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

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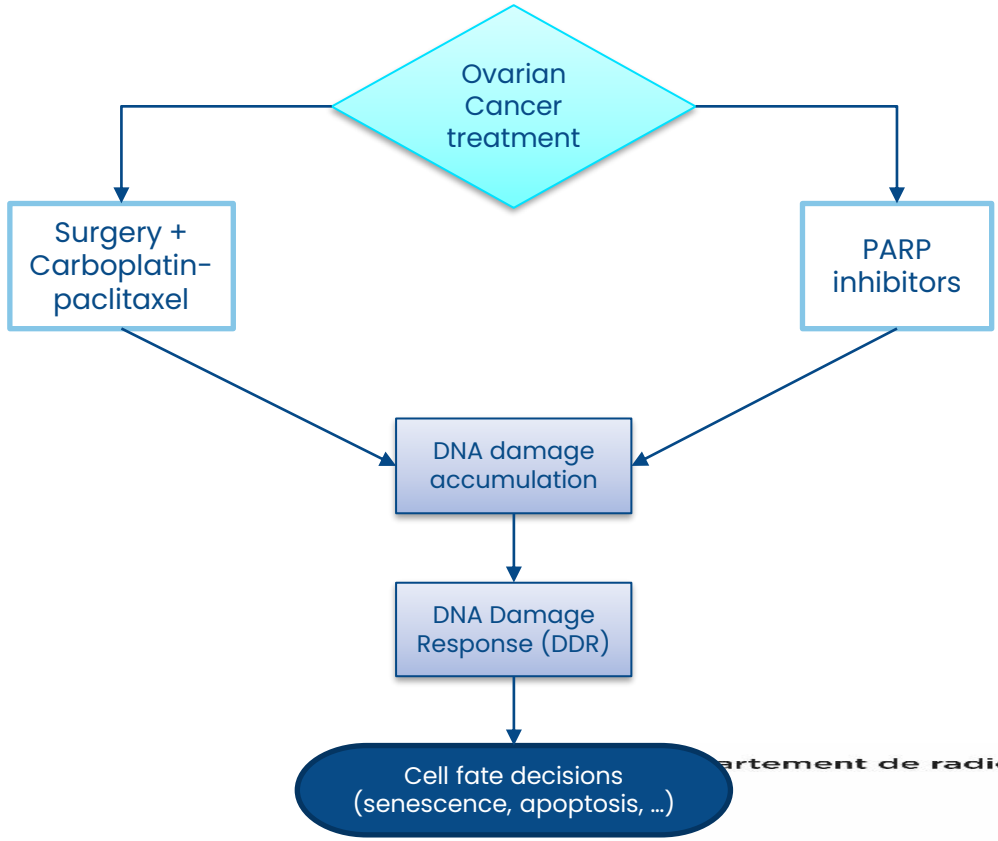
Introduction

Ovarian carcinoma is one of the most deadly gynecologic tumors, accounting for approximately 3.6% of all cancer cases and 4.3% of cancer deaths. First-line treatments for this cancer include debulking surgery combined with platinum and taxane chemotherapy (for example carboplatin and paclitaxel). Recently, poly (ADP-ribose) polymerase inhibitors (PARPi), like Olaparib, and radiation therapy have become new treatments for this type of cancer. These treatments can result in different cell fate decisions, involving not only mortality or survival of cancer cells, but also a treatment-induced arrest of proliferation called **senescence**.



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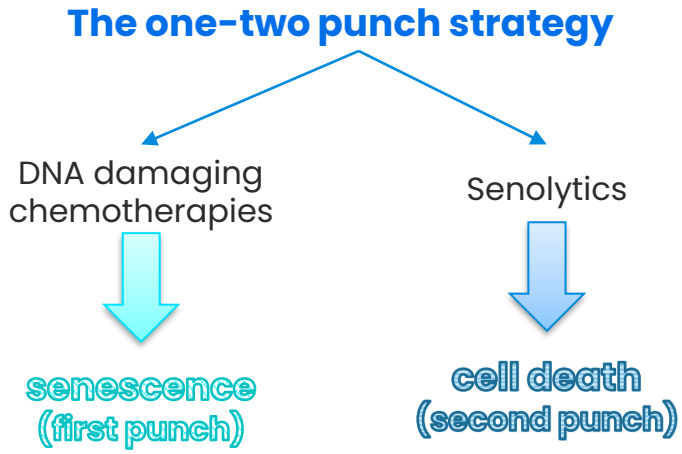
Introduction

