Nuclear Pore Complexes: A New Player in the Ageing Heart?

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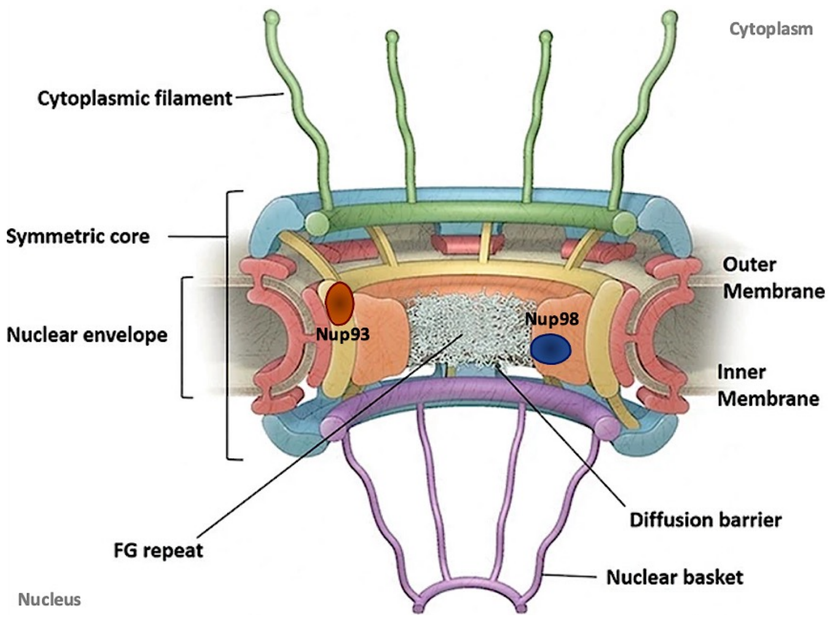
Background

- Nuclear pore complexes (NPCs) are large intracellular gateways that regulate macromolecule transport between the nucleus and cytoplasm.
- NPCs are integral to cellular homeostasis and have been implicated in age-related cell death in motoneurons, which similarly to cardiomyocytes lack appreciable cell division throughout life.
- Exploration of NPCs in the context of physiological heart ageing has been limited.

Aim: To determine whether age-related changes in the levels of long-lived nuclear envelope proteins is a feature of ageing human cardiomyocytes.

The Nuclear Pore Complex



Nucleoporins are the constituent proteins of NPCs.

NPCs consist of 30 unique, ~500 total proteins, with many different functions.

Figure 1:
Diagram of NPC structure, including two nucleoporins of interest: Scaffold nucleoporin Nup93, and FG-nucleoporin Nup98.
Image adapted from (1)

Some nucleoporins have very low rates of turnover during the life of a cell, subject to age-related protein damage.

Oxidative damage to nucleoporins has been shown to allow leak between the nucleoplasm and cytoplasm.

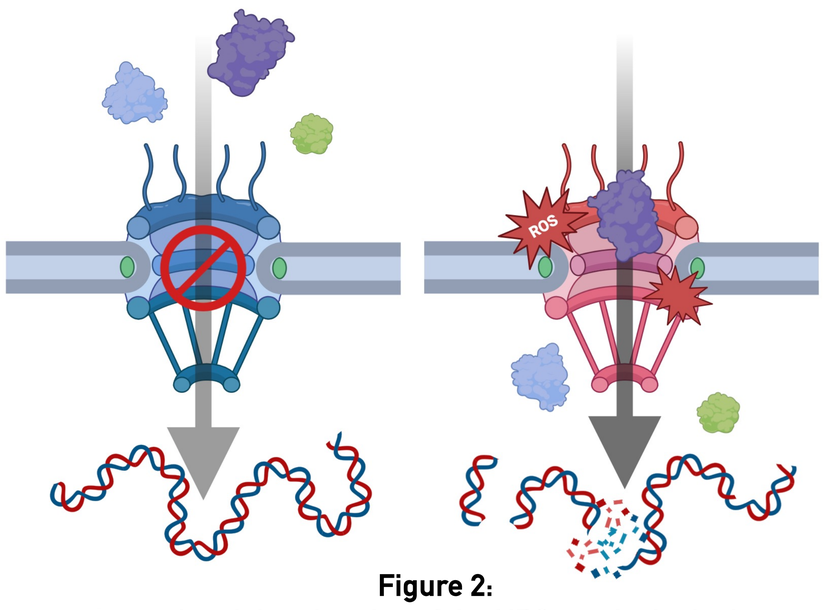
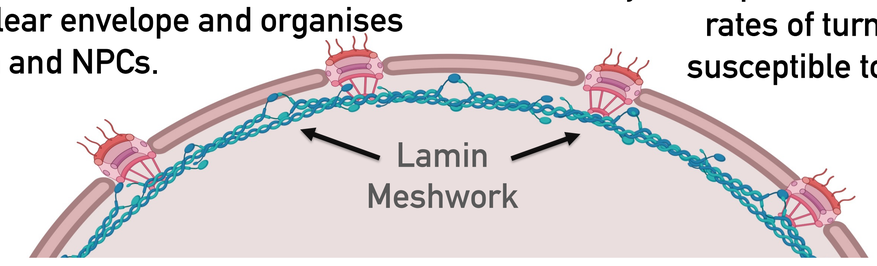


Figure 2:
Age-related deterioration of the NPC results in the loss the integrity of the nuclear barrier and DNA damage.

