peripheral neuropathies. The six coding exons of *MPZ* are indicated by shades of blue, and the transmembrane (TM) domain is encoded by exon 4. *Filled triangles*, +1 Frameshift mutations. *Open triangles*, +2 Frameshift mutations. *Arrows*, Altered region incorporated after frameshift mutations, with *X* demarcating the new stop codon. *Green*, CMT1B phenotype. *Red*, More severe DSN/CHN phenotype. Note that mutations associated with a severe DSN/CHN phenotype have either an early frameshift that disrupts the transmembrane domain or a net addition of a +1 frameshift in downstream exons while retaining the transmembrane domain, whereas CMT-associated mutations have a +2 frameshift in downstream exons. *B*, Wild-type and mutant MPZ protein sequences investigated in this study. *Green*, CMT-associated frameshift mutations. *Red*, DSN/CHN-associated mutations. *Black*, Wild-type sequence, with dots representing identity to the wild-type sequence. The TM domain (in rectangle) and RSTK motif (in boldface) are demarcated in wild-type MPZ.

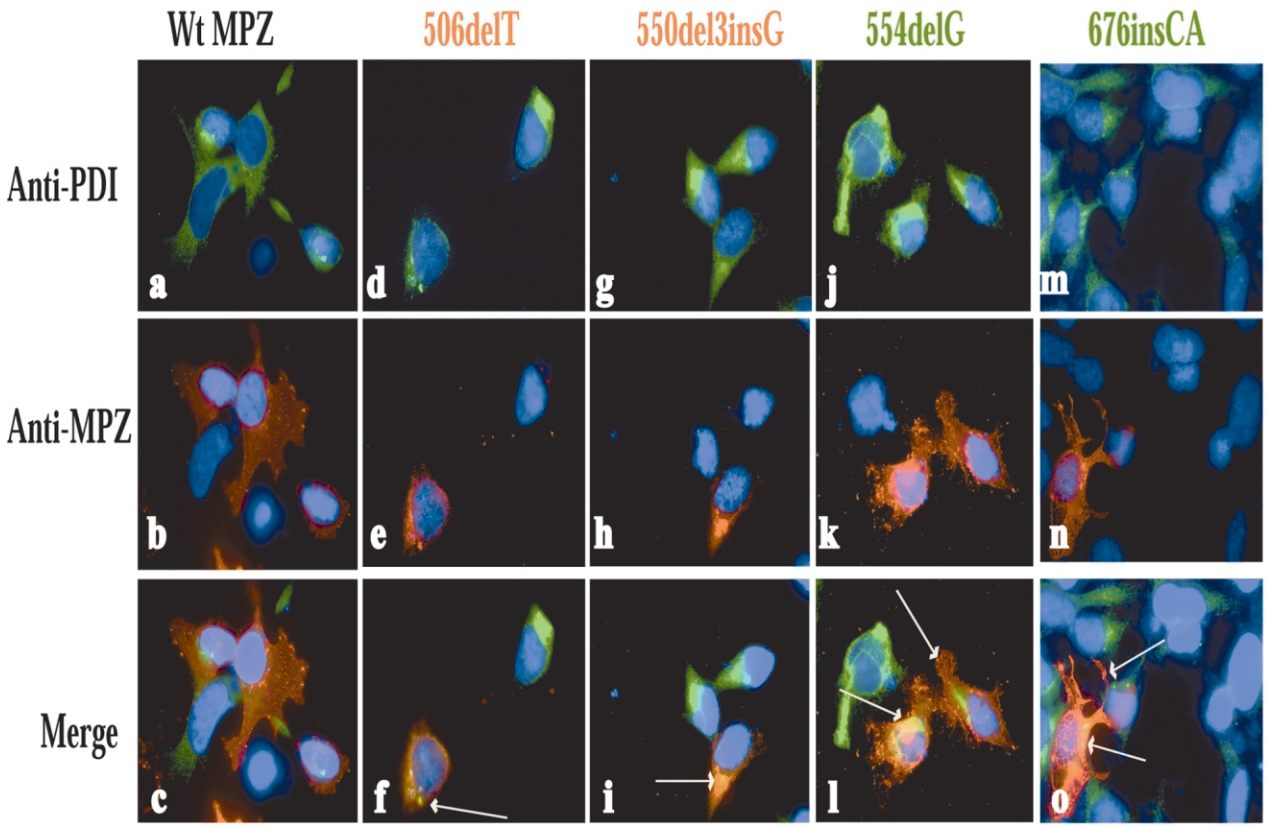


Figure 2 Subcellular localization of mutated MPZ proteins in HeLa cells. The HeLa cell line transiently transfected with wild-type *MPZ* shows the expression of wild-type MPZ protein on the plasma membrane. *Red,* DSN/CHN-associated mutations (*MPZ* mutants 506delT and 550del3InsG) are extensively retained in the ER, as evidenced by colocalization with PDI (*f* and *i, arrows*). Note that cells transiently transfected with these mutants display distinctive apoptotic morphology, including cell shrinkage. *Green,* CMT-associated *MPZ* mutations (554delG and 676insCA) are detected on the cell surface as well as in the ER by colocalizing with PDI (*l* and *o, arrows*).

NaCl, and 2.5 mM $CaCl_2$, at pH 7.4) containing a saturating concentration of annexin V–FITC and PI. After incubation, the cells were washed, pelleted, and analyzed in a FACScan analyzer (Becton Dickinson).

Curcumin Treatment

Curcumin was purchased from Sigma (catalogue number C7727). Curcumin stock was dissolved in dimethyl sulfoxide (DMSO) in accordance with conditions recommended by the supplier. Cells were pretreated with curcumin (10 μM, unless otherwise indicated) for 3 h before transfection.

Results

Mutant MPZ Is Retained in the ER

