Deregulation of LRSAM1 expression impairs the levels of TSG101, UBE2N, VPS28,
MDM2 and EGFR

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Running Head: Deregulation of LRSAM1 expression

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Abstract

CMT is the most common hereditary neuromuscular disorder of the peripheral nervous system with a prevalence of 1/2500 individuals and it is caused by mutations in more than 80 genes. LRSAM1, a RING finger ubiquitin ligase also known as TSG101-associated ligase (TAL), has been associated with Charcot-Marie-Tooth disease type 2P (CMT2P) and to date eight causative mutations have been identified. Little is currently known on the pathogenetic mechanisms that lead to the disease. We investigated the effect of LRSAM1 deregulation on possible LRSAM1 interacting molecules in cell based models. Possible LRSAM1 interacting molecules were identified using protein-protein interaction databases and literature data. Expression analysis of these molecules was performed in both CMT2P patient and control lymphoblastoid cell lines as well as in LRSAM1 and TSG101 downregulated SH-SY5Y cells. TSG101, UBE2N, VPS28, EGFR and MDM2 levels were significantly decreased in the CMT2P patient lymphoblastoid cell line as well as in LRSAM1 downregulated cells. TSG101 downregulation had a significant effect only on the expression of VPS28 and MDM2 and it did not affect the levels of LRSAM1. This study confirms that LRSAM1 is a regulator of TSG101 expression. Furthermore, deregulation of LRSAM1 significantly affects the levels of UBE2N, VPS28, EGFR and MDM2.

Keywords: LRSAM1, TSG101, interacting molecules, CMT2P
**Introduction**

Charcot-Marie-Tooth (CMT) disease is the most common inherited neuropathy, characterized by peripheral nervous system degeneration, with a relatively high frequency (1:2,500 individuals) (1, 2). CMT disease is classified into demyelinating, axonal and intermediate types based on electrophysiological findings (3). It is further sub-divided into an increasing number of genetic subtypes with more than 80 CMT genes currently known.

Leucine Rich Repeat And Sterile Alpha Motif Containing 1 (LRSAM1) has been associated with Charcot-Marie-Tooth disease type 2P (CMT2P). LRSAM1, is a RING finger ubiquitin ligase, which participates in a range of functions, including cell adhesion, signaling pathways and cargo sorting through receptor endocytosis (4, 5). It is a multidomain protein with an N-terminal leucine-rich repeat (LRR) domain, followed by an Ezrin-Radixin-Moezin (ERM) domain, a coiled-coil (CC) region, a SAM domain, a PSAP/PTAP tetrapeptide motif and a RING finger domain (6). LRSAM1, also known as TAL (TSG101-associated ligase), regulates the metabolism of *TSG101* (Tumor Susceptibility gene 101) by polyubiquitination (7). *TSG101* is a tumor suppressor gene component of the ESCRT machinery (Endosomal Sorting Complex Required for Transport), with a significant role in cell cycle regulation and differentiation (5, 8). LRSAM1 binds to TSG101, through a bivalent binding of the PTAP motif and the CC domain of LRSAM1 (5, 9). After binding, LRSAM1 attaches several monomeric ubiquitins to the C-Terminal of TSG101, thus regulating its function. LRSAM1 mutations impair the LRSAM1-TSG101 interaction (10).

We identified a dominant *LRSAM1* mutation (c.2047-1G>A, p.Ala683ProfsX3) located in the RING finger domain of LRSAM1 (11). Recently, we reported that downregulation of *LRSAM1* affects the proliferation and morphology of neuroblastoma SH-SY5Y cells, and, overexpression of wild-type *LRSAM1* rescues, while the c.2047-1G>A mutant fails to rescue the phenotype of the
cells (12). To date, eight LRSAM1 mutations have been associated with CMT neuropathy, seven of them associated with dominant and one with recessive inheritance (13).

