Targeting the Senescence Phenotype of Medulloblastoma with Novel Therapeutics

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Background

- Cellular senescence is defined as a state in which a cell no longer has the ability to proliferate.
- The cells remain irreversibly arrested in phase G1 of the cell cycle.
- Here they remain unresponsive to extrinsic stimuli with an altered gene expression profile but metabolically active and resistant to apoptosis. **(1,2)**

- Senescence has historically been thought of as a protective mechanism to abrogate external damages - DNA damage, strong mitogenic signals, and other non-genotoxic stresses, for example **[Figure 1]**. **(3)**

- However, evidence shows that such cells have a secretory phenotype that is deleterious to the tissue microenvironment. This creates surroundings that are permissive to tumour initiation, growth, recurrence and metastasis. **(4, 5, 6)**

- Senescent cells can be cleared with drugs called senolytics e.g., navitoclax, hence halting cancer progression and metastasis. **(7, 8)**
- We may therefore be able to utilise senolytics as a prophylactic drug to prevent cancer initiation and as an adjunct to existing therapies.
- We may also be able to induce and subsequently exploit senescence in tumours using existing therapies such as radiotherapy and chemotherapy. **(9)**

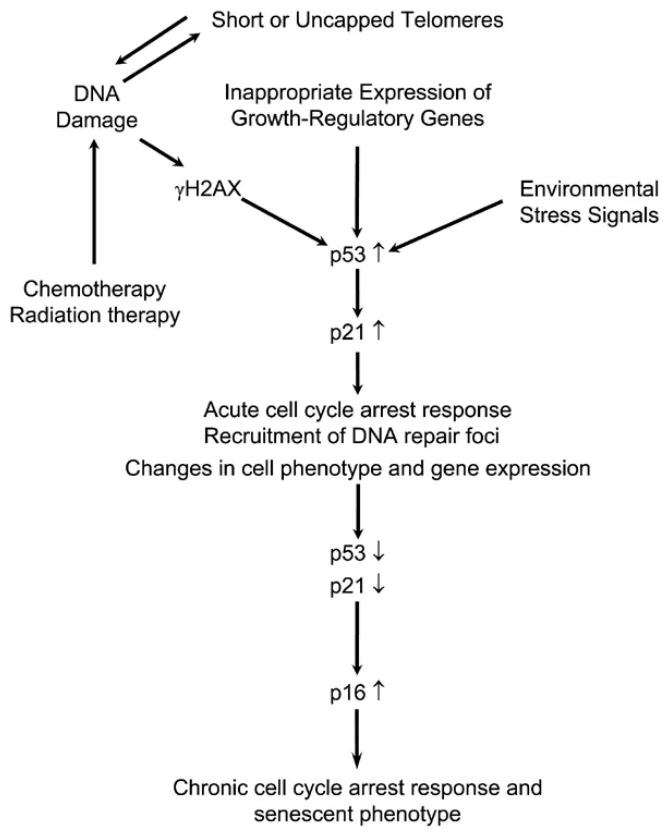


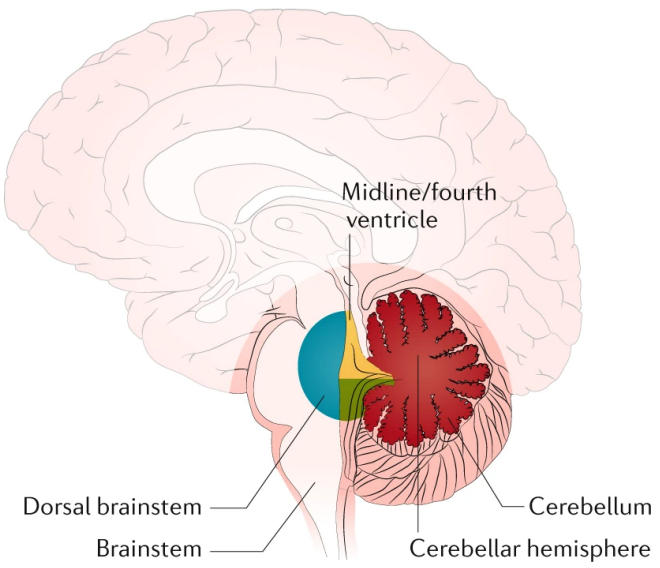
Figure 1 - Cellular Senescence Induction

Project Overview

- My project is investigating the presence and exploitability of senescence in medulloblastoma, as well as therapy-induced senescence. Can we induce senescence in medulloblastoma cells using chemotherapy, and if so can we target these cells with senolytics to improve prognosis and outcomes for children with this tumour?

