Exercises Cancer Bio 1 (week 2)

QUESTIONS:

Figure 1:

- Be able to explain the Figure.
- Why are PIR (parp-inhibitor resistant) clones also resistant to cisplatin, but not to docetaxel?
- Figure 1d/**Table 1**: is the Figure, which is shown representative?

Figure 2:

- Be able to explain the Figure.
- Understand principle of immunoprecipitation; Western blot analysis.
- Does the experiment tell if the truncated version of BRCA2 in CAPAN1 cells still binds to RAD51?

Table 2:

- By what mechanism do you think the deletions were arising?
- Do you see a way on how to suppress the evolution of resistance?

Figure 3:

- Be able to explain the Figure.
- Complementation experiment in CAPAN1 cells. Are the *BRCA2* mutations in CAPAN1 dominant or recessive?

Figure 4:

- Be able to explain the Figure.
- How do you think did drug resistance evolve? Why did cells with ORF-restoring secondary mutations accumulate in the patient?

General questions:

A) Why do heterozygous germline mutations in *BRCA2* predispose to cancer?

B) Would you expect that cancer cells carrying *BRCA2*-mutations would be sensitive to putative inhibitors of BRCA2; or putative inhibitors of BRCA1; or inhibitors of Pol θ (teta)?

C) Do you expect that the PARPi-resistant PIR-clones described in the paper would be sensitive to inhibitors of Pol θ (teta)?

D) Do you expect that BRCA2-deficient breast cancer cells become PARPi-resistant by loss of 53BP1?

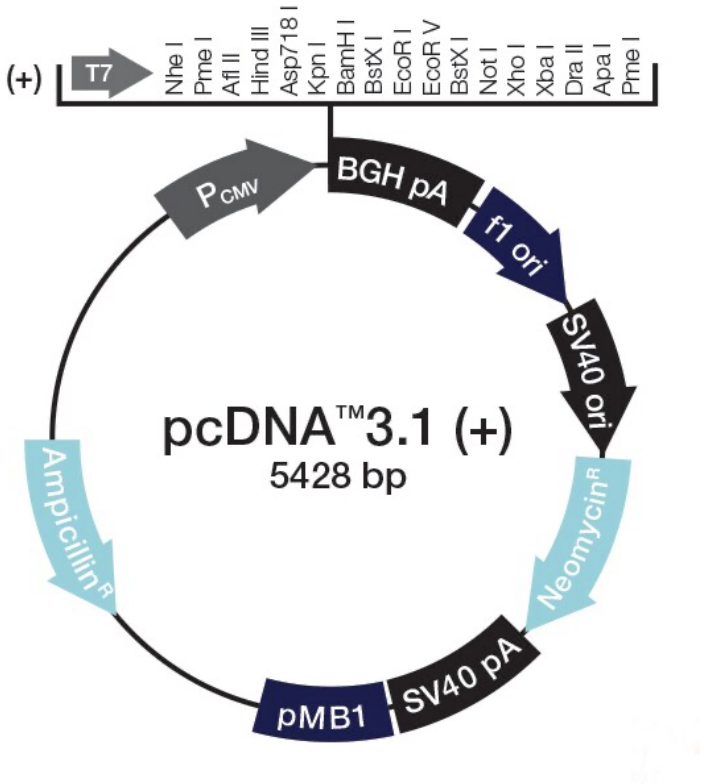
E) You obtain breast cancer cells from a patient with BRCA1-deficiency which became resistant to PARPi due to loss of 53BP1. How would you design a genetic screen to identify drug targets that could be used to specifically kill the PARPi-resistant cancer cells and not BRCA2/53PBP1-wild type cells?

