Developing an ovarian cancer tissue cell fate (TCFate) manipulation and detection tool

Marie Orléane Ada Ndong[1,3,4], Yu Zhan[3,4], Nicolas Malaquin[3,4] and Francis Rodier[2,3,4]

[1]Faculté de médecine, Université de Montréal ; [2]Département de Radiologie, radio-oncologie et médecine nucléaire, Faculté de médecine, Université de Montréal ; [3]Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM) ; [4]Institut du Cancer de Montréal (ICM).

Introduction

Ovarian cancer (OC) is one of the most common and deadly gynecological malignancy in the western world1. Generally, its standard treatment includes a maximal cytoreductive debulking surgery followed by DNA damaging platinum-based chemotherapies[1,2]. The incorporation of poly (ADP-ribose) polymerase inhibitors (PARPi) has resulted in a significant improvement in maintenance therapy[3]. We recently found that PARPi induce a particular state of senescence in OC cells (first punch), that can be driven to cell death with the use of senescent cell-killing drugs termed senolytics (second punch)[4]. This novel approach to treat ovarian cancer is called the "one-two punch strategy" (Fig. 1). However, senescence in OC is poorly characterized, in part due to limitations in our ability to measure cell fate in real time in cancerous tissue.

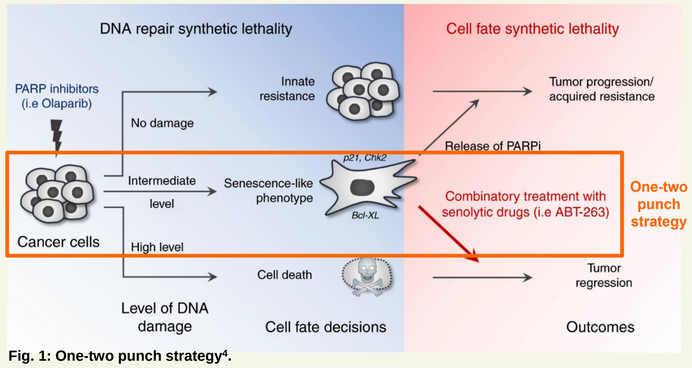

Fig. 1: One-two punch strategy[4].

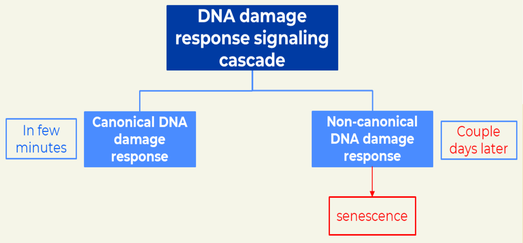

Fig. 2: DNA damage response signaling cascade induced by therapies leading to senescence.

Preliminary data

We demonstrated that the induction of DNA damage causing senescence leads to the activation of the DNA damage response (DDR) signaling cascade in two-phases (Fig. 2): firstly, a canonical and then a non-canonical DDR[5]. The p53 protein, known as the "guardian of the genome", is most often mutated in OC cells. Fortuitously, we have identified a segment of the p21 protein promoter, a p53 target, named p21SEN, that is only activated during non-canonical DDR associated with senescence, and in a p53-independent manner (Fig. 3).

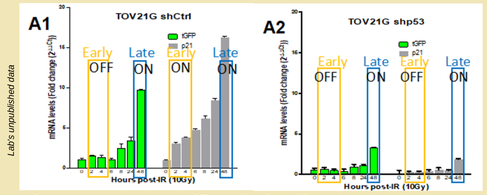

Fig. 3: Senescence specific activation of p21SEN promoter.

ENDOp21promoter — P21mRNA = endogenous p21 promoter

p21SENpromoter — tGFPmRNA = senescence-specific fragment of the p21 promoter

- **Endogenous p21:** decrease of the late activation, loss of the early activation = modulated and dependent on p53
- **p21SEN**: decrease but conservation of the late activation = **modulated but not dependent on p53**

Objectives

Our main objective is to develop and validate a novel and multimodal ovarian cancer tissue cell fate pre-clinical xenograft model to non-invasively track tissue cell fate decision.
The sub-objectives of our project are the following
1) Determine the senescence-specific and p53-independent regulatory and signaling pathways of the p21SEN promoter, our senescence reporter.
2) Verify that the dual luciferase system (DLS) works *in vitro* first then *in vivo*.

