Notch ligand Jagged1 in preserving DNA integrity

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CELL BIOLOGY

INTRODUCTION

The Notch signalling pathway is a highly conserved pathway, which has a significant role in development and in the maintenance of homeostasis. Notch regulates cellular apoptosis, proliferation and differentiation. Notch signalling has also been linked to different cancers either as an oncogenic or as a tumour suppressive factor.

Breast cancer is the most common cancer type among women worldwide. Hallmarks of cancer include an increased amount of DNA damage as well as changes in cellular proliferation.

Overexpression of the Notch ligand Jagged1 has been linked to aggressive breast cancer types with poor prognosis. The aim of this project was to investigate the role of Jagged1 in preserving DNA integrity during replication stress and mitosis. The tumor suppressor p53 has defined roles in G1/S and G2/M cell cycle checkpoints in response to a range of cellular stresses including DNA damage. One part of the project was also to validate a previous result of protein-protein interaction between Jagged1 and p53 observed in a proteomic screen for Jagged interactors during mitosis.

METHODS

Two different MDA-MB-231 breast cancer cell lines were used in this study: wild type (WT) and Jagged1 knockout (Jag1KO) cell lines.

To investigate the role of Jagged1 in the DNA damage response cells were treated with 2 mM hydroxyurea for 6h or 24h. The effect of treatment was studied using Western blotting and immunofluorescence staining using antibodies against DNA damage markers.

For the proliferation studies, equal amounts of cells were plated. The nuclei were stained with Hoechst 33342 -reagent and imaged at timepoints 0h, 24h, 48h and 72h.

To validate previous mass spectrometry results of protein-protein interaction in mitotic 293HEK (human embryonic kidney) cells, immunoprecipitation was used. Cell cycle synchronisation was done by using double thymidine block.

RESULTS

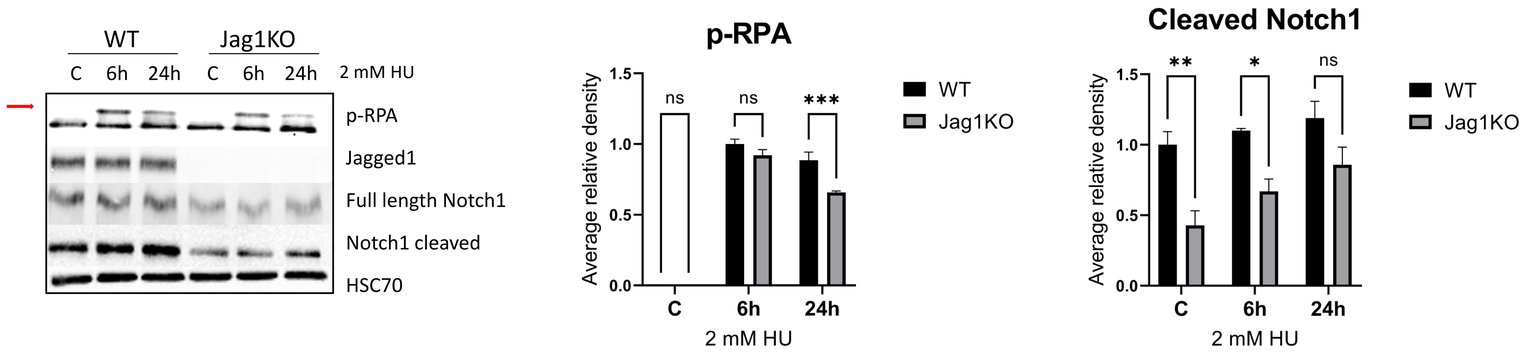

Figure 1. Jag1KO cells have a decreased replication stress response. WT and Jag1KO cells were treated with 2 mM hydroxyurea (HU) for 6h or 24h to induce replication stress. Phosphorylated replication protein A (p-RPA) was used as a marker for single stranded DNA damage in response to replication stress. In Jag1KO cells the replication stress response was decreased compared to WT cells. Jag1KO cells also demonstrated reduced Notch activity and levels of cleaved Notch1.

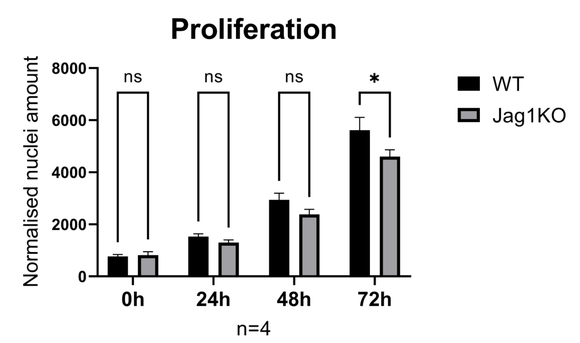

Figure 3. Jag1KO cells proliferate slower. Proliferation assay showed a decrease in the proliferation rate of Jag1KO cells compared to WT cells. Nuclei were stained and counted at 24, 48 and 72 hours after plating. At the 72h timepoint the difference in proliferation was significant (p=0.02).