We transiently transfected wild-type *MPZ* cDNA and disease-associated mutations (fig. 1*B*) in different cell lines to visualize the effects of each disease-causing mutation on its intracellular processing. We performed real-time PCR on cell lines after transfection and confirmed comparable levels of gene expression for the mutant and wild-type constructs (data not shown). In our control experiments, we verified that HeLa cell lines transfected with wild-type *MPZ* expressed the MPZ protein on the plasma membrane. We observed similar expression patterns in the HEK293 cell line (data not shown). Of note, all disease-causing mutants tested had a noticeably abnormal intracellular distribution in both cell lines (fig. 2 and data not shown). The distribution of CMT-associated mutants (*MPZ* 554delG and *MPZ* 676insCA) was different from the wild-type control, in that not only are the mutant proteins expressed on the cell surface but they are also detectable in the ER, as shown by colocalizing with the ER marker-protein PDI (fig. 2). Although these mutant proteins are expressed on the cell surface, they are likely to behave as null alleles, because of the disruption of the protein structure of the basic cytoplasmic domain (fig. 1*B*). Interestingly, *MPZ* mu-

tants 506delT and 550del3insG were detected only in the ER, and the staining pattern observed is clearly different from that seen for wild-type and CMT-associated mutants (fig. 2). The toxic effects of these mutant proteins perhaps do not require translocation to the cell membrane surface, where normal tetrameric MPZ proteins localize and function.

Severe Disease-Associated MPZ Mutants Cause Increased Apoptosis

When visualized by light microscopy, cells transfected with the severe *MPZ* mutant alleles, 506delT and 550del3insG, display distinctive apoptotic morphology, including cell shrinkage and detachment from the surface of the plate (data not shown). These observations led us to consider the possibility that such *MPZ* early frameshift mutations have a deleterious effect on cells and may induce apoptosis. To determine whether cell death of cells expressing disease-causing MPZ mutants are apoptotic, we performed TUNEL labeling on cells transfected with wild-type and *MPZ* mutants. We quantified the number of positive cells by counting all TUNEL-positive cells and DAPI-positive nuclei in 10 sections of the chamber slides. We found a large number of positive cells among those transfected with *MPZ* 506delT and 550del3insG (DSN- and CHN-associated mutations) compared with cells expressing either wild-type or CMT-associated mutations ($P = .015$ and $P = .018$, respectively) (fig. 3*A*).