The Nuclear Lamina

The nuclear lamina maintains the structure of the nuclear envelope and organises chromatin and NPCs.

Key lamin proteins also have low rates of turnover and are susceptible to age-related damage.

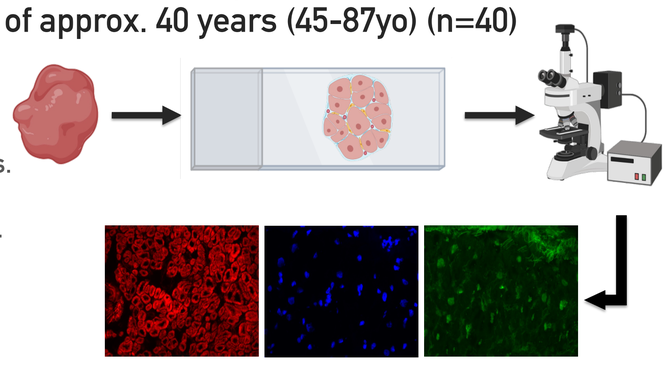


Methods

Right Atrial Appendage (RAA) tissue from the HeartOtago tissue bank were selected from participants across an age-range of approx. 40 years (45-87yo) (n=40)

Semi-quantitative immunohistochemistry

- Fluorescent labelling of nucleoporins of interest using IHC-F under constant conditions.
- Fluorescence intensity of each cardiomyocyte nucleus measured as a proxy for protein level.
- Included no-primary negative controls to normalise to background fluorescence.



4′,6-diamidino-2-phenylindole (DAPI) staining for chromatin and phalloidin-Alexa568 for cardiac actin allow specific identification of cardiomyocyte nuclei from the heterogeneous cell population of the myocardium.

Results

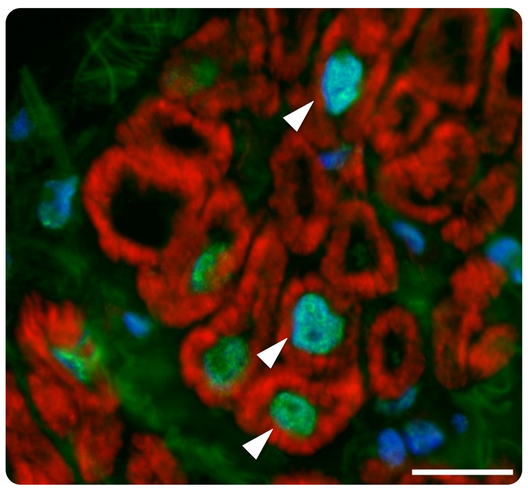


Figure 3: Composite representative immunostained RAA section
DAPI, Anti-Nup93, Phalloidin
Scale bar = 20μm

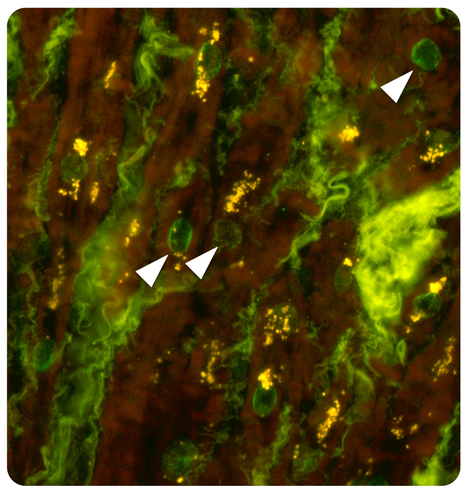


Figure 4: RGB representative immunostained RAA section
Anti-Nup98, Phalloidin
Arrows = Cardiomyocyte Nuclei

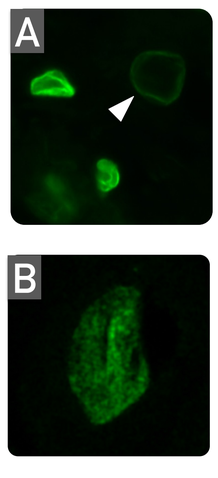


Figure 5:
A: Lamin A/C
B: Confocal Nup93

Data indicates no significant change in immunodetectable nuclear envelope protein levels with advancing age in human cardiomyocytes.

