Further evidence: UCHL1 regulated by Sp1 ameliorates cochlear hair cell senescence and oxidative damage

Sakura Watanabe, Marta Marchetti, Jonas Hartmann, Felix Hansen

Age-related hearing loss (ARHL) is the most common cause of hearing loss in the elderly. Ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) is a deubiquitinating enzyme involved in several types of human disease. The present study aimed to investigate the effect of UCHL1 on a hydrogen peroxide (H 2 O 2)-induced ARHL model in cochlear hair cells and uncover its underlying mechanism. Reverse transcription-quantitative (RT-q)PCR and western blot analysis were used to assess UCHL1 expression in HEI-OC1 cells exposed to H 2 O 2 . Following UCHL1 overexpression in H 2 O 2 -induced HEI-OC1 cells, cell activity was assessed by Cell Counting Kit-8 assay. The content of oxidative stress-associated markers including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and reactive oxygen species (ROS) was measured using corresponding commercial kits. Cell apoptosis was evaluated by TUNEL assay and western blot analysis. Cell senescence was assessed by senescence-associated ?-galactosidase staining and western blot analysis. RT-qPCR and western blot analysis were applied to measure mRNA and protein expression levels, respectively, of specificity protein 1 (Sp1) in H 2 O 2 -treated HEI-OC1 cells. In addition, the association between UCHL1 and Sp1 was verified by luciferase reporter and chromatin immunoprecipitation (ChIP) assay. The mRNA and protein expression levels of UCHL1 were also determined in Sp1-overexpressing cells by RT-qPCR and western blot analysis, respectively. Following Sp1 overexpression in UCHL1-overexpressing H 2 O 2 -treated HEI-OC1 cells, cell activity, oxidative stress, apoptosis and senescence were assessed. Finally, the expression levels of NF-?B signaling-related proteins p-NF-?B p65 and NF-?B p65 were detected using western blot analysis. The results showed that UCHL1 was downregulated in H 2 O 2 -treated HEI-OC1 cells. In addition, UCHL1 overexpression enhanced cell viability and promoted oxidative damage, apoptosis and senescence in H 2 O 2 -induced HEI-OC1 cells. Furthermore, Sp1 was upregulated in H 2 O 2 -treated HEI-OC1 cells. Additionally, luciferase reporter and ChIP assays demonstrated that Sp1 interacted with the UCHL1 promoter to inhibit UCHL1 transcription. Sp1 overexpression reversed the effect of UCHL1 overexpression on cell viability, oxidative stress, apoptosis, senescence and activation of the NF-?B signaling pathway in H 2 O 2- exposed HEI-OC1 cells. Collectively, the results suggested that UCHL1 transcriptional suppression by Sp1 protected cochlear hair cells from H 2 O 2 -triggered senescence and oxidative damage.

This is the demonstration PDF of the article.

