Supplemental Materials

Molecular Biology of the Cell

Caneus et al.

Supplementary Material:

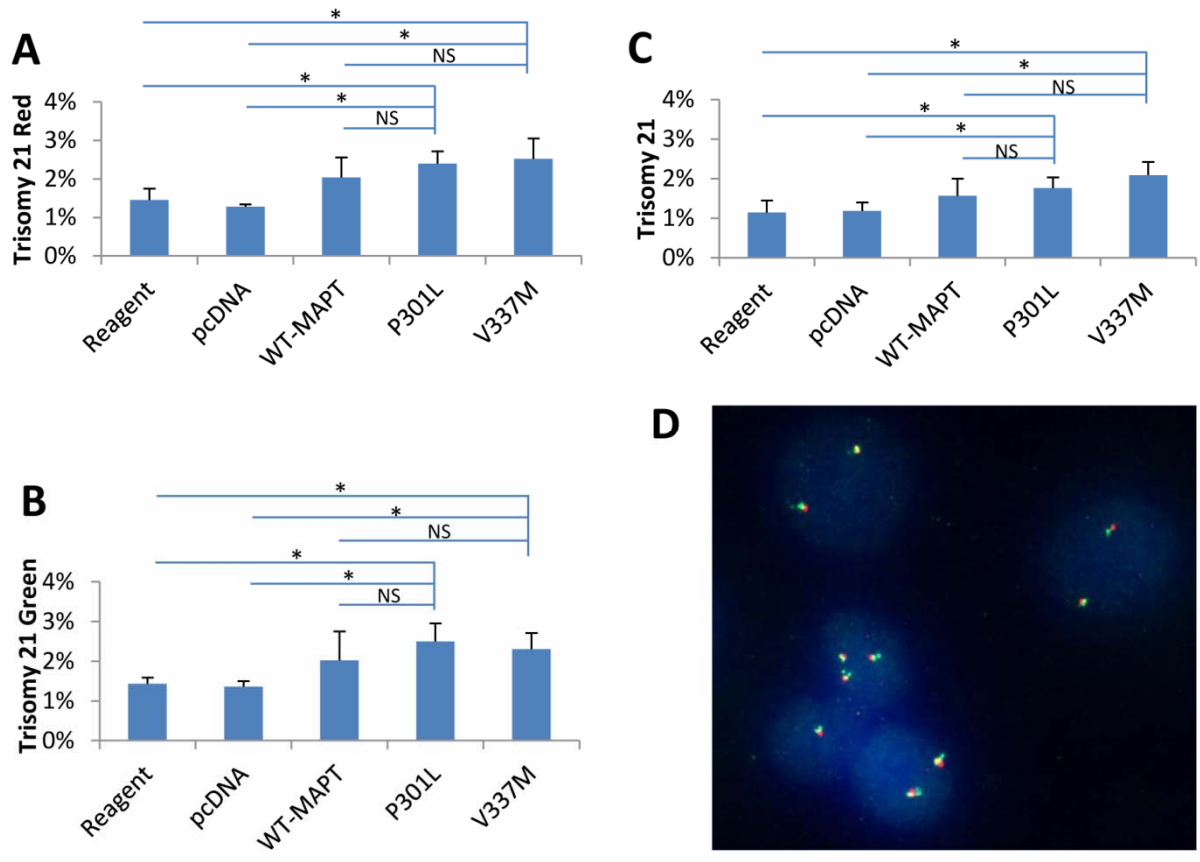
Supplemental Materials and Methods

FISH Analysis Using Two Independent Chromosome 21 Probes Together Confirms Increased Chromosome 21 Trisomy and Aneuploidy in hTERT Cells Transfected with Human Mutant MAPT genes

To further assess the levels of aneuploidy with minimal potential effects due to artifacts (e.g., signal mischaracterization, incomplete hybridization, broken chromosomes, etc.) and to corroborate our previous results, we carried out additional chromosomal FISH analyses using a probe mixture containing two independent probes that bind to two different, yet nearby, regions of human chromosome 21 with single nuclei suspensions prepared from hTERT cells transfected with mutant MAPT transgenes (at 48 h post-transfection), as described previously. Detection of probe signal, followed by statistical analysis revealed a significantly higher percentage of dual-labeled trisomy 21 (Supplemental Figure 1) cells in the samples expressing mutant MAPT relative to samples transfected with the pcDNA vector alone. These results further confirm data obtained previously using the dual color probe sets for detection of chromosomes 12 and 21 (Figures 5 and 6).

