CALR 52 bp Mutation Impairs Oxidative Stress Response and Increases Oxidative Stress-induced Apoptosis Level in UT-7 Cell Line

Roberta Vadeikienė[1], Rasa Ugenskienė[1,2], Elona Juozaitytė[3]

[1]Oncology Research Laboratory, Institute of Oncology, Lithuanian University of Health Sciences, Kaunas, Lithuania; [2]Department of Genetics and Molecular Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania; [3]Institute of Oncology, Lithuanian University of Health Sciences, Kaunas, Lithuania

7th Kaunas / Lithuania International Hematology / Oncology Colloquium
26 May 2022

Objective

BCR-ABL1-negative classic myeloproliferative neoplasms (MPN) include primary myelofibrosis, polycythemia vera, and essential thrombocythemia. Calreticulin (*CALR*) 52 bp deletion and 5 bp insertion were discovered to be involved in MPN pathogenesis, particularly in *JAK2* and *MPL* unmutated essential thrombocythemia and primary myelofibrosis. Calreticulin, a Ca^{2+}-binding chaperone, is implicated in Ca^{2+} homeostasis, protein folding, and response to oxidative stress. It is well known that oxidative stress induces the accumulation of reactive oxygen species (ROS) that damage membrane lipids, proteins, and DNA. Moreover, several studies demonstrated that MPN patients show high serum levels of intracellular ROS, which can lead to chronic inflammation and genomic instability. However, there is not much data on how mutated calreticulin affects oxidative stress response and oxidative stress-induced apoptosis. Therefore, we aimed to investigate the response to oxidative stress and apoptosis induction in UT-7 cells expressing either *CALR* WT or *CALR* 52 bp deletion.

Methods

- The UT-7 cell line was used in our study.
- CRISPR/Cas9 system was chosen for *CALR* 52 bp deletion initiation in cells. After the selection of potential DNA targets in gDNA, corresponding tabs were cloned into a vector (pSpCas9(BB)-2A-Puro (PX459) V2.0) that is optimized for Cas9 and RNA-guided expression in eukaryotic cells.
- Transfection of plasmid construct and HDR template into UT-7 cells was performed by electroporation. The following electrotransfection parameters were applied: 1 HV pulse of 1600 V/cm with 500 µs pulse duration and the electroporation system BTX T820 was used. The transfected cells were selected in puromycin (1 µg/ml).
- Further, UT-7 cells expressing WT and *CALR* 52 bp deletion were treated with H_2O_2 for 24 hours. The intracellular oxidative stress and apoptosis induced by H_2O_2 were measured by means of Muse® Oxidative Stress Kit and Annexin V & Dead Cell Kit, respectively. Cells were examined using the Muse® Cell Analyzer and at least 5000 events were detected for each sample.

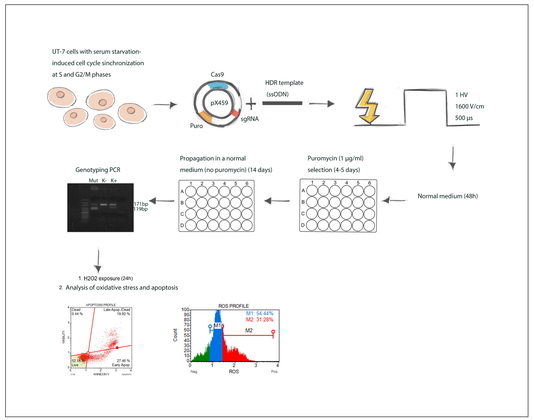

Figure 1. Overall schematic representation underlying experimental workflow.

Results

•To assess whether *CALR* 52 bp deletion can impact the response to oxidative stress and oxidative stress-induced apoptosis, UT-7 cells expressing either the WT or 52 bp deletion variants of *CALR* were treated with H_2O_2 for 24h. Our results showed that UT-7 cells expressing *CALR* 52 bp deletion exhibit higher levels of oxidative stress (i.e., ROS-positive cells) compared to cells expressing wild-type *CALR* (49.07% ± 2.96 *vs* 39.02% ± 4.38, p=0.015).

•These differences are more evident after cells were given 24 additional hours to reduce ROS accumulation induced by H_2O_2. After 24 h of repair, cells expressing *CALR* 52 bp deletion were unable to reduce ROS level. UT-7 cell line with mutated calreticulin exposed 51.53% ± 6.06 ROS positive cells. Whereas UT-7 cells with *CALR* WT were able to efficiently counteract the ROS accumulation (29.49% ± 1.65, p=0.003).

•Further, we analyzed whether the different level of oxidative stress has an impact on different ability to induce apoptosis. Our results revealed an increase in oxidative stress-induced apoptosis levels in UT-7 cells with *CALR* 52 bp deletion (27.5% ± 4,97) compared to *CALR* WT cells (25.00% ± 3.43).

•These *in vitro* data demonstrated that *CALR* 52 bp deletion impairs cell ability to respond to oxidative stress. Moreover, *CALR* Del52 cells can be characterized by a higher apoptosis level compared to *CALR* WT.

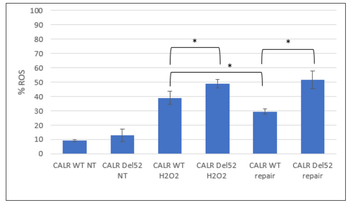

*Figure 2. Results of analysis of oxidative stress level in UT-7 cell line expressing either wild-type or mutated CALR after 24 h of treatment with H_2O_2 (400 µM) and after 24 h of repair. Data are displayed as a mean of the percentage of ROS-positive cells ± SD of three independent experiments. Differences between groups were evaluated using an independent sample t-test. Error bars indicate the SD of the mean. Significant differences at p<0.05 are presented as *. Abbreviations: NT – not treated, WT – wild-type, SD – standard deviation.*

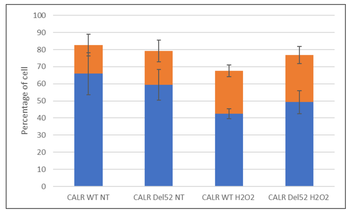

Figure 3. Representative bar graphs for oxidative stress-induced apoptosis in UT-7 cells expressing either wild-type or mutated CALR after 24 h of treatment with H_2O_2 (400 µM). Data are reported as mean ± SD of three independent experiments. Error bars indicate the SD of the mean. Abbreviations: NT – not treated, WT – wild-type, SD – standard deviation.

Conclusions

Our results demonstrated that the UT-7 cell line with *CALR* 52 bp deletion can be characterized by a greater increase of intracellular oxidative stress and have a higher apoptosis level compared to cells expressing wild-type *CALR* following the exposure to H_2O_2.

Key words

Myeloproliferative neoplasms; *CALR* 52 bp deletion; oxidative stress; apoptosis; UT-7 cell line; CRISPR/Cas9.