To exclude the possibility that the apoptosis may represent a nonspecific consequence of protein overexpression, we used the green fluorescent protein (GFP)/PI assay (Lamm et al. 1997) to analyze the DNA fragmentation in transiently transfected cells, with GFP as a marker. Coexpression of *MPZ* 506delT and 550del3insG resulted in an increase of apoptotic cells to a level ~20% higher than that seen with wild-type *MPZ* or CMT-associated mutations (fig. 3*B*). These findings are indicative of a mutation-specific increase in apoptosis and are inconsistent with a nonspecific effect from protein overexpression.

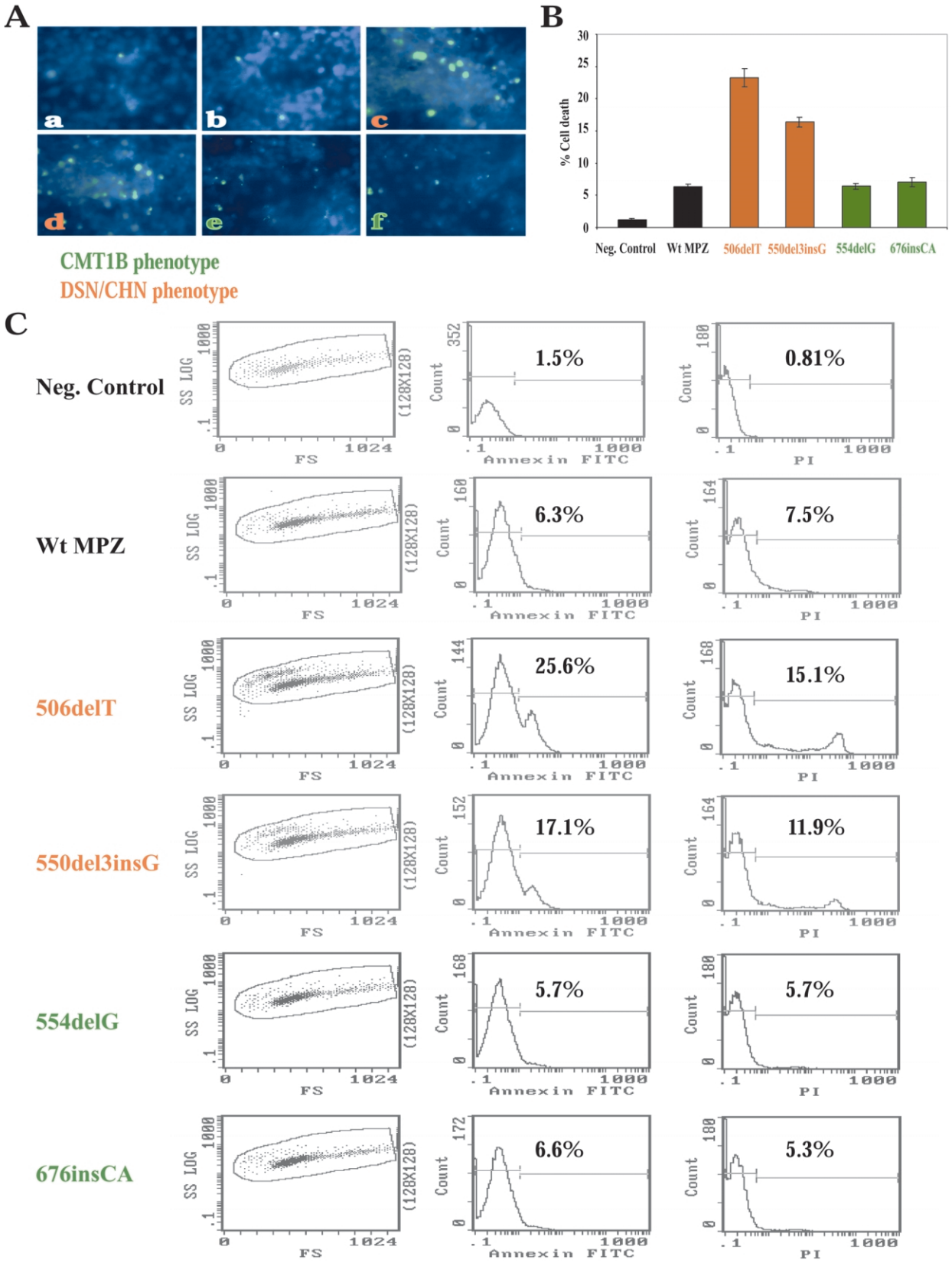
We also used a combined annexin V–PI staining to quantify cell apoptosis by an independent method. This objective assay measures apoptosis via the binding of FITC-labeled annexin V to phosphatidylserine, as analyzed by flow cytometry. Phosphatidylserine is normally confined to the inner leaflet of the plasma membrane and is externalized during apoptosis of many cell types (Preobrazhensky et al. 2001). The PI staining enables simultaneous determination of the associated loss of membrane integrity. Our analyses confirmed that *MPZ* 506delT and 550del3insG induce apoptosis, with the annexin V–positive cells reaching 25.6% and 17.1%, respectively (fig. 3*C*). In contrast, cells transfected with wild-type *MPZ* or CMT-associated mutations contained a minimal fraction of <7% (fig. 3*C*). These experiments clearly support the contention that the latter mutations conveying a milder CMT phenotype cause a less toxic effect on cells and, thus, likely act as loss-of-function alleles.