We recently found that DNA damaging chemotherapies 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)[1,2,3,4]. This novel approach to treat ovarian cancer is called the **"one-two punch strategy"**. In fact, previous data from our laboratory showed that, contrary to what has been described previously, the therapy-induced senescence (TIS) of PARPi in high-grade serous ovarian cancer (HGSOC) cells has all major signs of senescence but is reversible[4]. More importantly, HSGOC cells are sensitive to senolytics, which convert senescence to apoptosis, inducing a synergistic lethal effect[5,6,7]. This introduced the strategy in which the cell fate of ovarian cancer cells can be changed from an unstable senescence-like state induced by anti-cancer therapies, to an apoptotic state that can be caused by treatment with synergistic senolytic drugs.



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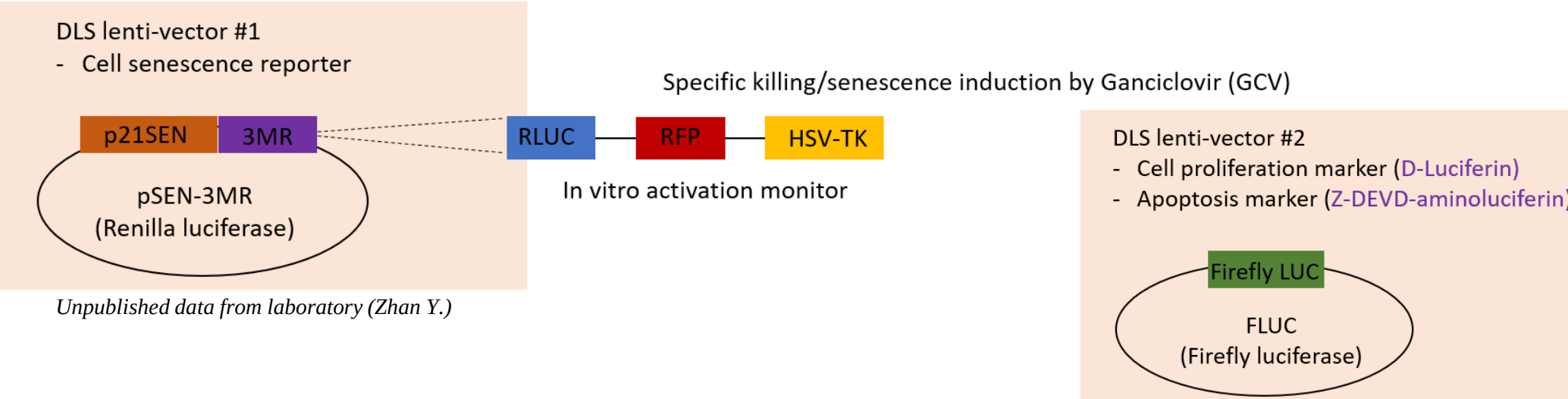
Objective

While cell fate decisions are key for the outcome of cancer therapy, there are **important limitations** in our ability to measure tissue cell fate in real time in cancer tissues. Essentially, because cell fate biomarkers are cumbersome to assess and require sequential invasive tissue biopsies that perturb experimental models. In addition, there are no ovarian cancer models that can provide non-invasive control of cell fates at selected times.

Thus, our main objective is to **develop and validate a novel and multimodal ovarian cancer tissue cell fate (TCFate) pre-clinical xenograft model to non-invasively track tissue cell fate decision**. Our model will be based on a dual luciferase system (DLS) containing a senescence reporter, the **p21SEN promoter**.

Materials and Methods

In our ovarian cancer TCFate model we use the senescence-specific p21 promoter fragment that we found and called p21SEN, to develop a DLS as a detector of cell fate decision and manipulator of senescence at *in vivo* and *ex vivo* levels. The proposed DLS contains **two major vectors**. The first one is a **fusion construct** composed by **the senescence reporter p21SEN** and a constitutively expressed **3-modality reporter (3MR)**. The 3MR consists of three components: a Renilla luciferase (RLUC) gene used to monitor *in vivo* senescence-induction, a red fluorescent protein (RFP) gene used to monitor *in vitro* activation, and a truncated herpes simplex virus 1 thymidine kinase (HSV-TK) gene offering a specific killing by Ganciclovir when activated[8]. The second part of the system is a **Firefly luciferase vector** that will be used to monitor tumor cell proliferation *in vivo* with D-Luciferin substrate, or as an apoptosis marker when given a substate which needed to be digest by Caspase-3.