In order to study the role of LRSAM1 and the effect of the c.2047-1G>A mutant E3 ligase domain, we identified molecules that possibly interact with LRSAM1. Expression levels of selected molecules were evaluated in the LRSAM1 c.2047-1G>A CMT2P patient lymphoblastoid cell line and also in LRSAM1 knocked down SH-SY5Y cells. Since, TSG101 is the only currently well characterized interactor of LRSAM1 (5), we also performed TSG101 downregulation in SH-SY5Y cells and evaluated the levels of selected molecules in these cells as well.

**Results**

**Deregulation of LRSAM1 affects TSG101 expression**

Since TSG101 is an already known interactor of LRSAM1, we evaluated TSG101 levels in the CMT2P patient lymphoblastoid cell line. Western blot analysis of the CMT2P patient and control lymphoblastoid cell lines confirmed the presence of the wild-type and the mutant truncated LRSAM1 proteins in the patient sample, as previously reported (4), and revealed a more than 50% reduction of TSG101 levels in the patient lymphoblastoid cell line as compared with the controls (Figure 1).

With another approach, double transfection of LRSAM1 siRNA in neuroblastoma SH-SY5Y cells caused a >70% decrease of LRSAM1 levels which also resulted to an approximately 50% reduction of TSG101 levels. Double transfection of TSG101 siRNA caused a 70% decrease on TSG101 levels, while it did not affect the LRSAM1 levels (Figure 2).

**Protein-protein interaction network extraction of LRSAM1 possibly interacting molecules**

TSG101, UBE2N, VPS28, EGFR and MDM2 that possibly interact with LRSAM1 were identified using the protein-protein interaction database STRING (Figure 3) and IntAct. After literature
based evaluation these molecules were selected for further analysis. Furthermore, the AP-3 was selected for analysis since in a recent study LRSAM1 immunoprecipitated with AP-3 (14).

**RNA expression analysis of interacting molecules in lymphoblastoid cell lines**

We investigated RNA expression of candidate interacting molecules (TSG101, UBE2N, VPS28, EGFR, MDM2 and AP-3) in the CMT2P patient and control lymphoblastoid cell lines. mRNA levels of TSG101, UBE2N, VPS28, MDM2 and EGFR were significantly reduced in the patient lymphoblastoid cell line as compared to the control, while AP-3 presented an equal expression in both patient and control lymphoblastoid cell lines. Specifically, the patient sample showed significantly lower mRNA levels of TSG101 (43%), UBE2N (45%), VPS28 (32%), EGFR (41%) and MDM2 (35%) as compared to the control sample. In contrast AP-3 (98%) showed a similar expression between the patient and control samples (Figure 4).

**RNA expression analysis of interacting molecules in LRSAM1 or TSG101 downregulated SH-SY5Y cells**

RNA expression of the above candidate molecules was evaluated in LRSAM1 or TSG101 downregulated cells as well. Ninety-six hours after the first LRSAM1 siRNA transfection, we observed an approximately 70% reduction of endogenous LRSAM1 RNA levels, reproducing the previously reported effect (5). Downregulation of LRSAM1 also caused a significant decrease in approximately 50% of TSG101, UBE2N, VPS28, MDM2 and EGFR expression. In agreement with findings in the patient derived lymphoblastoid cell line, AP-3 levels were not affected by the LRSAM1 downregulation. Ninety-six hours after the first TSG101 siRNA transfection, we observed an approximately 70% reduction of endogenous TSG101 RNA levels. Downregulation of TSG101 caused an approximately 50% reduction of VPS28 and a 20% decrease of MDM2 levels. Normal levels of LRSAM1, UBE2N, EGFR and AP-3 were observed after TSG101 down
regulation (Figure 5). Although, \textit{LRSAM1} downregulation caused a significant reduction of TSG101 levels (55%), \textit{TSG101} downregulation did not affect the expression of \textit{LRSAM1}.