Curcumin Enhances Mutant MPZ Processing in the ER

These data are consistent with the contention that MPZ early frameshift mutations associated with severe disease (fig. 1*A*) convey a more deleterious effect to the cell. The severity of the DSN/CHN phenotype is mainly caused by the more pronounced demyelination or disruption of the axon–Schwann cell interactions that leads to axonal loss. This could be due to a misfolding and mislocalization of MPZ mutants in the ER, possibly by mechanisms involving certain ER chaperones or a decrease in the amount of MPZ available for myelin compaction in Schwann cells.

To evaluate a potential reagent's ability to relieve the toxic effect associated with severe disease-causing *MPZ* frameshift mutations, we investigated the effect of curcumin, a dietary supplement, on MPZ mutants. Curcumin modulates a number of cellular messenger pathways, including NF-kB and intracellular calcium (Egan et al. 2004; Sarkar and Li 2004). Recently, Egan et al. (2004) have shown that curcumin can apparently rescue misfolded proteins in both cell cultures and a homozygous ΔF508-CFTR mouse model by presumably interfering with the function of ER calcium-dependent chaperones (Egan et al. 2004). We hypothesized that curcumin could have a protective effect by causing MPZ-misfolded and MPZ-aggregated mutants to be released from the ER, potentially relieving the toxic effect associated with these mutations in cells. This hypothesis was experimentally addressed by treating cells expressing mutant and wild-type control MPZ with curcumin.

When curcumin was present at low doses (2 μM and 5 μM), we observed a limited number of cells having partial ER release of the severe disease-associated mutants (*MPZ* 506delT and 550del3insG) (data not shown). However, after increasing the concentration of curcumin to 10 μM, we observed a greater apparent release from ER retention, mostly to the cytoplasm and the cell surface (fig. 4*A*). Notably, we also observed a significant decrease in apoptosis after treating cells with 10 μM of curcumin (fig. 4*B* and 4*C*). The percentage of apoptotic cells after curcumin treatment is essentially the same as that observed for the control wild-type MPZ protein and is significantly different from that observed for these mutants without curcumin treatment (fig. 3*C*). These data indicate that curcumin treatment abrogates the ER retention and aggregation-induced apoptosis as-


A
a
b
c
d
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CMT1B phenotype
DSN/CHN phenotype
B
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30
25
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Neg. Control
Wt MPZ
506delT
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C
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SS LOG
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Count
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1.5%
0.81%
6.3%
7.5%
25.6%
15.1%
17.1%
11.9%
5.7%
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sociated with neuropathy-causing MPZ-truncating mutants observed in cells.