BACKGROUND INFORMATION:

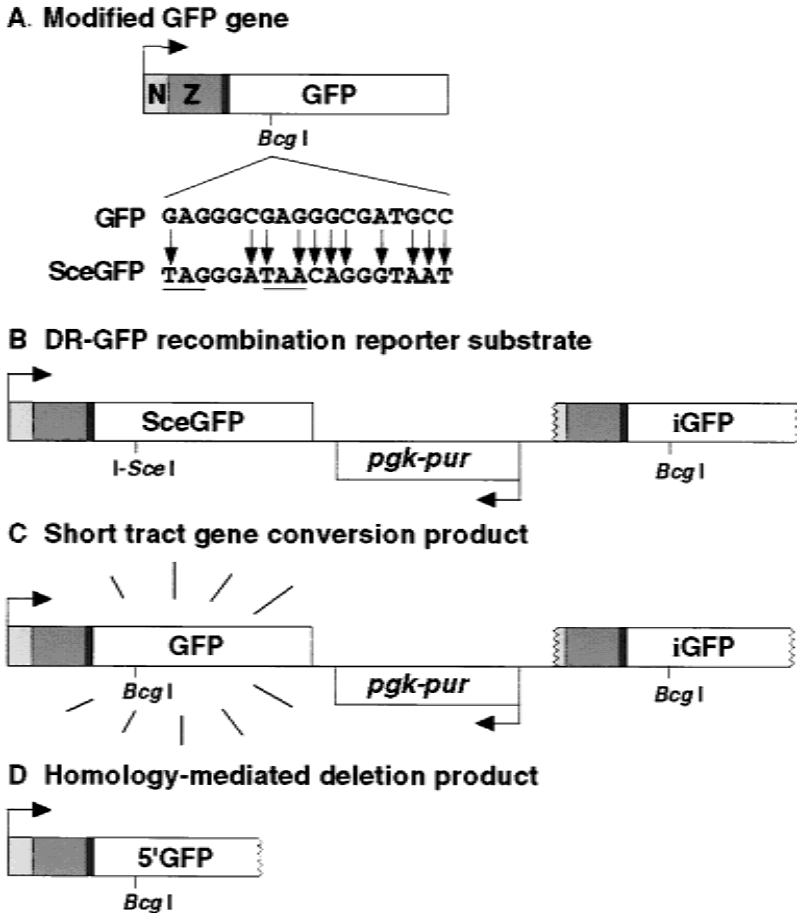
γ-H2AX : H2AX constitutes about 2-25% of the H2A histones in mammalian chromatin. H2AX becomes phosphorylated by ATM and ATR kinases on serine 139 at DNA double-strand breaks (DSB) forming foci that can be seen under the microscope. The phosphorylated form of H2A is called γH2AX. It is used as a marker for DSB.

BRCA2-proficient BxPC3 cells are commonly used as a control for BRCA2-deficient Capan-1 cells. Both cell lines stem from pancreatic cancers.

Mitomycin C (MMC), an antibiotic isolated from Streptomyces caespitosus, is an alkylating agent covalently binding DNA and inducing inter- and intrastrand cross-links.



Expression vector used in Figure 3c,d to express wt and mt BRCA2 from cDNAs.



Principle of Reporter assay used in Fig 3d:

Figure 1. GFP expression plasmids. (*A*) Modified *GFP* gene. The modified *GFP* gene encodes the EGFP protein fused to a nuclear localization signal (N) and zinc finger domain (Z). It is expressed from a hCMV enhancer/chicken b-actin promoter (arrow) on a spliced message (not shown). ***GFP* is modified to *SceGFP* so as to contain an I-*Sce*I site and in-frame termination codons (underline)**. (*B*) DR–GFP recombination substrate. **Downstream of the *SceGFP* gene is *iGFP*, a 5' and 3'-truncated *GFP* gene**. (*C*) (STGC) product. **In a STGC, a DSB at the I-*Sce* I site is repaired from the *iGFP* gene on the same chromatid or sister chromatid, to result in a functional GFP gene.** (*D*) Homology-mediated deletion product.

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Links for background information on techniques you should be familiar with from previous courses (also covered in text books; e.g. Mol Biol of the Cell (Alberts et al.))

RNA interference
https://en.wikipedia.org/wiki/Small_interfering_RNA

https://horizondiscovery.com/en/applications/rnai/sirna-applications?gclid=EAIaIQobChMIkpaT4N-3-gIV6oODBx1G7Q1pEAAYAiAAEgLjiPD_BwE

Western blot
https://en.wikipedia.org/wiki/Western_blot

Immunoprecipitation
https://en.wikipedia.org/wiki/Immunoprecipitation
https://www.youtube.com/watch?v=OrVVZ8X3n6k

PCR
https://en.wikipedia.org/wiki/Polymerase_chain_reaction