**Discussion**

\textit{LRSAM1} has been associated with CMT disease and currently its role in neurodegenerative disorders and in CMT pathogenesis is still unclear. In the present study we found that deregulation of \textit{LRSAM1} impaired \textit{TSG101}, \textit{UBE2N}, \textit{VPS28}, \textit{MDM2} and \textit{EGFR} levels. \textit{TSG101} downregulation had an effect on \textit{VPS28} and \textit{MDM2} levels.

The E3 ligase activity of \textit{LRSAM1} performs ubiquitination of TSG101, regulating its expression (5). TSG101 levels are also modulated by other mechanisms beyond \textit{LRSAM1} E3 regulation. \textit{MDM2}, another E3 ligase, shares an autoregulatory loop with TSG101 (15). TSG101 prevents its ubiquitination and increases the levels of \textit{MDM2} thus promoting ubiquitin degradation of p53 (15, 16). Over expression of truncated TSG101 in U20S cells caused reduction of \textit{MDM2} levels (15), while over expression of normal TSG101 doubled the short half-life of \textit{MDM2} and elevated its expression levels (15). Notably we detected that 70% downregulation of \textit{LRSAM1} caused 55% decrease in TSG101 and also 55% decrease in \textit{MDM2}, whereas 70% downregulation of TSG101, which did not affect \textit{LRSAM1}, caused only 20% decrease in \textit{MDM2} expression. These results indicate that TSG101 exerts a small effect on \textit{MDM2} expression (20%) relative to the effect of \textit{LRSAM1} (55%), the later through an unknown mechanism.

Interaction of TSG101 (yeast VPS23) with VPS28, VPS37 and MVB12 is necessary for the formation of the ESCRT-I complex (17). Binding between TSG101 and ESCRT-I subunits protects TSG101 degradation (9). VPS28 binds to the C-Terminal of TSG101 and blocks the interaction with \textit{LRSAM1}, thus subsequently decreases the ubiquitination of TSG101 (18). Reduced levels of VPS28 were observed in cells that lack TSG101 (18), while over expression of VPS28 did not affect the levels of TSG101 (9). In our study, we observed decreased levels of...
VPS28 in the CMT2P patient lymphoblastoid cell line and showed that down regulation of LRSAM1 or TSG101 caused a significant reduction in VPS28 levels. It is thus possible that mutant or knockdown LRSAM1 affects VPS28 levels directly or through the impaired TSG101 levels. TSG101 and/or VSP28 deficiency possibly affect the formation of the ESCRT-I complex. The ESCRT-I complex along with ESCRT-0, ESCRT-II and ESCRT-III form the endosomal sorting complexes required for transport (ESCRTs). Each ESCRT complex, consists of VPS proteins, and regulates biogenesis of the Multivesicular Body (MVB) and cargo sorting within the lysosomes. Besides endocytic trafficking, tumor suppression, autophagy, miRNA function and cancer, the ESCRT has been associated with neurodegenerative diseases (19, 20). In the central nervous system endosomal trafficking and lysosomal functions are necessary processes for the survival of neurons. A recent study showed that, lack of Hrs (component of ESCRT-I) caused accumulation of ubiquitinated protein and neurodegeneration in the central nervous system of a mouse model (21). In addition, mutated CHMP2B (component of ESCRT-III) leads to Frontotemporal dementia linked to chromosome 3 (FTD-3) because of autophagosome and ubiquitinated protein accumulation (20). It is thus possible that dysregulation of any ESCRT complex can affect the normal function of the ESCRT machinery and the central nervous system.

