Does Inflammation induce DNA Damage in Neurons?

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Introduction

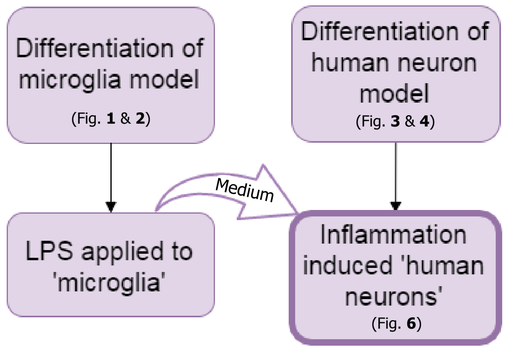
- Oxidative stress and inflammation are major contributors to ageing and neurodegenerative diseases such as Alzheimer's disease
- Compared to age-matched controls, there are elevated levels of DNA strand breaks in Alzheimer's patients [1]

- It is known that oxidative stress causes DNA damage in neurons but it has not been shown for inflammation yet
- Neurons are not directly targeted by inflammation
- Inflammation in neurons is mediated by microglia cells, which are the resident macrophages of the brain
- To model human neurons, I will differentiate SH-SY5Y neuroblastoma cells and to model the microglia cells I will differentiate U937 lymphoma cells into macrophage-like cells

Aims

- To investigate whether a medium containing pro-inflammatory cytokines will induce DNA damage in my human neuron model
- The medium will be harvested from macrophage-like cells that have been treated with LPS, a bacterial virulence factor, to initiate an inflammatory response
- I will then analyse the neurons for DNA damage in the form of phosphorylation to the histone H2AX

Methods



- Immunostaining of treated SH-SY5Y cells (Fig. **5** & **6**)
  - Primary antibody: γH2A.X rabbit
  - Secondary antibody: Alexa Fluor 594
  - DAPI nuclei staining
- Images taken using Leica fluorescence and light microscopes

Results

- The differentiated SH-SY5Y cells developed long neurotic processes and formed a network, similar to human neurons
- The differentiated U937 cells formed irregular clumps which is characteristic of macrophages

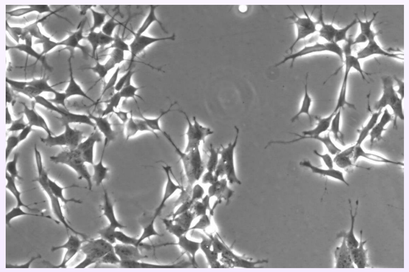


Fig. 1 Undifferentiated SH-SY5Y neuroblastoma cells

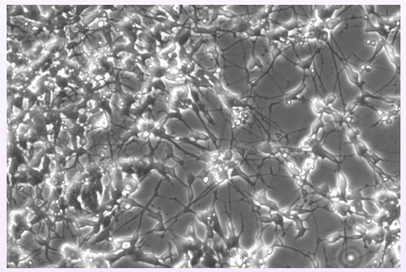


Fig. 2 SH-SY5Y 10 days after differentiation **Human neuron model**

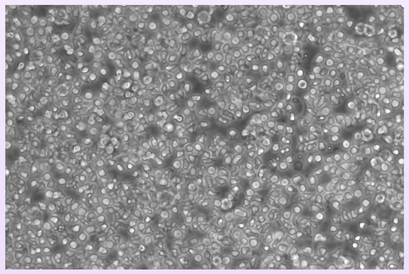


Fig. 3 Undifferentiated U937 lymphoma cells

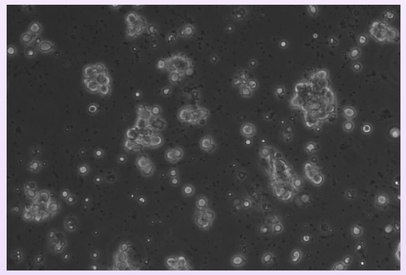


Fig. 4 U937 cells 2 days after differentiation **Microglia model**

- Medium from LPS-treated U937 cells was used to mimic inflammation in the neurons
- As positive controls for damage, the neurons were also treated with hydrogen peroxide to cause oxidative stress
- **Red** spots indicate **DNA damage** in the **blue**-stained **nuclei**

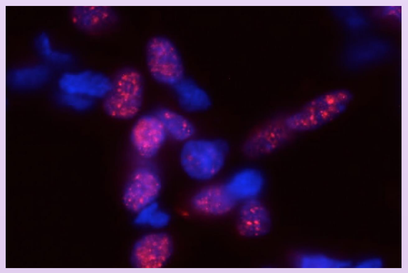


Fig. 5 H_2O_2 treatment of undifferentiated SH-SY5Y cells

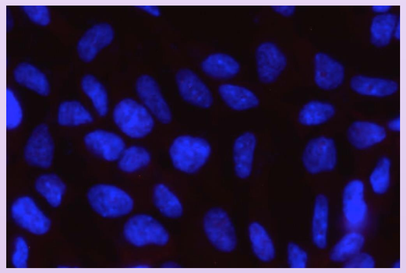
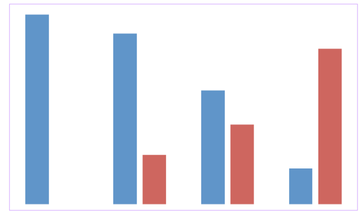


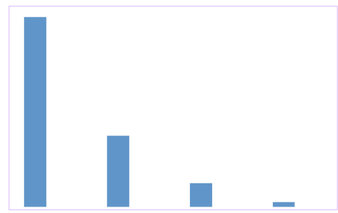
Fig. 6 Pro-inflammatory medium applied to differentiated SH-SY5Y cells

Discussion

- DNA damage signals of phosphorylated histone H2AX **do not** occur in differentiated SH-SY5Y cells (Fig. **2**), but it does occur in the undifferentiated cells (Fig. **1**)
- It is well known that hydrogen peroxide treatment causes DNA damage and the signals were present in my undifferentiated neurons (Fig. **5**)



3 hour hydrogen peroxide treatment
Fig. 7 Undifferentiated SH-SY5Y cells



3 hour hydrogen peroxide treatment
Fig. 8 Differentiated SH-SY5Y cells

- More cell death occurred with increasing concentrations of hydrogen peroxide in both types of neurons, however there was no DNA damage signal in the differentiated neurons (Fig. **8**)
- There were DNA damage signals in the inflammation-treated undifferentiated neurons but not in the differentiated neurons (Fig. **6**)
- Future work could investigate why there is cell death in these differentiated cells without a DNA damage signal

Acknowledgements

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Keywords

- Neurons – *Nerve cells*
- Neurodegenerative disease – *A disease in which there is a progressive loss of neuron structure or function*
- Oxidative stress – *Damage that occurs from reactive oxygen*
- Microglia - a type of Macrophage – *Immune cells that digest foreign substances*
- Pro-inflammatory cytokines – *Chemicals that induce inflammation*
- Differentiation – *One cell type changing into another*

References

1. Madabhushi, R., et al. (2014). "DNA damage and its links to neurodegeneration." Neuron **83**(2): 266-282.
2. Image edited from https://www.corcell.com/wp-content/uploads/dna-163710_640.jpg