Obtaining universal neurons and cardiomyocytes

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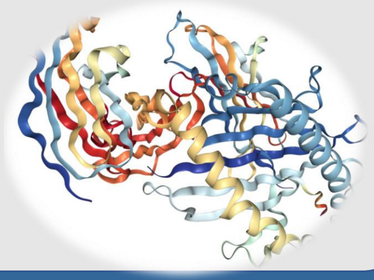
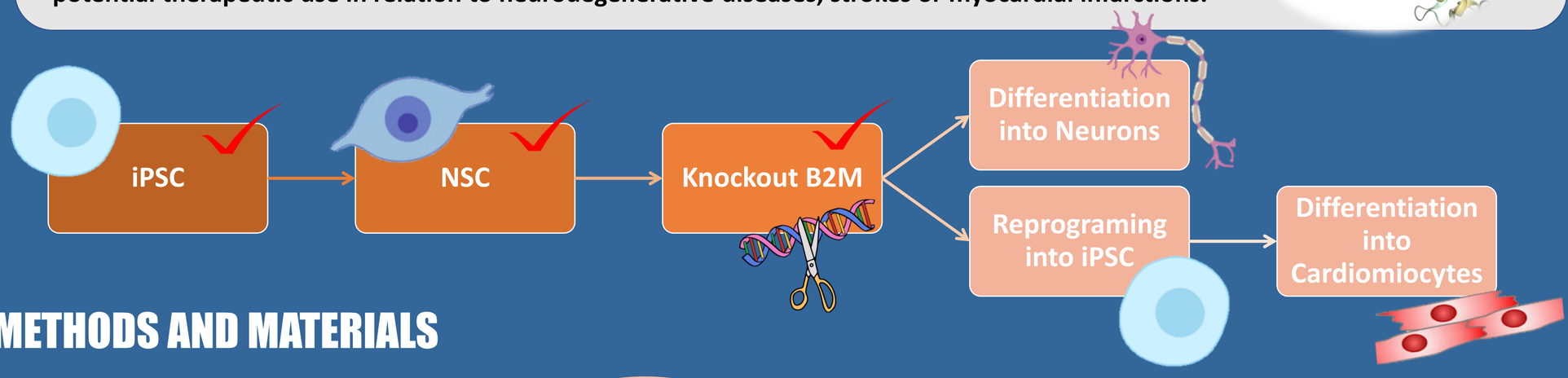
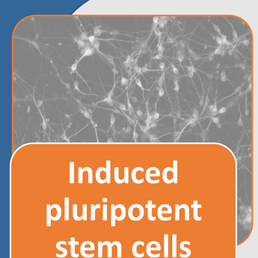
INTRODUCTION

β-2 microglobulin is part of the MHC (major histocompatibility complex), which is responsible for binding antygen derived from own proteins or from pathogens and transferring the antigen presentation to the cell surface for recognition by appropriate T cells. Removal (knock-out) of the gene encoding β-2 microglobulin (B2M) causes the lack of an immune response from the immune system in relation to cells with B2M, thanks to which the cells acquire the feature of universality with potential therapeutic use in relation to neurodegenerative diseases, strokes or myocardial infarctions.





METHODS AND MATERIALS



Induced pluripotent stem cells (iPSCs) were differentiated into NSCs.

The CRISPR method based on a lentiviral expression system was used to delete the B2M gene in NSCs.

- Plasmids with appropriate gRNA sequences were multiplied in immuno- competent cells
- lentiviral vectors were obtained and NSC cells were transduced.
- Transduced cells were subsequently selected by antibiotic selection.