Materials and methods

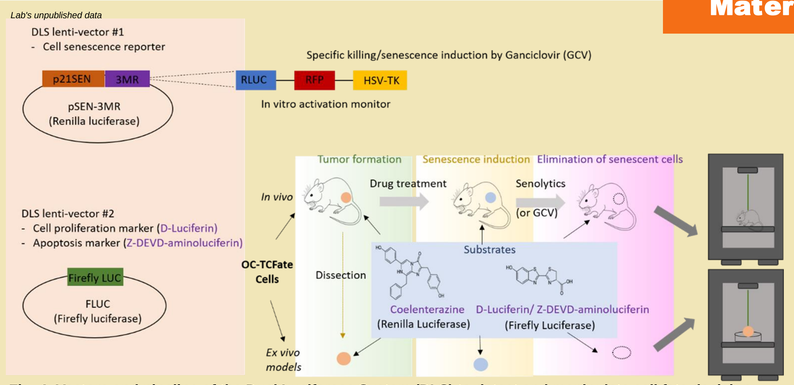

Fig. 4: Vectors and pipeline of the Dual Luciferase System (DLS) to detect and manipulate cell fate decision.

The proposed model uses bioluminescence generated by a dual luciferase system to assess tumor growth and tissue cell fate decision. Our system consists of :

1) a p21SEN-3MR fusion construct that expresses a 3-modality reporter (3MR) driven by a senescence reporter (p21SEN),

2) a Firefly luciferase construct used to monitor tumor proliferation with the substrate D-luciferin and apoptosis with the Caspase3-activated substrate, Z-DEVD-aminoluciferin.

Results

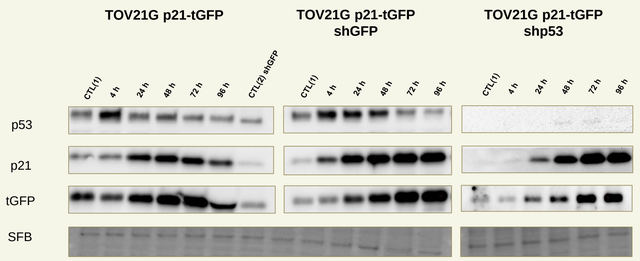

Fig. 5: Activations of p53, p21 and tGFP in a p53-knockdown ovarian cancer cell line after ionizing radiation (IR).

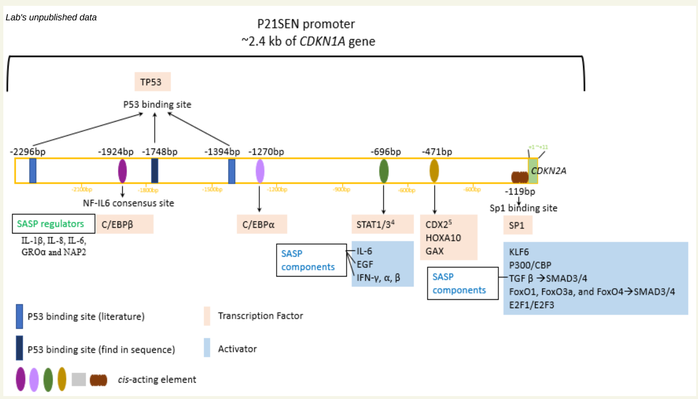

Fig. 6: p21SEN promoter map reveal potential pathways regulating p21SEN.

Conclusion and perspectives

Our current results confirm that there is indeed a p53-independent activation of p21 in OC lines, and the data obtained suggest proteins related to pathways that may be responsible for the regulation of p21 during senescence and in the absence of p53. These pathways remain to be investigated to confirm the key players in this regulation.
Finally, the OC-TCFate model will enable non-invasive live-tracking of senescence/death and, with our senescence reporter p21SEN, make it possible to manipulate the tissue cell fate decision in order to improve the treatment of ovarian cancer.

References :
1. Cortez, A.J., Tudrej, P., Kujawa, K.A. et al. Advances in ovarian cancer therapy. Cancer Chemother Pharmacol 81, 17–38 (2018).
2. A. Chandra, C. Pius, M. Nabeel et al., "Ovarian cancer: current status and strategies for improving therapeutic outcomes," Cancer Medicine, vol. 8, no. 16, pp. 7018–7031, 2019.
3. Lheureux S, Braunstein M, Oza AM. Epithelial ovarian cancer: Evolution of management in the era of precision medicine. CA Cancer J Clin. 2019; 69:280–304.
4. Fleury, H., et al., Exploiting interconnected synthetic lethal interactions between PARP inhibition and cancer cell reversible senescence. Nat Commun, 2019. 10(1): p. 2556.
5. Malaquin, N., et al., Non-canonical ATM/MRN activities temporally define the senescence secretory program. EMBO Rep, 2020: p. e50718.

Contact : marie.orleane.ada.ndong@umontreal.ca