Discussion

More than 90 different point mutations in *MPZ* that result in a spectrum of inherited demyelinating neuropathies, including CMT, DSN, and CHN (Inherited Peripheral Neuropathies Mutation Database), have been identified. The identification and evaluation of these mutations in patients with different clinical severities not only has provided insights into the role of MPZ in myelin structure (Warner et al. 1996) but has also enabled genotype/phenotype correlations (Inoue et al. 2004; Shy et al. 2004). Various nonsense and frameshift mutations in *MPZ* result in both mild and severe forms of neuropathy, and we previously demonstrated that mutations in ORFs could dictate disease severity by mechanisms other than effects on the protein product (Inoue et al. 2004).

Most *MPZ*-truncating mutations associated with a more severe form of peripheral neuropathy result in premature termination codons within the terminal or penultimate exons and are thus not detected by the nonsense-mediated decay surveillance pathway. Such mRNA is translated into mutant proteins with potential dominant-negative activity. However, a subset of premature termination codon mutations at the 3′ end of *MPZ* escapes the nonsense-mediated decay pathway and the mRNA is translated into mutant protein, but a mild peripheral neuropathy phenotype results. Here, we provide experimental evidence that the escape from nonsense-mediated decay does not necessarily result in a more severe phenotype and that the phenotypic outcome depends on the function of mutant proteins. Furthermore, we demonstrate that curcumin, a dietary supplement, apparently stimulates the translocation of intracellularly retained mutant MPZ from the ER to the plasma membrane, clearly reducing cytotoxicity of the mutant protein, as evidenced by a decreased percentage of apoptotic cells (fig. 4).

CMT-associated mutants are expressed on the cell surface but convey only a minor toxic effect, as evidenced by apoptosis assays, and, thus, may function as loss-of-function alleles. The mild phenotype produced by these mutants suggests that they do not interfere with tetramer formation and do allow wild-type MPZ molecules to partly restore myelin function. CMT-associated mutants thus likely act as null alleles or possess a reduced level of activity because of the disruption of the protein structure of the basic cytoplasmic domain. The cytoplasmic domain of MPZ is extremely basic and has been shown to stabilize adhesion between the intracellular components of the plasma membrane in myelin by interacting with the apposing anionic lipid bilayer to help in the formation of the major dense line (Ding and Brunden 1994; Martini et al. 1995*a*). The cytoplasmic domain contains a PKC target motif (RSTK), and this property of the intracellular domain can be extensively affected by changes in some amino acids critical to homophilic interactions (Xu et al. 2001).

In contrast, mutations associated with severe DSN/CHN cause a more deleterious effect to the cells by being associated with ER retention (fig. 2) and apparent aggregation-induced apoptosis (fig. 3). Such MPZ mutants may also indirectly affect wild-type MPZ cell-targeting to myelin. In fact, $Mpz^{+/-}$ heterozygous knockout mice show normal myelination at an early age because of the partial expression of wild-type Mpz molecules, whereas a lack of MPZ expression could account for the severe phenotype, which has been observed in null $Mpz^{-/-}$mice (Giese et al. 1992; Martini et al. 1995*b*). The gene dosage dependence of wild-type MPZ for proper myelin maintenance has also been observed in heterozygous *MPZ*-truncated mutations in parents who presented with a CMT1 phenotype, whereas the homozygous children had a more severe DSN phenotype (Warner et al. 1996).

We provide evidence that, by treating cells with curcumin, we could abrogate the ER retention of selected MPZ mutants enough to ameliorate the toxic effect, as determined by apoptosis assays, associated with these mutations (fig. 4). Previous studies have shown that some mutant MPZ aggregates colocalize with BiP, an HSP70 chaperone in the lumen of the ER, but not with calnexin (Matsuyama et al. 2002; Shames et al. 2003).