- Wild type – WT control NSCs and ko B2M NSCs using one gRNA sequence (gRNA1 and gRNA3) were used for analysis.
- As a positive control for RT-PCR, monocytes isolated from the peripheral blood of a patient with acute monocytic leukemia - THP-1 - were used

Real-time PCR

Western blotting (WB)

- Wild type – WT control NSCs and ko B2M NSCs using one gRNA sequence (gRNA2as well as ko B2M cells using 3 different gRNAs were used for the analysis
- To verify the effectiveness of ko, cells were treated with INF-γ to further enhance B2M expression

REFERENCES

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RESULTS

Real-time PCR:

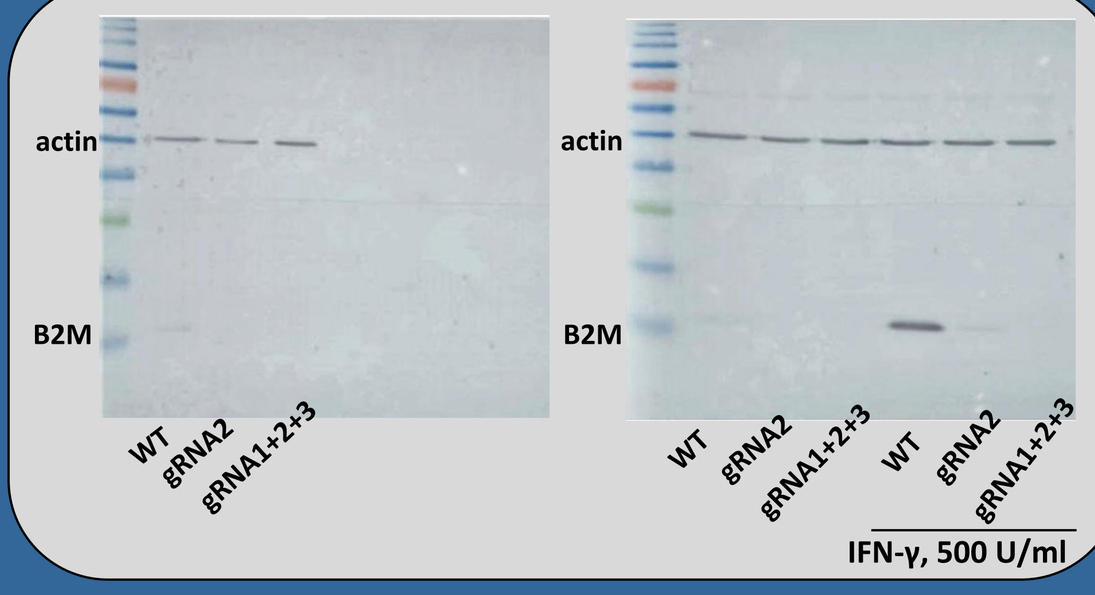
Based on the obtained results, both the decrease in B2M expression in cells after knock-out with gRNA1 and decrease in B2M expression in cells after knock-out with gRNA3 were be observed. It is also worth noting that B2M expression itself in NSC-WT cells was significantly lower compared to control THP cells.

Results of Real-time PCR: Relative expression with Pfaffl method

	Pfaffl	Ct			
Sample	B2M	TBP	TBP	B2M	B2M
NSC wt	1,00	18,75	18,65	22,02	21,92
THP-1	3,78	17,23	16,00	17,64	17,72
NSC KO gRNA1	0,21	22,35	21,67	27,80	27,85
NSC KO gRNA3	0,41	27,51	27,55	32,95	31,79

Western Blot:

The result obtained confirms the results obtained by RT-PCR that B2M expression in NSC-WT cells was at a low but noticeable level, whereas expression in ko B2M cells was not observed. Protein expression was determined relative to actin. To verify whether the addition of cytokines increases B2M expression in the knock-out cells, interferon gamma (IFN-γ, 500 U/ml) was added to culture for 24 hours and the entire analysis was repeated



CONCLUSIONS

- **Based on the obtained result, it can be seen that the addition of IFN-γ increases B2M expression in NSC-WT cells.**
- **B2M knock-out was also confirmed when all gRNAs were used simultaneously and partial silencing of B2M using only gRNA 2.**
- **The above results confirm that it was possible to obtain neural stem cells with the B2M gene removed, which is the first step to obtain universal neurons and universal cardiomyocytes.**

SCIENTYFIC ACHIVEMENTS

Role of Senescence in Tumorigenesis and Anticancer Therapy Stoczyńska-Fidelus Ewelina, Węgierska Marta, Kierasińska Amelia [i in.], Journal of Oncology, 2022, vol. 2022, s.1-23, Numer artykułu:5969536. DOI:10.1155/2022/5969536.
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