- Medulloblastoma is a primary central nervous system tumour derived from neuroepithelial cells. It comprises four subtypes of embryonal tumours of the cerebellum (**WNT-activated**, **SHH-activated**, **Group 3**, and **Group 4**) **[Figure 2]**. The five-year survival rate is 72%, though many factors can affect prognosis. **(10, 11)**

Figure 2 - Localisation of Medulloblastoma Sub-Types



Methods

- To investigate senescence induction in medulloblastoma cells, a novel chemotherapy combination was used over the course of nine days. Cells were seeded on day one and treated every second day.

- Cyclophosphamide and methotrexate were kept at constant concentration, cisplatin concentration was varied (1µmol, 3µmol, 10µmol).
- Three wells of approximately fifty-thousand cells were used per condition.

- On day nine, to lyse the cells and extract their RNA whilst maintaining viability, RLT-buffer and β-mercaptoethanol were used. RNA was then isolated and purified.

- To confirm presence or absence of senescence in the cells and quantify the difference between conditions, a variety of primers were used to target cell-cycle inhibitor genes and senescence-associated secretory phenotype genes.
- GAPDH and β-actin were used as housekeeping genes and normalised against each other.

Results

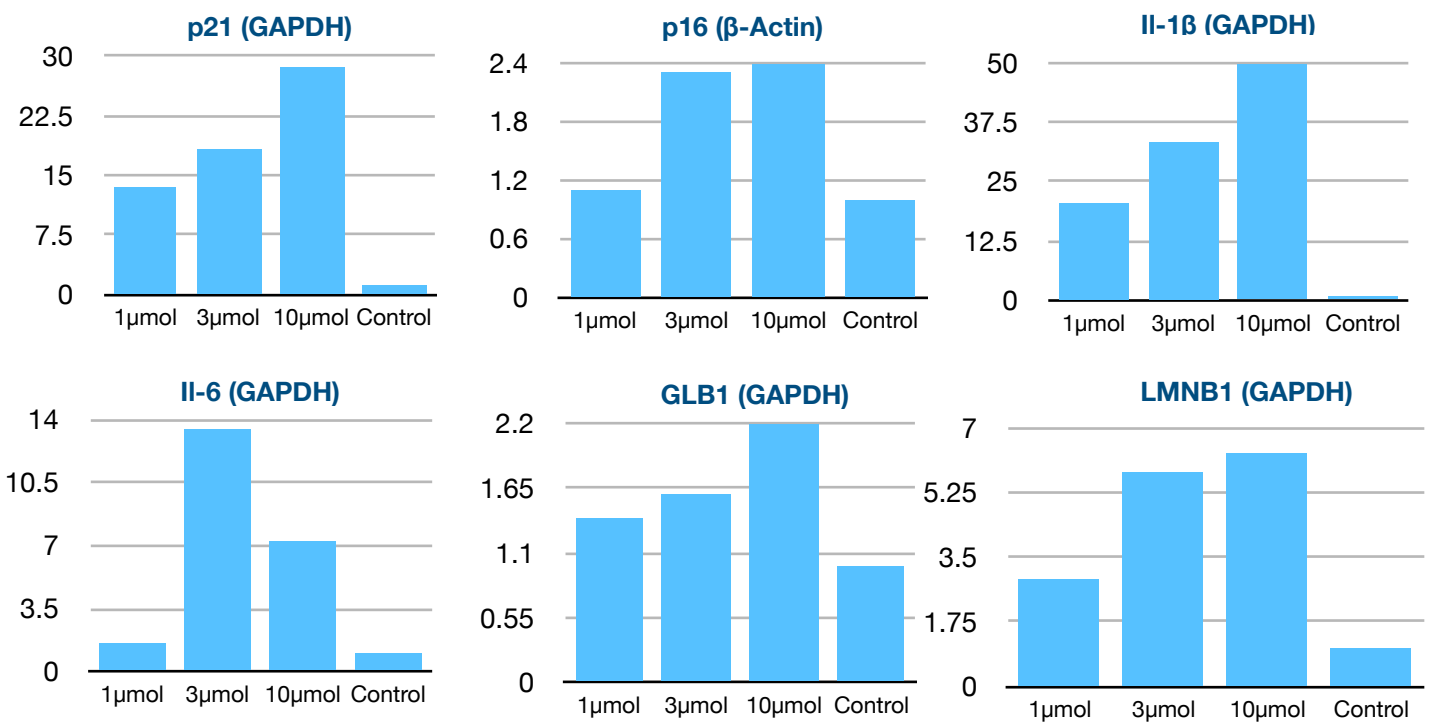


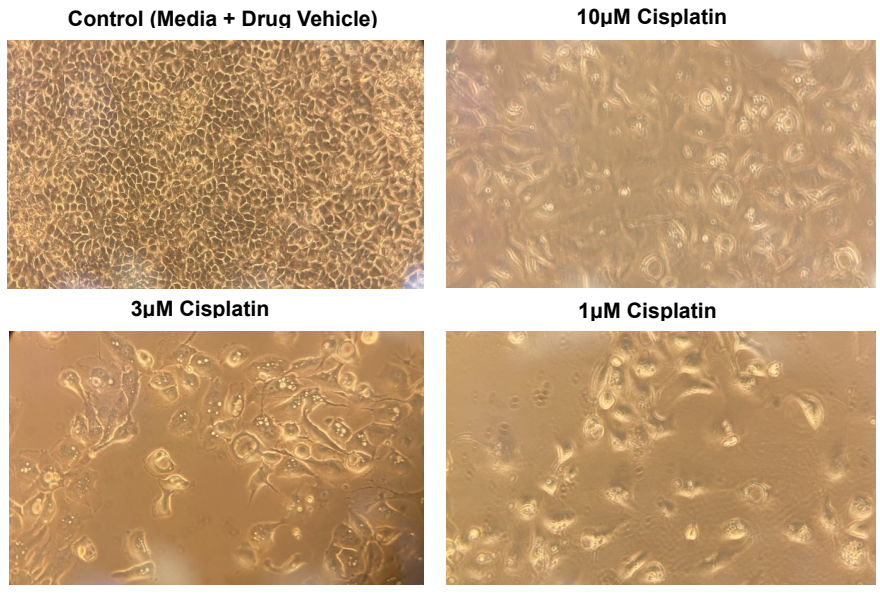
Figure 3 - Relative Up-Regulation of Senescence-Associated Genes following Chemotherapy

- qPCR analysis using primers targeting cell-cycle inhibitors revealed up-regulation of p16 and p21 following chemotherapy. This was in a stepwise manner with increased concentration of cisplatin **[Figure 3].**

- Similarly, there was up-regulation of a variety of senescence-associated secretory phenotype genes. Again in a stepwise manner in the majority of cases **[Figure 3].**

- Images taken from the light microscope after the chemotherapy regimen showed morphology characteristic of senescence, optimised at µmol cisplatin **[Figure 4]**.

Figure 4 - Morphology of ONS-76 Cells following Chemotherapy



Future Research

- The initial experiment confirmed chemotherapy-induced senescence in the ONS-76 medulloblastoma cell line. This needs to be confirmed with further repeats and additional experiments investigating other senescence markers e.g., senescence-associated β-galactosidase and EDU.

- RNA bulk sequencing will reveal the gamut of genes unregulated following the chemotherapy regimen and potential targetable proteins.