Expression of FTLD-Causing Forms of MAPT in hTERT-HME1 Cells Does Not Induce Oxidative Stress

In this experiment, hTERT-HME1 cells were plated on a 96-well plates and allowed to grow to 50-75% confluency. The cells were transfected with the pcDNA3.1 vector alone or with the pcDNA3.1 vector with wild-type MAPT (WT-MAPT) or with FTLD mutant MAPT genes (*P301L* or *V337M*) in replicates of six using the FuGENE6 transfection reagent (Promega, Cat. E2691). Negative controls included untransfected cells (Cell), cells treated with the transfection reagent alone (Reagent), and cells transfected with the pCDNA3.1 vector alone. The samples were then incubated at 37°C for 72 h following transfection. After the transfection period, the cells were harvested and prepared for oxidative stress analysis using the Cellular ROS/Superoxide Detection Assay Kit (Abcam, cat. Ab139476). A portion of the wells were used as positive control samples. According to the manufacturer's recommended procedure, the positive control cells were treated with pyocyanin (PC) and incubated for 30 min under normal cell culture conditions. Following the incubation period, all of the samples were rinsed with wash buffer, treated with the ROS/Superoxide Detection mixture in growth media, and then incubated for 1 h at 37°C, 5% CO_2. The samples were then retrieved, and fluorescence intensity was measured using a Synergy™ H4 Hybrid Multi-Mode Microplate Reader from BioTek simultaneously across all of the different samples to obtain measurements of oxidative stress levels, including ROS (2A) and superoxide (2B). This experiment was repeated three more times, and the average fluorescence intensity was computed to plot the differences between all the different samples. One-way ANOVA with Bonferroni correction and paired student t-test were used to assess statistical significance among all the different samples.