We observed a decreased expression of UBE2N in the patient lymphoblastoid cell line and also in LRSAM1 downregulated cells. A recent study showed, using a ubiquitination assay, that LRSAM1 interacts with UBC13, a mouse homolog of UBE2N and that mutations of LRSAM1 prevent this interaction, abolishing the ubiquitination activity of LRSAM1(10). UBE2N belongs to the family of Ubiquitin-conjugating enzymes (E2) and it is the only known enzyme that facilitates the creation of K63-linked polyubiquitination chains on proteins which are selected for proteasomal degradation (22, 23). E2 along with E1 and E3 enzymes consist the UPS system which removes the misfolded and abnormal proteins within the cells. Alzheimer, Parkinson and Huntington neurodegenerative diseases are caused by the formation of inclusions in the brain.
because of the accumulation of misfolded proteins in neurons (23), indicating a possible pathogenic role of the UPS system in neurodegeneration. Expression of UBE2N was found to be elevated in synaptosomes in tissues obtained from aged monkey brain while UPS activity was found to be depressed. The increased level of UBE2N in aged monkey brain or the over expressed UBE2N in HEK293 cells, caused the accumulation of mutant huntingtin (HTT). In contrast, the decreased level of UBE2N in aged monkey brain, HEK293 and PC12 cells caused reduction of mutant HTT accumulation (23). Knockdown of UBE2N in Parkin transfected HeLa cells inhibited the K63-linked polyubiquitination at mitochondrial sites suggesting a contribution of UBE2N in Parkin-mediated mitophagy (24).

The RING finger domain of LRSAM1 also plays a role in the regulation of EGFR expression (5). We observed that the LRSAM1 c.2047-1G>A mutation, located in the RING finger domain, results in impaired EGFR expression. Transfection of an inactive mutant-RING LRSAM1 has been reported to lead to the reduction of EGFR expression, while transfection of wild type LRSAM1 improved EGFR expression (5). Also in the presence of mutant TSG101 in NIH 3T3 and SL6 cells, EGFR moved from the early endosomes and MVBs to the plasma membrane for recycling (25). In our study, downregulation of TSG101 did not affect the expression of EGFR. The EGF receptor is a family member of the ErbB receptors of kinases and regulates essential signaling pathways including cell proliferation and differentiation, cell cycle and migration (26). Upregulation of EGFR has been associated with both oncogenesis and neurodegeneration. Expression of EGFR is activated in many malignancies such as breast cancer, prostate cancer and gliomas (27) and stimulates growth and differentiation of neurons (28). Increased levels of the EGFR ligand promoted neuronal death, while high EGFR immunoreactivity was detected in neurites near the neuritic plaques of Alzheimer disease. Lack of Presenilin (PS1), a regulator of EGFR expression, increased EGFR levels resulting to abnormal cell cycle re-entrance (27, 28).
AP-3 has been reported to form a heterotetrameric complex, targeting cargos of lysosomes and lysosome related organelles (29). Recently LRSAM1 was identified as an AP-3 interactor, because it immunoprecipitated with AP-3. It has been proposed, that while LRSAM1 is bound to AP-3, it regulates the ESCRT-I sorting function, by ubiquitinating certain cargoes (14). We found normal expression of AP-3 in the CMT2P patient lymphoblastoid cell line as well as after LRSAM1 downregulation in SH-SY5Y cells. These data, suggest that the interaction between the two molecules is probably controlled by AP-3 and not LRSAM1.

In conclusion, we confirm that TSG101 expression is regulated by LRSAM1. Decreased levels of UBE2N and EGFR were observed only after LRSAM1 knockdown thus indicating that these two molecules are regulated by a mechanism in which only LRSAM1 is involved. Decreased expression of VPS28 and MDM2 after either LRSAM1 or TSG101 downregulation demonstrates that the levels of these two molecules are regulated by a mechanism in which both LRSAM1 and TSG101 may be involved. Further investigation of the above molecules may enable delineation of the pathways that lead to CMT2P pathology.

**Materials and Methods**

In this study, we selected possible LRSAM1 interacting molecules and investigated their expression levels in lymphoblastoid cell lines as well as in LRSAM1 and TSG101 downregulated neuroblastoma SH-SY5Y cells.

