Profiling cellular senescence: insights into biomarkers and senolytic therapeutics

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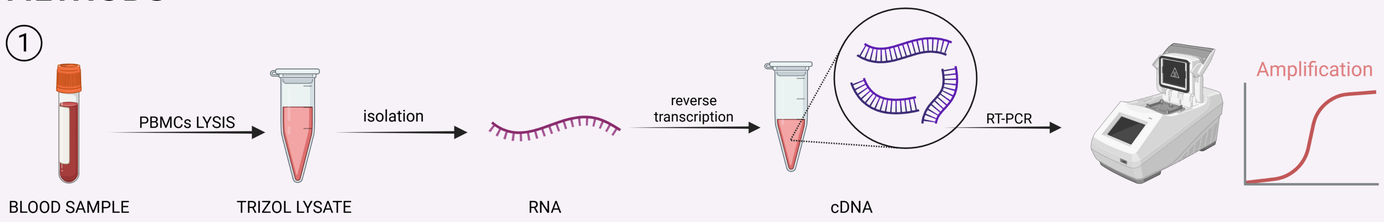
INTRODUCTION

Cellular senescence is a state of permanent cell cycle arrest triggered by various stressors resulting in DNA damage. Although senescent cells lose their proliferative capacity, they remain metabolically active and undergo characteristic morphological and metabolic changes, such as Senescence-Associated Secretory Phenotype (SASP) that includes pro-inflammatory cytokines, chemokines, and growth factors. While senescence serves as a protective mechanism preventing the replication of damaged cells, its accumulation over time promotes inflammation and tissue dysfunction.

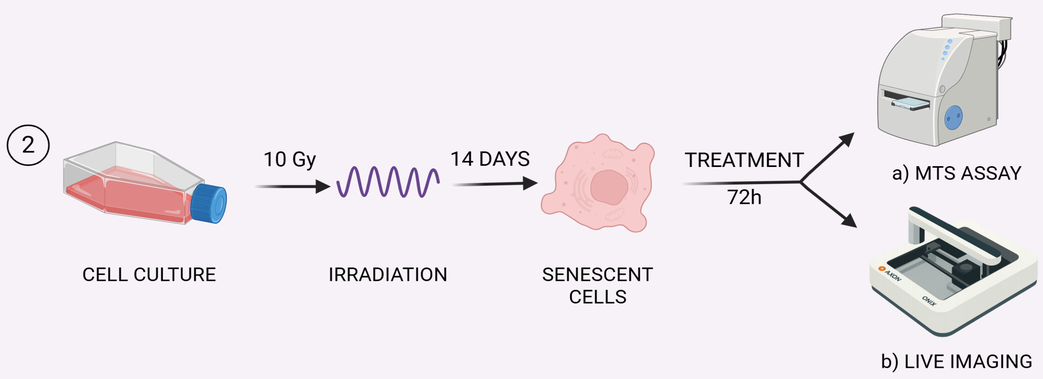
Key regulators and biomarkers of senescence include cyclin-dependent kinase inhibitors p16 (*CDKN2A*) and p21 (*CDKN1A*), which inhibit specific cyclin-dependent kinases. These molecules are considered biomarkers of biological aging. In this study, we measured p16 and p21 expression in blood samples from a cohort of healthy individuals and compared the levels to chronological age to explore potential correlations.

Senolytics are drugs that selectively target and remove senescent cells, potentially reducing their harmful effects. We generated senescent cell lines and treated them with Navitoclax, MCOPPB, and Piritramide to asses the effects of the compounds on the viability of the cells. To evaluate the functional impact of these treatments, HCT116 cells were monitored during the treatment period to measure changes in cell confluency. This approach allowed us to directly quantify how each compound affects the survival of senescent versus non-senescent cells.

METHODS



RNA was isolated from trizol lysates prepared from blood samples of 450 healthy donors. After reverse transcription, RT-PCR was done using probes for *CDKN2A* and *CDKN1A* genes. Number of gene copies per 1 µg of RNA was quantified using standard curved method.