Unpublished data from laboratory (Zhan Y.)

Figure 1: Vectors composing the Dual Luciferase System (DLS).

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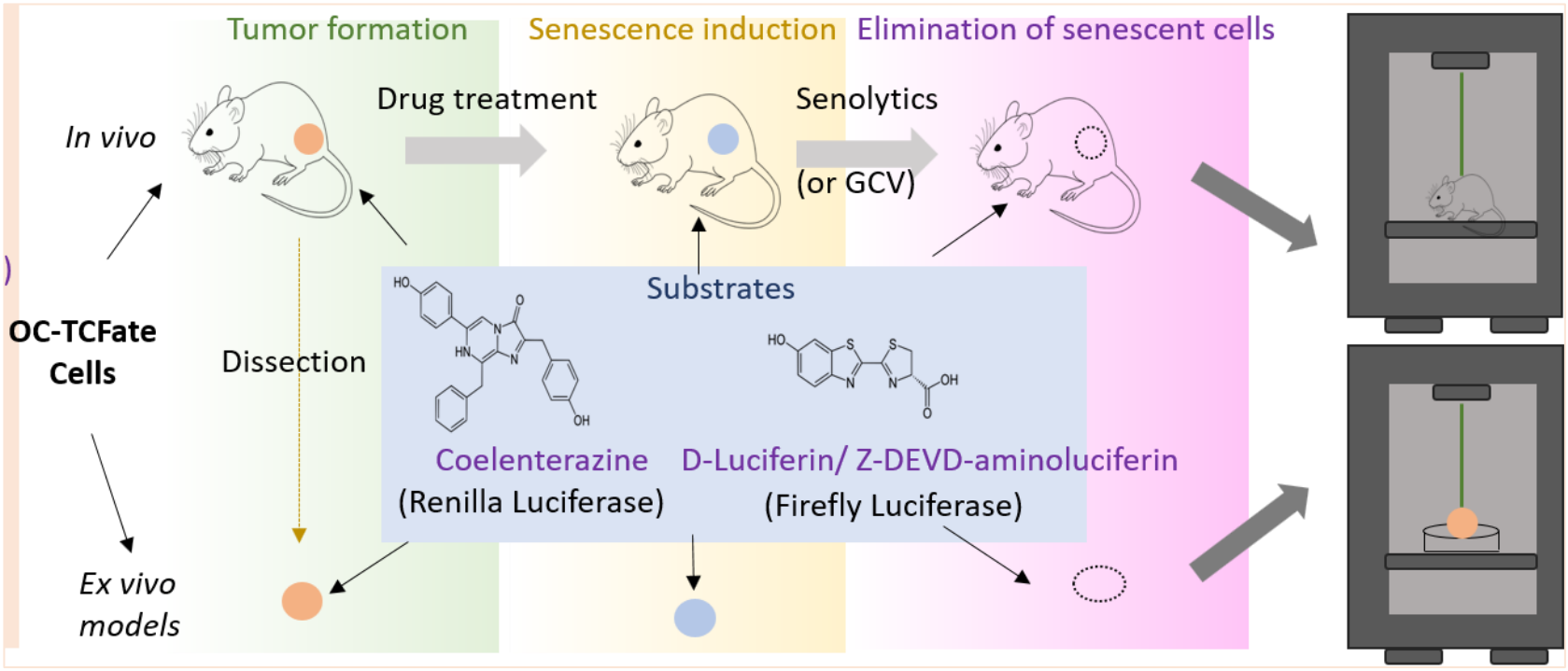
Materials and Methods

We will make the xenograft model with ovarian cancer cell lines expressing Firefly luciferase and p21SEN-3MR, and then detect the bioluminescence signal at multiple time points by injection of different substrates, after PARPi treatment or ionizing radiation. Additionally, when senescence will be induced, we will be able to remove senescent cancer cells with senolytic treatments like Navitoclax (ABT-263).



Unpublished data from laboratory (Zhan Y.)