**Cell culture**

**a. Lymphoblastoid cell cultures**

Lymphoblastoid cell lines were established from a CMT2P patient and two normal control individuals using peripheral blood. Lymphocytes were collected from peripheral blood using
Selected lymphocytes were infected with the Epstein-Barr virus (EBV) and were cultured in DMEM medium supplemented with 2% FBS.

b. Human SH-SY5Y neuroblastoma cells culture

Human SH-SY5Y neuroblastoma cells (ECACC, Sigma-Aldrich, U.S.A.), were cultivated in Dulbecco's Modified Eagle Medium DMEM (Invitrogen, U.S.A.) growth medium without L-glutamine. The DMEM medium was supplemented with 10% FBS (Invitrogen, U.S.A.), 2% GlutaMAX™ (Gibco, U.S.A.) and 1% Penicillin-Streptomycin 100X Solution (Invitrogen, U.S.A.). Medium was changed every 2 or 3 days and 0.25% Trypsin-EDTA (Life Technologies, U.S.A.) was used for routine splitting of the cell culture. Both cell lines were incubated in a humidified atmosphere under 5% CO₂ at 37°C.

Whole human LRSAM1 constructs

The pIRES2-EGFP-LRSAM1 wild-type and mutant constructs were purchased from Eurofins (Germany) as previously described (12). The mutant LRSAM1 cDNA construct included a G base deletion at the first base of exon 25, creating the frame shift at the RNA level (11).

Downregulation of LRSAM1 or TSG101 in neuroblastoma SH-SY5Y cells

Transfections were performed using the Lipofectamine® 3000 (C3019H, Life Technologies, U.S.A.) The siRNAs against LRSAM1 or TSG101 (Life Technologies, USA) were double-transfected into SH-SY5Y cells as previously described (12) and according to the manufacturer’s instructions. The appropriate amount of siRNA and Lipofectamine® 3000 were dissolved separately in the Opti-MEM® reduced serum medium (Life Technologies, U.S.A.) without FBS and antibiotics. Negative control siRNA (Life Technologies, USA), lipofectamine only and untransfected cells were used as controls of the experiments. Twenty-four hours after each transfection, medium was replaced with fresh DMEM medium. Cells were harvested 96 hours after the first transfection for protein and RNA extraction.
Protein-protein interaction database

In silico analysis was carried out using the STRING9.05&10.0 (http://string-db.org/) and IntAct (http://www.ebi.ac.uk/intact/) databases. Extracted LRSAM1 possibly interacting molecules were selected for RNA expression analysis after literature evaluation.

RNA isolation and cDNA synthesis from experimental SH-SY5Y cells and lymphoblastoid cell lines

Cells were collected in PBS and total RNA was isolated using the Qiagen RNeasy kit (Qiagen, U.S.A.). 1% β-Mercaptoethanol (Sigma-Aldrich, U.S.A.) was added in lysis buffer before use. Whole cDNA was synthesized using the ProtoScript® First Strand cDNA Synthesis Kit (New England Biolabs, U.K.) using the oligo-dT primer d(T)23VN according to the manufacturer instructions.

RNA expression levels

Expression levels of TSG101, UBE2N, VPS28, MDM2, EGFR and AP-3 were evaluated by cDNA PCR amplifications. At least two sets of primers specific for each molecule and also for the GAPDH housekeeping gene were used. For each set of primers a standard curve was plotted in order to define the optimum number of PCR reaction cycles that fall within the exponential phase of the amplification reaction (Supplementary Figure 1, available upon request). RNA levels of the patient and control samples as well as of the LRSAM1 and TSG101 knocked down cells were normalized using the GAPDH housekeeping RNA levels. Gel bands were measured using the ImageJ analysis software.
Protein extraction from experimental SH-SY5Y cells and lymphoblastoid cell lines