Senescent cells were generated in HCT116, MRC5, and U2OS cell lines by exposure to 10 Gy irradiation. After a 14-day senescence period, senescent cells (SEN) were treated with selected compounds for 72 h. a) Cell viability and compound-specific IC_{50} values were determined using the MTS assay. b) Confluency changes during treatment were monitored and evaluated in HCT116 cells by live-cell imaging and it's corresponding software.

RESULTS

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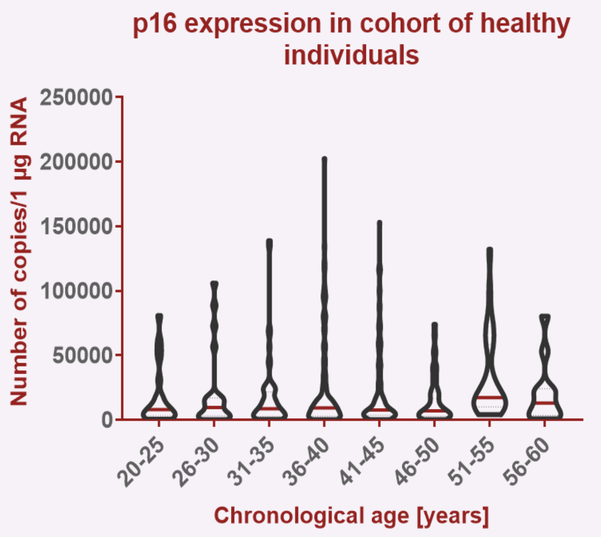


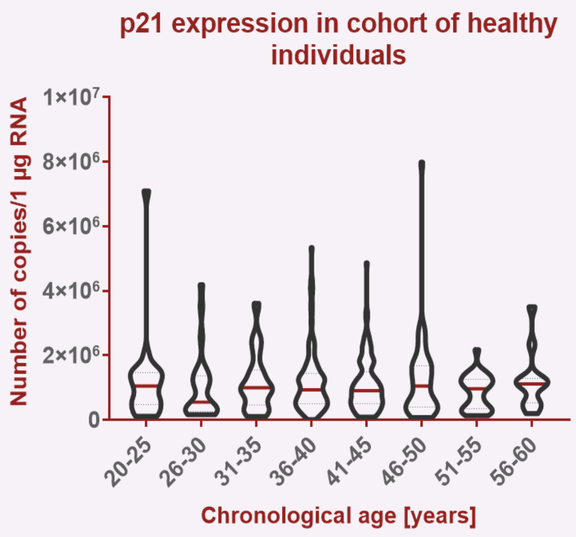
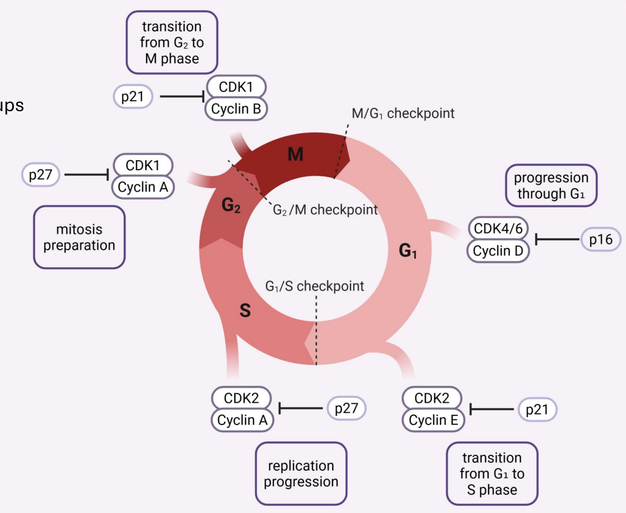


Table 1: Distribution of individuals across the age groups

Year range	Number of individuals
20-25	22
26-30	26
31-35	65
36-40	120
41-45	126
46-50	50
51-55	29
56-60	12



2 a)

Table 2.1: IC_{50} values for Navitoclax and MCOPPB measured in senescent and non-senescent (control) HCT116, MRC5, and U2OS cells.

	HCT116 IC_{50} (µM)				MRC5 IC_{50} (µM)				U2OS IC_{50} (µM)			
	SEN	SD	CONTROL	SD	SEN	SD	CONTROL	SD	SEN	SD	CONTROL	SD
Navitoclax	5,56	3,37	27,39	8,77	2,10	0,35	30,13	1,90	2,40	0,18	44,40	5,36
MCOPPB	18,76	8,51	44,73	6,95	17,72	2,87	21,28	1,35	26,17	11,44	>50	0,00

Table 2.2: IC_{50} values for Piritramide measured in senescent and non-senescent (control) HCT116 cells.

