Replication stress in cortical organoids reveals potential common fragile sites involved in neuronal disorders

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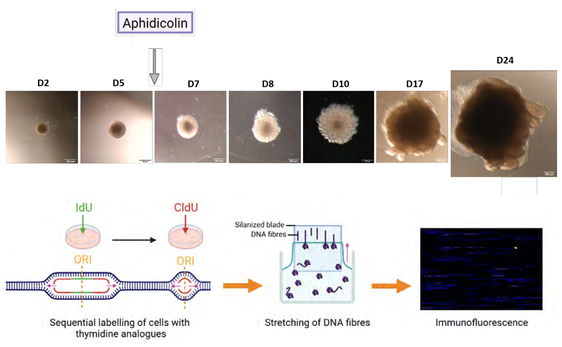
Summary

During human brain development, neuronal precursors divide thousands of times to produce about 80 billion neurons. The rapid divisions can cause DNA breaks and genomic damage that can lead to the initiation of brain cancer or its abnormal development. Experiments in mice have shown that neuronal precursors are indeed the site of high genetic instability under replicative stress conditions, with recurrent DNA break sites (RDCs)[1]. They correspond to long genes that are specifically expressed during neurogenesis and replicate at the end of S phase, three general features also observed in common fragile sites (CFSs). We purpose to determine whether the previously mapped recurrent DNA break sites correspond to regions that, following replicative stress such as a slowing down of replication fork progression, are likely to fail to complete their duplication in time before entry into mitosis[2]. We investigated this by differentiating human induced pluripotent stem cells (hiPSCs) into cortical organoids. Neuronal precursors were exposed to a low dose of aphidicolin (APH), which slows down replication forks, at an early stage of active division. We determined

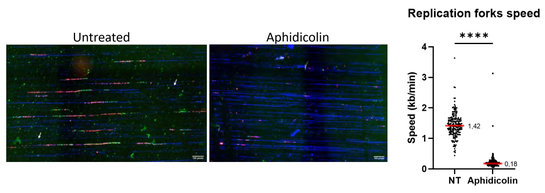
potential CFSs (pCFSs). 26.5% of these are true CFSs, because they have been identified as RDCs in previous studies. These pCFSs are involved in neuronal disorders, such as autism, schizophrenia and bipolarity. Finally, we observed that genes up-regulated under replicative stress are involved in innate immune response pathways.

Aphidicolin penetrates and acts in organoids

Approach



Results

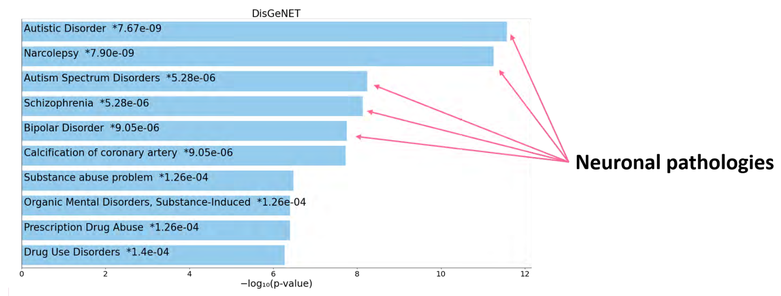


The replication fork speed is significantly slowed down with aphidicolin.

→ Aphidicolin penetrates and acts on organoids.

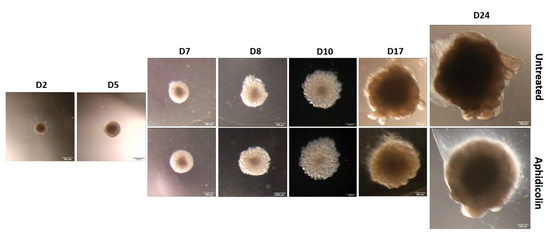
→ We can use aphidicolin to induce a replicative stress.

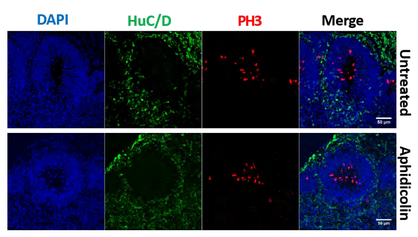
pCFSs are enriched in neuronal pathologies



- The pCFSs are enriched in neuronal pathologies like autistic disorder, narcolepsy, autism spectrum disorders, schizophrenia or bipolar disorder.
- If those genes are indeed broken during the brain development, it could lead to neuronal pathologies.

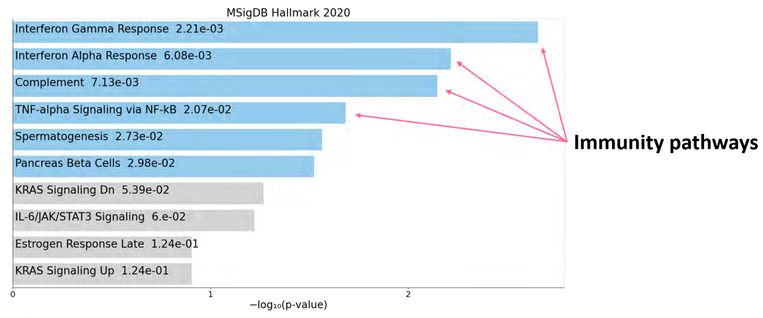
Organoids, treated or not with aphidicolin, have similar phenotypes at D24





→ These results indicate that replicative stress during early development does not appear to affect organoid development in the long term.

Genes up-regulated under replicative stress are involved in innate immunity pathways



Differential expression analysis on nascent RNA (APH vs NT)

- Some cell's innate immune response pathways are enriched in presence of a replicative stress.

→ If breaks are generated during brain development, maybe it induces innate immunity pathways in response to the presence of damaged DNA.

Identification of potential CFSs

Conclusion

→ Aphidicolin penetrates and acts on organoids.

→ The new method to identify potential CFSs is working

→ Potential CFSs are enriched in neuronal diseases.

→ In replicative stress condition, the cell's innate immune response pathways seem to be activated probably in response to the presence of damaged DNA.

References

[1] Wei, P.-C. *et al.* Long Neural Genes Harbor Recurrent DNA Break Clusters in Neural Stem/Progenitor Cells. *Cell*, 2016.

[2] Brison, O. *et al.* Transcription-mediated organization of the replication initiation program across large genes sets common fragile sites genome-wide. *Nat. Commun.*, 2019.