Figure 2: Pipeline of Dual Luciferase System (DLS) to detect cell fate decision and manipulate senescence.

Preliminary results

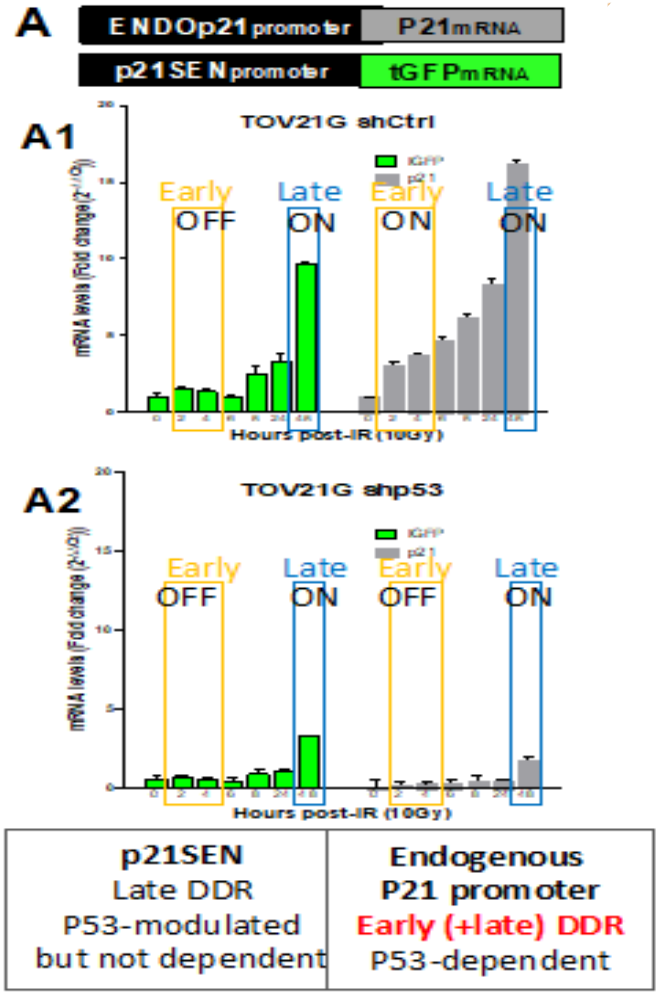
Previously, we demonstrated that the induction of DNA damage causing senescence leads to the activation of the DNA damage response (DDR) signaling cascade in two-phases: firstly, a canonical DDR activated in a few minutes and secondly a non-canonical DDR linked to senescence a couple days later[9].

Moreover, the transcription factor p53 has target genes such as p21, a well-established cyclin-dependent kinase inhibitor playing an important role in cell cycle arrest, making it a potential senescence biomarker[10,11]. But, p21 becomes active shortly after DNA damage in p53 wild-type cancer cells, and can also be activated in p53-deficient cancer cells, limiting its utility as a specific senescence reporter[1,11].

Fortuitously, we have identified a segment of the p21 protein promoter, **p21SEN**, that **is only activated during non-canonical DDR associated with senescence, and in a p53-independent manner** (Fig. 3A).

Finally, we hypothesized that the p21SEN promoter can serve as a specific senescence reporter, independently of p53, in the context of the "one-two punch" strategy.

Figure 3: Validation of p21SEN as a senescence reporter. A) mRNA levels of endogenous p21 (gray bars) and p21SEN-driven tGFP (green bars) in TOV21G shCTL (top) and shp53 (bottom). Time course includes control and after irradiation (IR) 2h and 4h (early), 6h, 8h, 24h and 48h (late). The early/late ON/OFF state of gene expression is indicated.



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Perspectives and conclusion

The ovarian cancer tissue cell fate (OC-TCFate) model will not only allow live and non-invasive monitoring of senescence and cell death, but also simplify the process of preclinical screening of new senomorphic or senolytic drugs, or drug combinations. In addition, microdissected OC-TCFate tumors can be used as *ex vivo* tumor models in microfluidic devices, and the cells can be used for spheroids or organoids derivation.

With our new senescence reporter p21SEN, our system will make it possible to manipulate the tissue cell fate decision in order to improve the treatment of ovarian cancer but also of other cancers such as breast cancer for example, or even of diseases related to aging.

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To all my laboratory members

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