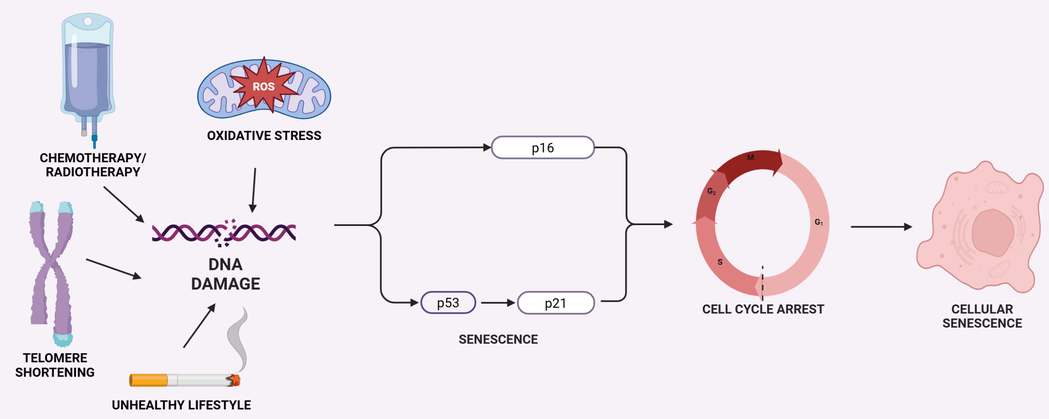
	HCT116 IC_{50} (µM)			
	SEN	SD	CONTROL	SD
Piritramide	38,43	9,11	>50	0,00

- Tables 2.1 and 2.2 summarize the IC_{50} values of Navitoclax, MCOPPB, and Piritramide across senescent and control cell populations. Together, these results demonstrate the difference in sensitivity of senescent cells to the treatments compared to their non-senescent counterparts.
- Table 3 outlines the confluency changes of HCT116 cells over 72 h following treatment with Piritramide, Navitoclax, or MCOPPB. The values represent the difference in confluency between 0 h and 72 h for senescent and control cells, with untreated cells included for comparison.

Table 3: Confluency change of senescent and non-senescent (control) HCT116 cells after 72 h incubation.

b)

c [µM]	Confluency change of HCT116 cells after 72 h incubation [%]							
	Piritramide		Navitoclax		MCOPPB		Untreated cells	
	SEN	CONTROL	SEN	CONTROL	SEN	CONTROL	SEN	CONTROL
50,00	5,75	66,93	-6,33	3,68	-6,80	3,03	24,00	74,10
12,50	39,57	78,87	22,48	65,45	34,08	78,53		
3,13	32,50	77,77	27,35	75,77	36,62	79,68		
0,78	34,32	71,15	35,42	74,57	36,68	79,37		
0,20	34,97	75,98	34,10	75,38	43,35	79,45		
0,05	30,62	75,03	32,52	71,33	41,43	79,88		
0,01	27,63	74,68	30,33	74,93	31,47	78,13		



CONCLUSION

① No correlation was observed between p16/p21 expression and chronological age in the 450 analysed blood samples. Expression levels showed high variability within age groups, with similar median values across the cohort. This might be due to different distribution of individuals in each age group, genetics, or also by different lifestyles of individuals as p16 and p21 are more closely linked to tissue-specific biological age rather than chronological age.

② Testing the effects of various compounds on viability of senescent and control (non-senescent) cell lines confirmed the senolytic activity of Navitoclax and MCOPPB – known senolytic agents. It also revealed potential senolytic effects of opioid analgesic Piritramide. These findings were further explored using live imagining system which showed decrease in confluency of senescent HCT116 cells treated with 50 µM Navitoclax or MCOPPB. Furthermore senescent HCT116 cells treated with 50 µM Piritramide displayed smaller increase in confluency compared to both control and untreated cells. These findings will require further investigation.

ACKNOWLEDGEMENTS

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