Age-related hearing loss (ARHL) is the most common cause of hearing loss in the elderly. Ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) is a deubiquitinating enzyme involved in several types of human disease. The present study aimed to investigate the effect of UCHL1 on a hydrogen peroxide (H 2 O 2)-induced ARHL model in cochlear hair cells and uncover its underlying mechanism. Reverse transcription-quantitative (RT-q)PCR and western blot analysis were used to assess UCHL1 expression in HEI-OC1 cells exposed to H 2 O 2 . Following UCHL1 overexpression in H 2 O 2 -induced HEI-OC1 cells, cell activity was assessed by Cell Counting Kit-8 assay. The content of oxidative stress-associated markers including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and reactive oxygen species (ROS) was measured using corresponding commercial kits. Cell apoptosis was evaluated by TUNEL assay and western blot analysis. Cell senescence was assessed by senescence-associated ?-galactosidase staining and western blot analysis. RT-qPCR and western blot analysis were applied to measure mRNA and protein expression levels, respectively, of specificity protein 1 (Sp1) in H 2 O 2 -treated HEI-OC1 cells. In addition, the association between UCHL1 and Sp1 was verified by luciferase reporter and chromatin immunoprecipitation (ChIP) assay. The mRNA and protein expression levels of UCHL1 were also determined in Sp1-overexpressing cells by RT-qPCR and western blot analysis, respectively. Following Sp1 overexpression in UCHL1-overexpressing H 2 O 2 -treated HEI-OC1 cells, cell activity, oxidative stress, apoptosis and senescence were assessed. Finally, the expression levels of NF-?B signaling-related proteins p-NF-?B p65 and NF-?B p65 were detected using western blot analysis. The results showed that UCHL1 was downregulated in H 2 O 2 -treated HEI-OC1 cells. In addition, UCHL1 overexpression enhanced cell viability and promoted oxidative damage, apoptosis and senescence in H 2 O 2 -induced HEI-OC1 cells. Furthermore, Sp1 was upregulated in H 2 O 2 -treated HEI-OC1 cells. Additionally, luciferase reporter and ChIP assays demonstrated that Sp1 interacted with the UCHL1 promoter to inhibit UCHL1 transcription. Sp1 overexpression reversed the effect of UCHL1 overexpression on cell viability, oxidative stress, apoptosis, senescence and activation of the NF-?B signaling pathway in H 2 O 2- exposed HEI-OC1 cells. Collectively, the results suggested that UCHL1 transcriptional suppression by Sp1 protected cochlear hair cells from H 2 O 2 -triggered senescence and oxidative damage.

This is the demonstration PDF of the article.

Age-related hearing loss (ARHL) is the most common cause of hearing loss in the elderly. Ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) is a deubiquitinating enzyme involved in several types of human disease. The present study aimed to investigate the effect of UCHL1 on a hydrogen peroxide (H 2 O 2)-induced ARHL model in cochlear hair cells and uncover its underlying mechanism. Reverse transcription-quantitative (RT-q)PCR and western blot analysis were used to assess UCHL1 expression in HEI-OC1 cells exposed to H 2 O 2 . Following UCHL1 overexpression in H 2 O 2 -induced HEI-OC1 cells, cell activity was assessed by Cell Counting Kit-8 assay. The content of oxidative stress-associated markers including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and reactive oxygen species (ROS) was measured using corresponding commercial kits. Cell apoptosis was evaluated by TUNEL assay and western blot analysis. Cell senescence was assessed by senescence-associated ?-galactosidase staining and western blot analysis. RT-qPCR and western blot analysis were applied to measure mRNA and protein expression levels, respectively, of specificity protein 1 (Sp1) in H 2 O 2 -treated HEI-OC1 cells. In addition, the association between UCHL1 and Sp1 was verified by luciferase reporter and chromatin immunoprecipitation (ChIP) assay. The mRNA and protein expression levels of UCHL1 were also determined in Sp1-overexpressing cells by RT-qPCR and western blot analysis, respectively. Following Sp1 overexpression in UCHL1-overexpressing H 2 O 2 -treated HEI-OC1 cells, cell activity, oxidative stress, apoptosis and senescence were assessed. Finally, the expression levels of NF-?B signaling-related proteins p-NF-?B p65 and NF-?B p65 were detected using western blot analysis. The results showed that UCHL1 was downregulated in H 2 O 2 -treated HEI-OC1 cells. In addition, UCHL1 overexpression enhanced cell viability and promoted oxidative damage, apoptosis and senescence in H 2 O 2 -induced HEI-OC1 cells. Furthermore, Sp1 was upregulated in H 2 O 2 -treated HEI-OC1 cells. Additionally, luciferase reporter and ChIP assays demonstrated that Sp1 interacted with the UCHL1 promoter to inhibit UCHL1 transcription. Sp1 overexpression reversed the effect of UCHL1 overexpression on cell viability, oxidative stress, apoptosis, senescence and activation of the NF-?B signaling pathway in H 2 O 2- exposed HEI-OC1 cells. Collectively, the results suggested that UCHL1 transcriptional suppression by Sp1 protected cochlear hair cells from H 2 O 2 -triggered senescence and oxidative damage.

This is the demonstration PDF of the article.