Proteins were extracted using a protein lysis buffer, that contained 1M NaCl, 10mM Tris-Cl (pH=7.5), 10% glycerol (G5516, Sigma-Aldrich, U.S.A), 1% Tween²⁰ (Affymetrix, U.S.A.), 10Mm β-Mercaptoethanol (Sigma-Aldrich, U.S.A) and 1x EDTA-free Protease Inhibitor Cocktail (Promega, U.S.A.). Cell lysates were centrifuged at 4°C and supernatants were collected. Lysates were sonicated and denatured at 95°C. Then proteins were diluted in 1x Sodium Dodecyl Sulfate 20% (ThermoFisher Scientific, U.K.). The Coomassie Plus (Bradford) protein assay (23236, ThermoFisher Scientific, U.K.) was used to determine the protein concentration.

Western blot analysis

Proteins were loaded and separated onto SDS-PAGE 12% polyacrylamide gels and were transferred to Hybond-P hydrophobic polyvinylidene difluoride (PVDF) membranes (Millipore, Germany). Membranes were blocked for 1 hour at room temperature and were incubated overnight at 4°C with the respective specific primary antibody for each protein [mouse anti-LRSAM1/Abcam ab73113 (1:400), rabbit anti-LRSAM1/Novus Biological H00090678-D01 (1:750), mouse anti-TSG101/Novus Biological NB200-11 (1:500) and mouse anti-β-ACTIN/Sigma-Aldrich A2228 (1:4000)]. Then, incubation with the appropriate secondary antibodies was performed for 2 hours and followed by incubation with the visualization LumiSensor™ Chemiluminescent HRP Substrate Kit (Genscript, U.S.A.) at room temperature. Membranes were visualized using the UVP imaging system (BioRad, U.S.A.). Quantification of Western blots was performed using the ImageJ program (https://imagej.nih.gov). The protein quantity ratio was estimated relative to β-Actin.
Statistical analysis

Quantitative data (ratio %) from three independent experiments were analyzed using the two-tailed Student’s paired t-test. Thresholds of P-value < 0.05 were considered statistically significant. All the data are expressed as the mean% ± standard deviation (SD) from three independent experiments. The mean of quantitative data of the control samples was set to 100%.

Acknowledgements

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Conflict of Interest Statement

The authors declare no conflict of interest.
References


Legends to Figures

Figure 1: Investigation of TSG101 levels in lymphoblastoid cell lines. Western blot analysis of TSG101 levels in the patient and control (1, 2) cell lines. β-ACTIN was used as an internal control. Quantification of TSG101 was performed relative to β-ACTIN. Control 1 values were set to 100%. **TSG101 levels**: Patient lymphoblastoid cell line (45±4.02%, p<0.03998), Control 1 lymphoblastoid cell line (103±1.24%, p<0.02147), Control 2 lymphoblastoid cell line (98±2.49%).

Figure 2: Efficiency of LRSAM1 and TSG101 downregulation. (A) Western blot analysis of LRSAM1 and TSG101 levels, compared with the controls (Negative control siRNA, Lipofectamine only, Untransfected cells) 96 hours after the first transfection. β-ACTIN was used as an internal control. (B) Quantification of LRSAM1 and TSG101 was performed relative to β-ACTIN. Untransfected cells values (LRSAM1 and TSG101) were set to 100% and reflect the normal protein levels within the 5 days of the experimental procedure. **LRSAM1 levels**: LRSAM1 siRNA transfected cells (25±3.94%, p<0.004), TSG101 siRNA transfected cells (91±2.47%, p<0.0244), Negative control siRNA transfected cells (90±2.89%, p<0.009), Lipofectamine only transfected cells (94±2.15%, p<0.008) and Untransfected cells (100±1.36%). **TSG101 levels**: LRSAM1 siRNA transfected cells (53±2.72%, p<0.007), TSG101 siRNA transfected cells (32±1.64%, p<0.0174), Negative control siRNA transfected cells (84±2.20%, p<0.027), Lipofectamine only transfected cells (93±2.49%, p<0.040) and Untransfected cells (100±4.51%).

