p21SEN: A CDKN1A Promoter Fragment Exclusively Active in Senescence

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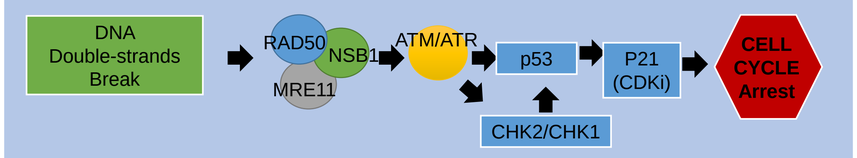
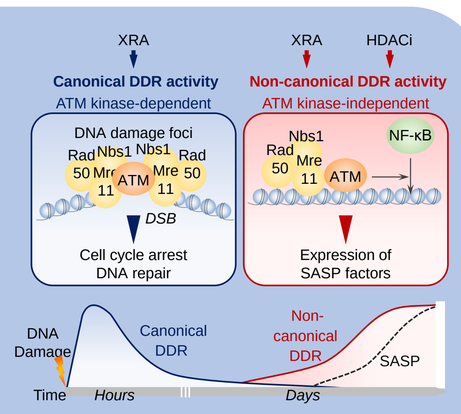
Introduction

1. DNA double strand breaks activates the DNA damage response (DDR)



2. The DDR occurs in two phases :
- An early phase whithin hours following DNA damage
- A late phase within the following days
- The DDR in the second phase is non-canonical and it' responsible for NF-kB activation and SASP production



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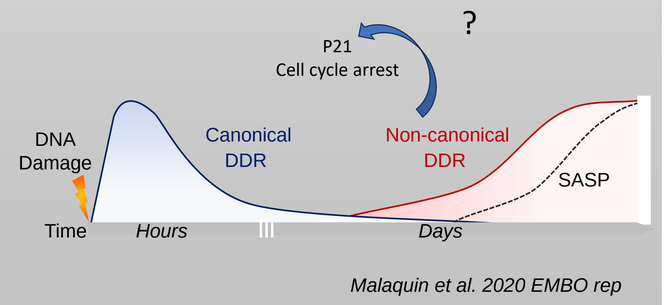
3. How p21 is regulated in senescence ?



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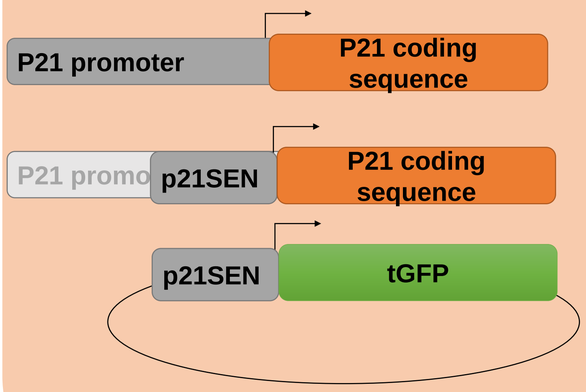
Material and Methods : p21SEN-tGFP a reporter of p21 promoter late activity

Cell line : TOV21G (Ovarian cancer cell line; p53wt)

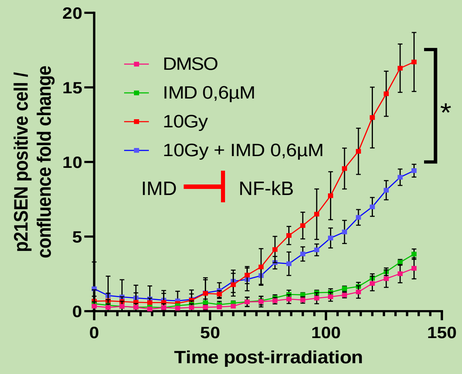


1. **p21SEN identification: a part of p21 promoter responding to DNA damaging agent**
2. **Fusion of p21SEN with the coding sequence of tGFP allowing to follow the p21SEN promoter activity**

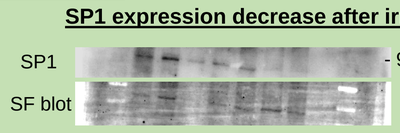
Result 2 : NF-kB and SP1 are regulator of p21SEN activity

Cell line : TOV21G shP53

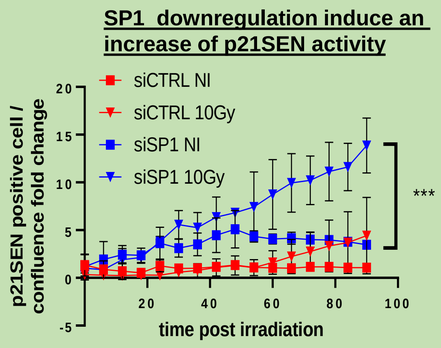
NF-kb inhibition decreases the p21SEN activity