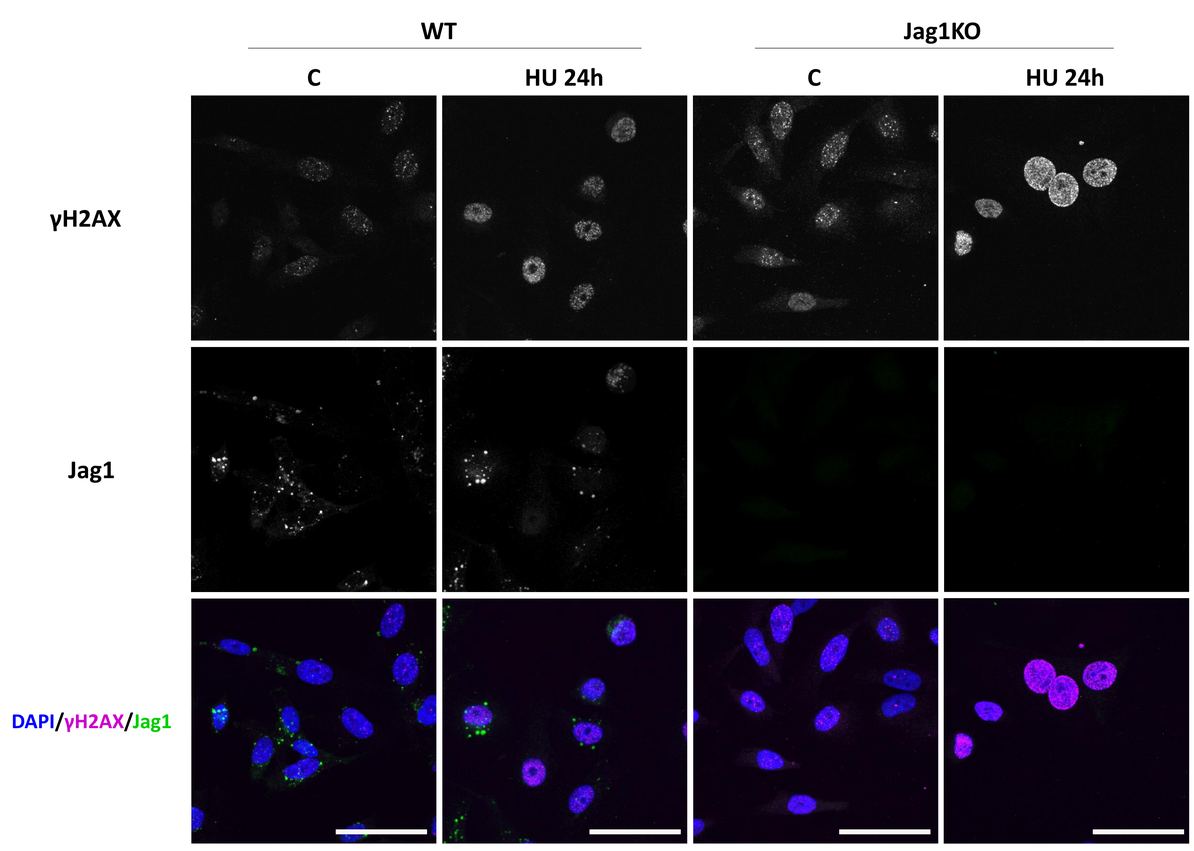

Figure 2. Loss of Jagged1 causes more DNA damage in MDA-MB-231 breast cancer cells. Cells were treated with 2 mM hydroxyurea (HU) for 24h and stained for microscopy. γH2AX is a biomarker for double stranded DNA damage. Scale bar = 50 µm.

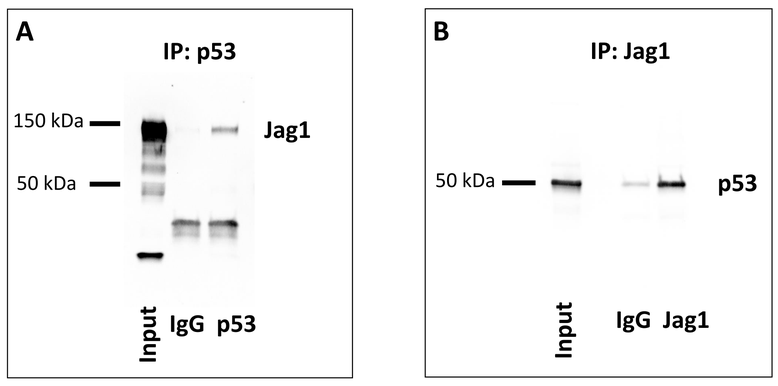

Figure 4. Jagged1 interacts with p53 in mitotic 293HEK cells. The protein-protein interaction between Jagged1 and p53 observed in a proteomic screen for Jagged interactors in mitotic cells was validated with immunoprecipitation (IP). A) p53-IP and Jagged1 blotted. B) Jagged1-IP and p53 blotted. IgG functions as a control in both. These IP results validated the interaction between Jagged1 and p53 in mitotic cells.

CONCLUSIONS

- **Loss of Jagged1 decreases replication stress response**
- **Loss of Jagged1 causes more DNA damage in MDA-MB-231 breast cancer cells**
- **Jag1KO cells proliferate slower than wild type cells**
- **Jagged1 and p53 interact with each other in mitotic human embryonic kidney cells, 293HEK cells**

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