Figure 3 Increased cell death in HeLa cell lines after transfection with mutant *MPZ*. *A,* Apoptosis induced in HeLa cell lines after transient transfection with *MPZ* 506delT (*c*) and 550del3insG (*d*). Cell lines transfected with wild-type *MPZ* (*b*), *MPZ* 554delG (*e*), and *MPZ* 676insCA (*f*) show a significantly lower number of positive cells. Data from TUNEL assays revealed the presence of more positive cells only after transfection with DSN/CHN-associated mutations (*c* and *d*). A negative control (*a*) is also shown. *B*, DSN/CHN-associated mutations (*MPZ* 506delT and 550del3insG) increased the number of apoptotic cells, compared with wild-type *MPZ*. Bar graph shows results for severe (*red*) and relatively mild (*green*) mutant alleles ±SD for each of four independent experiments ($n = 4$). *C,* Representative study of the flow-cytometric analysis of apoptosis after transfecting cells with wild-type *MPZ* and disease-associated mutations. Significant differences were observed in the percentage of cells undergoing apoptosis when transfected with 506delT and 550del3insG (DSN/CHN-associated mutations), whereas CMT-associated mutations show a less toxic effect on cells. Note that overexpression of wild-type *MPZ* induces measurable apoptosis, compared with the negative control (representative data from one of three independent experiments with comparable results; FS = flow-sorted cells and PI = propidium iodide).