- Further investigations will show the effects of a senolytic compound on these cells. This will first be done with Navitoclax and then with a small-molecule library.

- Alongside the cellular biology work, a CDX mouse model of medulloblastoma will be used to lineage trace senescent cells within the tumour(s), investigate the effects of chemotherapy on the tumour(s) in vivo, and the effects of senolytic on overall tumour burden (with and without chemotherapy).

- The ultimate aim of the work is to provide pre-clinical underpinning of a clinical trial aimed at improving the prognosis and outcomes for children with medulloblastoma.

References

1. Collado M, Blasco M, Serrano M. Cellular Senescence in Cancer and Aging. Cell. 2007;130(2):223-233.

2. Hoare M, Das T, Alexander G. Ageing, telomeres, senescence, and liver injury. Journal of Hepatology. 2010;53(5):950-961.

3. Shay J, Roninson I. Hallmarks of senescence in carcinogenesis and cancer therapy. Oncogene. 2004;23(16):2919-2933.

4. Coppé J, Desprez P, Krtolica A, Campisi J. The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression. Annual Review of Pathology: Mechanisms of Disease. 2010;5(1):99-118.

5. Gonzalez-Meljem J, Apps J, Fraser H, Martinez-Barbera J. Paracrine roles of cellular senescence in promoting tumourigenesis. British Journal of Cancer. 2018;118(10):1283-1288.

6. Gonzalez-Meljem J, Haston S, Carreno G, Apps J, Pozzi S, Stache C et al. Stem cell senescence drives age-attenuated induction of pituitary tumours in mouse models of paediatric craniopharyngioma. Nature Communications. 2017;8(1).

7. Kirkland J, Tchkonia T, Zhu Y, Niedernhofer L, Robbins P. The Clinical Potential of Senolytic Drugs. Journal of the American Geriatrics Society. 2017;65(10):2297-2301.

8. Kirkland J, Tchkonia T. Senolytic drugs: from discovery to translation. Journal of Internal Medicine. 2020;288(5):518-536.

9. Northcott P, Robinson G, Kratz C, Mabbott D, Pomeroy S, Clifford S et al. Medulloblastoma. Nature Reviews Disease Primers. 2019;5(1).

10. Medulloblastoma Diagnosis and Treatment [Internet]. National Cancer Institute. 2021 [cited 1 October 2021]. Available from: https://www.cancer.gov/rare-brain-spine-tumor/tumors/medulloblastoma

11. Haston S. Investigation of the tumour promoting effects of cellular senescence during pituitary and lung tumourigenesis [PhD]. University College London; 2019.