The Relationship Between DNA Damage Response and Accumulation of DNA Damage in LRRK2 G2019S Parkinson's Disease

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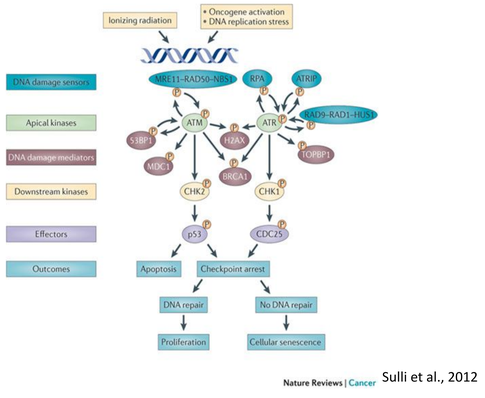
Abstract

Parkinson's disease (PD) is a neurodegenerative disease that affects 7-10 million people worldwide. Among people with PD, an accumulation of DNA damage has been noted, with one possible explanation for this accumulation being a dysregulated DNA damage response. Studying this DNA damage can open doors for new therapeutic targets of PD. The goal of the current study was to evaluate the role of ataxia telangiectasia mutated (ATM), a protein involved in DNA damage response, and leucine rich repeat kinase 2 (LRRK2), a kinase protein, in the accumulation of DNA damage in PD. I hypothesized that the implication of ATM and LRRK2 activation in LRRK2 G2019S PD indicates a relationship between a DNA damage response through ATM, LRRK2 kinase activity, and the accumulation of DNA damage. Western blot and immunocytochemistry (ICC) experimental methods were used to test this hypothesis in healthy control and LRRK2 G2019S lymphoblastoid cell lines (LCLs). The results revealed increases in the phosphorylation of H2AX, a proxy for DNA double-strand breaks (DSBs), in LRRK2 G2019S cells. Increased baseline phosphorylation of ATM in LRRK2 G2019S cells as well as a decrease in H2AX phosphorylation following inhibition of ATM kinase activity suggested a relationship between a DNA damage response through ATM and the accumulation of DNA damage. Interestingly, the results also showed a reversal in DNA damage following inhibition of LRRK2 kinase activity, implicating LRRK2 kinase activity in the accumulation of DNA damage in PD. Ultimately, the results of this study indicate a relationship between the accumulation of DSBs in PD, the DNA damage response via ATM, and LRRK2 kinase activity, although the exact nature of this relationship is still unknown.

Background

DNA Damage Response Pathways



Nature Reviews | Cancer Sulli et al., 2012

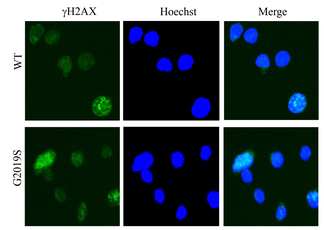
- H2AX phosphorylation is increased in PD models involving alpha-synuclein, indicating a possible accumulation of DNA double-strand breaks (Milanese et al., 2018)
- ATM activation is increased in synucleinopathy models of PD (Milanese et al., 2018)

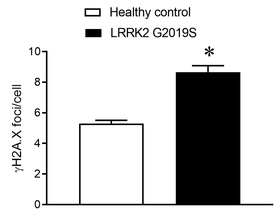
Methods

- Cell Culture and Treatment: Two lines each of healthy control and LRRK2 G2019S PD lymphoblastoid cell lines (LCLs) were used in this study. ATM kinase activity was inhibited through treatment with KU-60019 and LRRK2 kinase activity was inhibited through treatment with Mli-2.
- Western Blotting was used to measure baseline phosphorylation of ATM and its substrates P53 and CHK2, with beta-actin as a control.
- Immunocytochemistry (ICC) was used to measure phosphorylation of H2AX at a baseline and following inhibition of ATM or LRRK2.

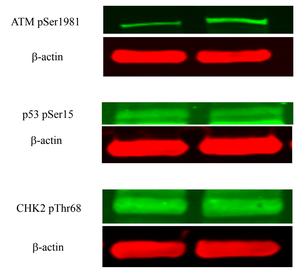
Results

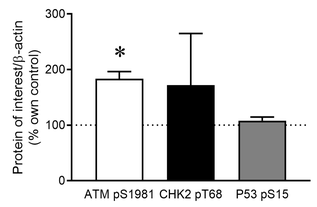
Increased phosphorylation of H2AX due to LRRK2 G2019S



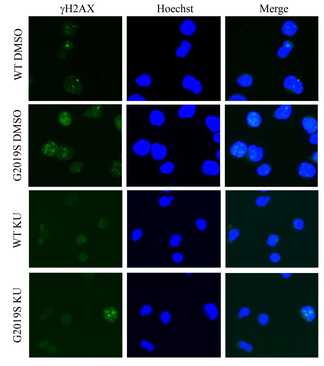


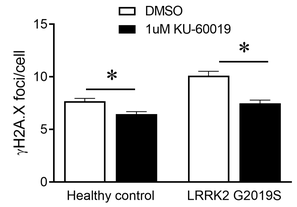
Activation of ATM kinase due to LRRK2 G2019S, but no significant differences in P53 and CHK2 activation





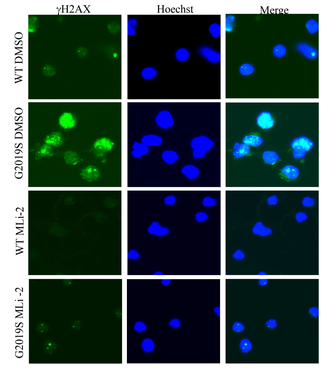
Inhibition of ATM kinase activity results in reversal of increased H2AX phosphorylation related to LRRK2 G2019S

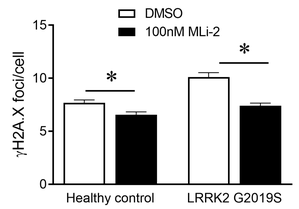




Results

Inhibition of LRRK2 kinase activity results in reversal of increased H2AX phosphorylation related to LRRK2 G2019S





Conclusions

- Baseline levels of DNA damage, measured through H2AX phosphorylation, are greater in LRRK2 G2019S cell lines.
- Reversal of H2AX phosphorylation following inhibition of LRRK2 kinase activity implicates LRRK2 kinase in DNA damage accumulation in PD.
- ATM phosphorylation is greater in LRRK2 G2019S cell lines, but there is no significant difference in P53 or CHK2 phosphorylation between LRRK2 G2019S cell lines and healthy controls. An ATM-mediated DNA damage response pathway might be activated in PD, but this could not be confirmed in this study.
- Reversal of H2AX phosphorylation following inhibition of ATM kinase activity implicates ATM in DNA damage accumulation in LRRK2 G2019S PD.

Future Directions

- Explore the exact role of LRRK2 in DNA damage accumulation and DNA damage response
- Investigate the involvement of other DNA damage response pathways in PD and the role of LRRK2 kinase activity in these pathways
- Expand this study to include other cell types (ex. neurons)

Acknowledgements

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References

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