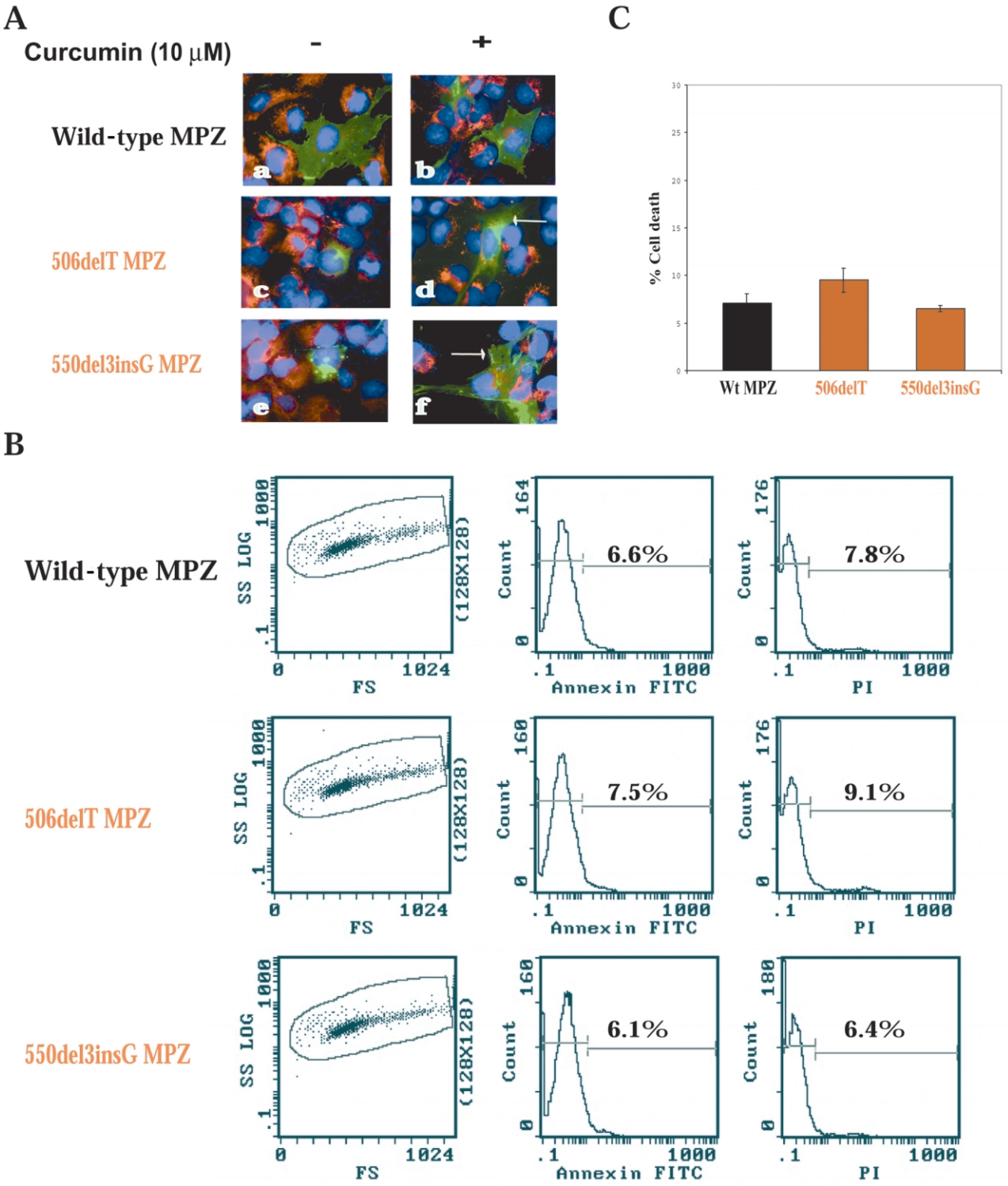

Figure 4 Release of ER-retained MPZ mutants with curcumin treatment. *A,* Curcumin treatment of HeLa cells rescues *MPZ* 506delT (*d*) and 550del3insG (*f*) mutants (in green) retained in the ER (PDI markers in red) to the cytoplasm. Interestingly, mutant *MPZ* 506delT (*d, arrow*) was detectable only in the cytoplasm, mainly because of the disruption of the transmembrane domain in this mutant protein. Note that cells treated with 10 μM curcumin showed reduced ER retention and mostly cytoplasmic localization for MPZ mutants (*d* and *f, arrows*). *B,* Apoptosis analysis of cells transiently transfected with wild-type *MPZ* mutations 506delT and 550del3insG after curcumin treatment. *C,* Bar graph of wild-type (*black*) and severe (*red*) alleles treated with curcumin, showing average $\pm$SD from three independent experiments, as shown in panel B. Cells undergo less apoptosis when treated with curcumin ($n = 3$). DMSO alone, at the same concentration used in our curcumin preparation, had no toxic effect on cells (data not shown).

The involvement of mutant proteins with BiP is often associated with activation of the "unfolded protein response," which leads to the upregulation of certain ER chaperones and interference with folding of newly synthesized proteins that, under stress conditions, could be detrimental to cell growth and survival (Little and Lee 1995; Gething 1999). Although the mechanism through which curcumin corrects the processing and function of misfolded or aggregated mutant proteins in cells is not yet determined, it is hypothesized that it may interfere with the function of the ER calcium-dependent chaperones by altering the calcium levels in the ER (Egan et al. 2004). However, one study brings into question this purported mechanism for the curcumin effect (Song et al. 2004). Nevertheless, other recent reports document the correction of impaired folding mutations (Teijido et al. 2004) and the inhibition of aggregation formation (Yang et al. 2004) after curcumin treatment. We now document in cell culture the apparent release of proteins with severe *MPZ* alleles from ER retention, and we demonstrate less apoptosis for curcumin-treated misfolding mutants. These findings suggest that curcumin treatment may be sufficient to relieve the toxic effects associated with severe disease-causing MPZ mutations in whole animals.

Our observations could potentially be relevant for patients with severe peripheral neuropathies that result from protein misfolding in the ER, including those caused by MPZ, Cx32 (Deschenes et al. 1997), PMP22 (Naef et al. 1997; D'Urso et al. 1998; Dickson et al. 2002; Ryan et al. 2002), or a host of other proteins encoded by disease-associated CMT genes (Saifi et al. 2003; Szigeti and Lupski, in press). Although curcumin shows utility in cell culture, further studies are required in transgenic mouse models to evaluate its potential use for therapy of peripheral neuropathy disorders.

Acknowledgments

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Web Resources

The URLs for data presented herein are as follows:

Inherited Peripheral Neuropathies Mutation Database, http://www.molgen.ua.ac.be/CMTMutations/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for CMT1B, DSN, and CHN)

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