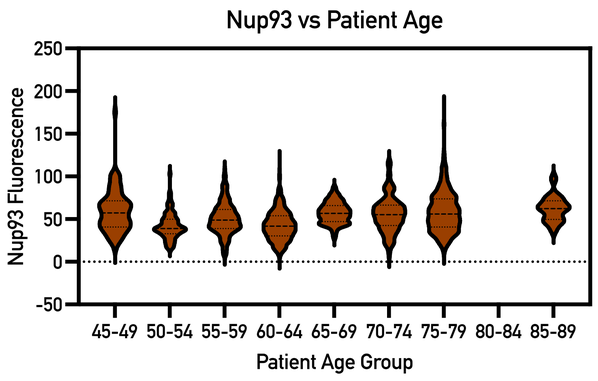


Figure 6: Distributions of Nup93 fluorescence intensity across patient ages, normalised to negative controls (n=35; p<0.05, p=0.26).

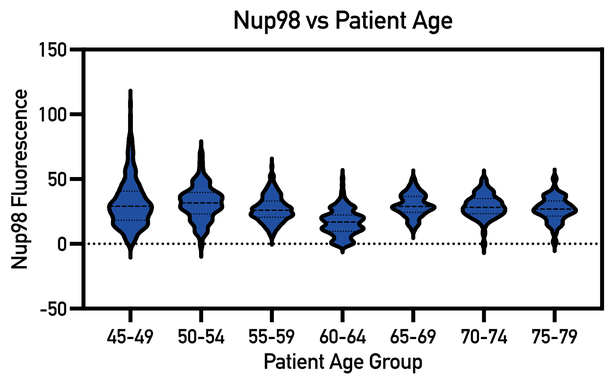


Figure 7: Distributions of Nup98 fluorescence across patient ages, normalised to negative controls (n=34; p<0.05, p=0.30).

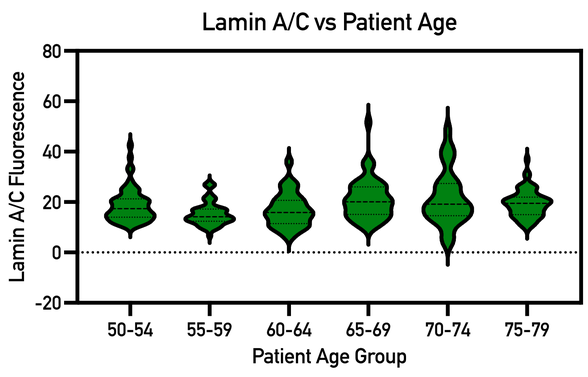


Figure 8: Distributions of lamin A/C fluorescence across patient ages, normalised to negative controls (n=26; p<0.05, p=0.52).

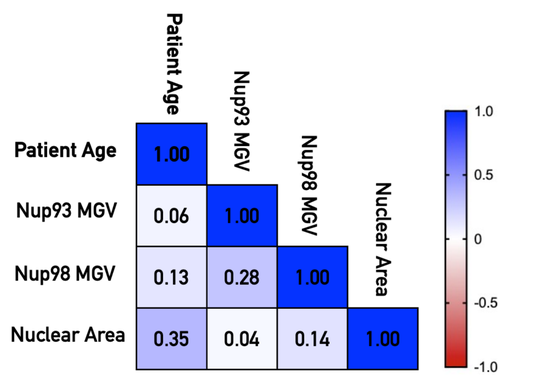


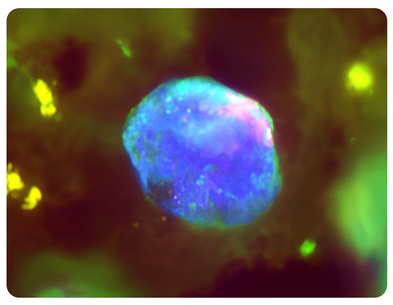
Figure 9: Spearman correlation matrix of relationships between key nucleoporin levels and patient age.

Conclusions

No significant change in immunodetectable Nup93, Nup98, or Lamin A/C as a function of patient age.

- Current data suggests that the overall immunodetectable level nuclear envelope components in human cardiomyocytes are not lost at appreciable rates after 40 years of age.
- These findings could indicate that ageing human cardiomyocytes have a mechanism for piecemeal repair of nuclear envelope components, or that age-related damage is not conveyed by a decline in protein level, but of functional decline or modification.

Future Directions - NPCs and DNA Damage



Future work will explore colocalisation between NPCs and nuclear envelope defects with DNA damage.

Figure 10: Composite Immunofluorescence image showing the relationship between DNA damage and a nuclear envelope defect in human RAA.
DAPI, Nup93, γH2AX (DNA-damage marker)

References

1. https://www.the-scientist.com/infographics/infographic-the-nuclear-pore-complex-32456

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