A
Trisomy 21 Red
4%
3%
2%
1%
0%
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NS
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Reagent
pcDNA
WT-MAPT
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V337M
B
Trisomy 21 Green
4%
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C
Trisomy 21
4%
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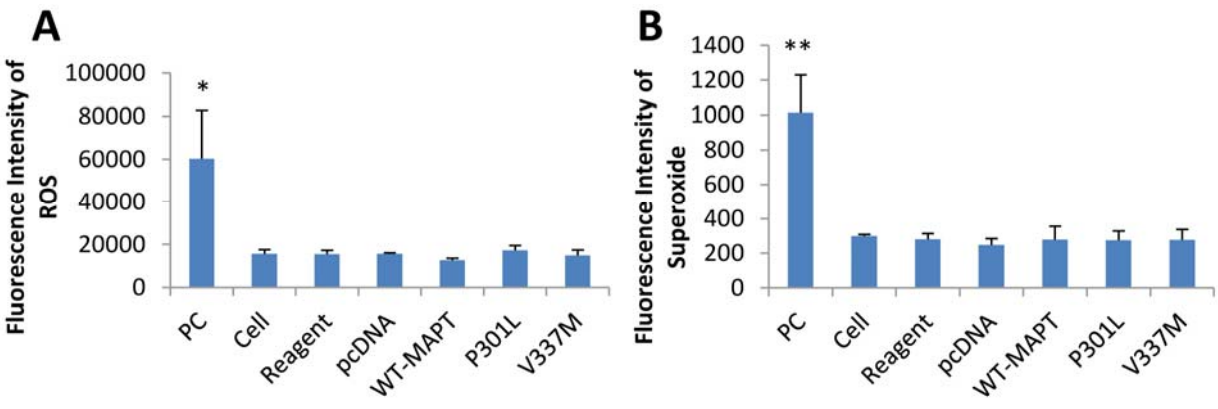
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Fluorescence Intensity of ROS
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80000
60000
40000
20000
0
*
PC
Cell
Reagent
pcDNA
WT-MAPT
P301L
V337M
B
Fluorescence Intensity of Superoxide
1400
1200
1000
800
600
400
200
0
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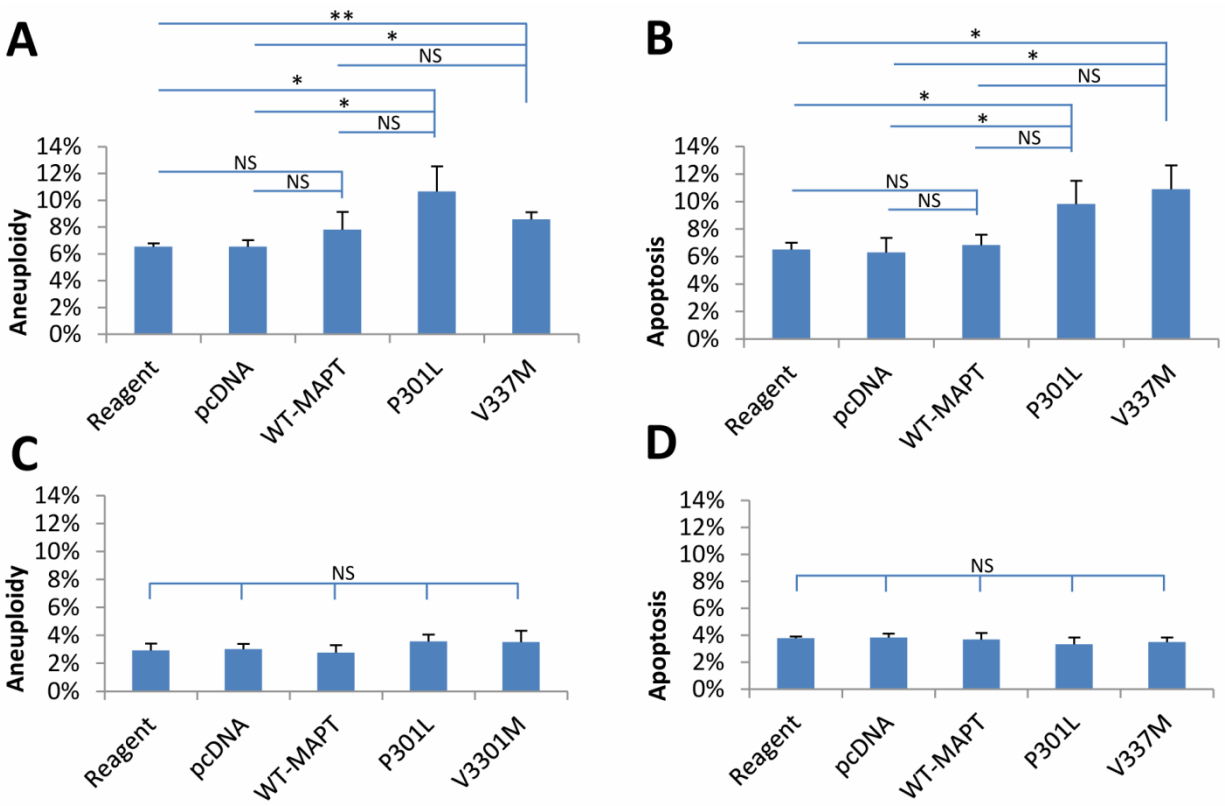
PC
Cell
Reagent
pcDNA
WT-MAPT
P301L
V337M


A
Aneuploidy
14%
12%
10%
8%
6%
4%
2%
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NS
NS
Reagent
pcDNA
WT-MAPT
P301L
V337M
B
Apoptosis
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NS
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C
Aneuploidy
NS
Reagent
pcDNA
WT-MAPT
P301L
V3301M
D
Apoptosis
NS