Figure 3: Protein–protein interaction network determined by STRING analysis for LRSAM1 protein. The interaction sources text mining, experiments, databases, co-expression, neighborhood, gene fusion and co-occurrence were used for the selection of the proteins.

Figure 4: Investigation of LRSAM1 interacting molecules in the CMT2P patient lymphoblastoid cell line. (A) RNA expression analysis of most likely interacting molecules in patient and control samples. GAPDH was used as a housekeeping internal control. (B) Quantification of the expression levels of the molecules relative to the control sample, which was set to 100. Values were obtained as ratios of the RNA of interest over GAPDH control. Specifically, the patient lymphoblastoid cell line showed significantly lower levels of TSG101 (43%±0.28%, p<0.005), UBE2N (45%±0.97%, p<0.0109), VPS28 (32%±3.28%, p<0.031), EGFR (41%±1.88%, p<0.012) and MDM2 (35%±2.01%, p<0.025) than the control sample. In contrast AP-3 (98%±3.47%, p<0.041) showed similar expression between the patient and control. Amplification of each primer set was repeated in triplicate.

Figure 5: Investigation of LRSAM1 interacting molecules in LRSAM1 or TSG101 downregulated cells. Quantification of the RNA expression levels of the interacting molecules relative to the Untransfected control cells, which was set to 100 96h after the first siRNA transfection. Values were obtained as ratios of the RNA of interest over GAPDH control. Specifically, **LRSAM1 downregulation** significantly decreased the levels of LRSAM1 (30%±3.86%, p<0.0275), TSG101 (45%±2.19%, p<0.0092), UBE2N (52%±3.05%, p<0.0158), VPS28 (47%±3.62%, p<0.0336), EGFR (49%±3.38%, p<0.0125) and MDM2 (45%±2.44%, p<0.096) compared to the Untransfected control cells. In contrast, AP-3 (96%±2.59%, p<0.0109) showed similar expression between the LRSAM1 knocked down and Untransfected cells. **TSG101 downregulation** significantly decreased the levels of TSG101 (33%...
±1.11%, p<0.0068) and VPS28 (55%±2.86%, p<0.0154) and also caused a small decrease of MDM2 (79%±3.37%, p<0.0217) compared to the Untransfected cells. Expression levels of LRSAM1 (96% ±4.41%, p<0.0427), UBE2N (94%±2.97%, p<0.0172), EGFR (96%±3.96%, p<0.0298) and AP-3 (99%±4.57%, p<0.0462) were similar to the Untransfected cells.
### Abbreviations

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<td>Adaptor Protein 3</td>
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<td>CC</td>
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<td>CMT</td>
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<td>DMEM</td>
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<td><img src="image17" alt="Image" /></td>
</tr>
</tbody>
</table>

Legend:
- **LRSAM1 siRNA**
- **TSG101 siRNA**
- **Negative control siRNA**
- **Lipofectamine only**
- **Untransfected cells**
A. 

<table>
<thead>
<tr>
<th>Gene</th>
<th>Blank</th>
<th>Patient</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRSAM1</td>
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<tr>
<td>TSG101</td>
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<td></td>
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<tr>
<td>UBE2N (E2)</td>
<td></td>
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<tr>
<td>MDM2</td>
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<tr>
<td>VPS28</td>
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<tr>
<td>EGFR</td>
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<tr>
<td>AP-3</td>
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<td></td>
</tr>
<tr>
<td>GAPDH</td>
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</tr>
</tbody>
</table>

B. 

RNA Levels (%)

- **Patient Lymph. cell line**
- **Control Lymph. cell line**

- **LRSAM1**
- **TSG101**
- **UBE2N (E2)**
- **MDM2**
- **VPS28**
- **EGFR**
- **AP-3**

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