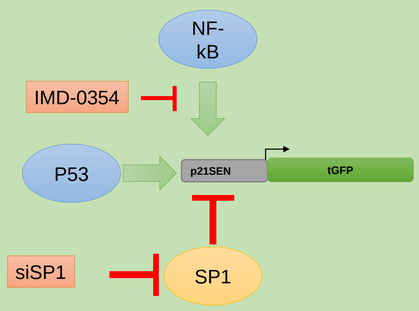


SP1 expression decrease after irradiation



SP1 downregulation induce an increase of p21SEN activity



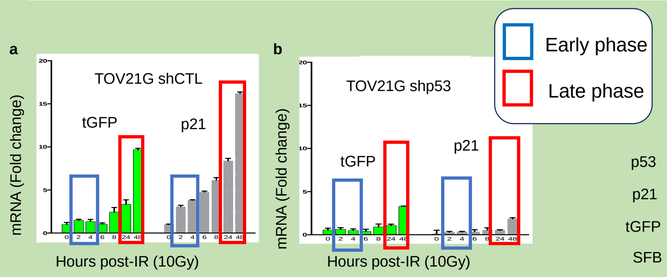


Result 1 : p21SEN characterisation

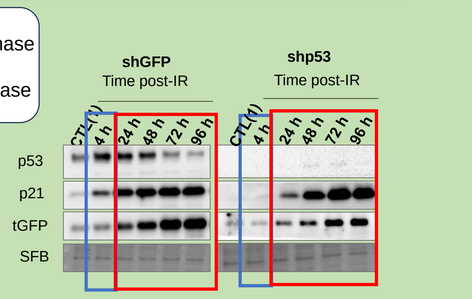
A. p21 is expressed in different cell lines independently of p53 status during senescence

Cancer type	Cell Line	TP53 status	treatment	P21 expression during senescence (mRNA and/or protein)
Ovarian cancer	OV1946 OV1369 OV90 OV4453	Mutated	Olaparib	↑
	SKOV3		Irradiation (10Gy)	↑
	TOV21G	Wild-type	Olaparib Irradiation (10Gy) Carboplatine/Paclitaxel (C/P)	↑

B. p21SEN promoter is activated only during senescence and independtly of p53



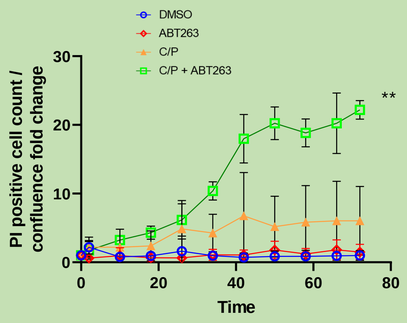
tGFP and late p21 mRNA expression correlate after irradiation (IR)

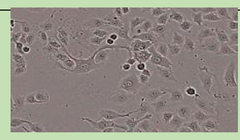
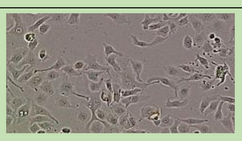
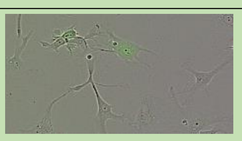
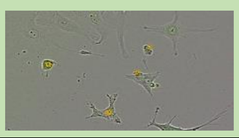


tGFP and late p21 protein expression correlate after irradiation (IR)

Result 3 : p21SEN positive cell are targetable by senolytics

Objectives :
- Induce senescence with a chemotherapeutic an agents combination used in clinic : Carboplatine/Palitaxel (C/P)
- Induce senescence cell death by a senolytic agent (ABT263)



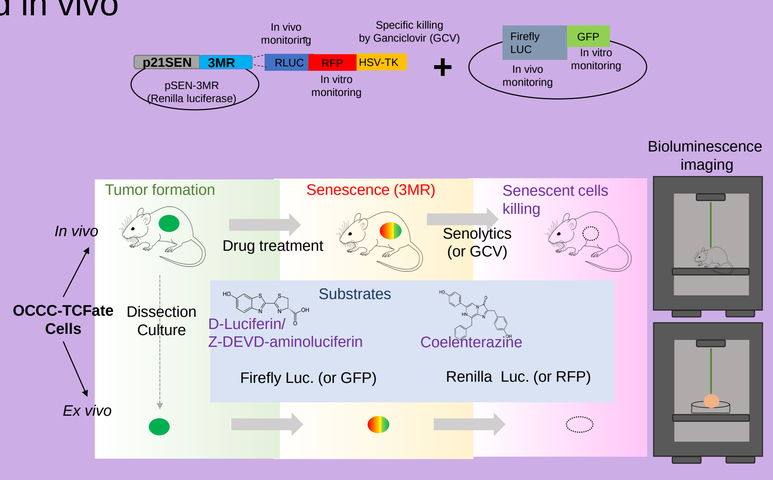
	72hrs
DMSO	
ABT263	
C/P	
C/P+ ABT263	

Conclusion

- P21 is expressed in two phases after DNA-damage similarly to Canonical : 1st phase : few hours after DNA damages, 2nd phase occurring the following days after similarly to senescence. The second phase is not dependent of p53
- p21SEN, a fragment of p21 promoter, is only activated during the second phase and it's not dependent of p53
- p21SEN activity is regulated by NF-kB and SP1
- p21SEN positive cells are a target of senolyic (ABT263)

Perspective

- Use p21SEN reporter to develop senolytic bases therapy in vitro and in vivo



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