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.
- What do you conclude from Figure 2 about the expression of BRCA2 from putative additional allele(s) of *BRCA2* in CAPAN1-cells?
- 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?

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 with mutations in MMEJ factors would be sensitive to PARP inhibitors? Explain your reasoning.

C) Would you expect that cancer cells carrying *BRCA2*-mutations would be sensitive to putative inhibitors of BRCA2; or putative inhibitors of BRCA1; or putative inhibitors of Pol θ (teta) which is involved in microhomology-mediated end joining?

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.

Principle of Reporter assay used in Fig 3d :

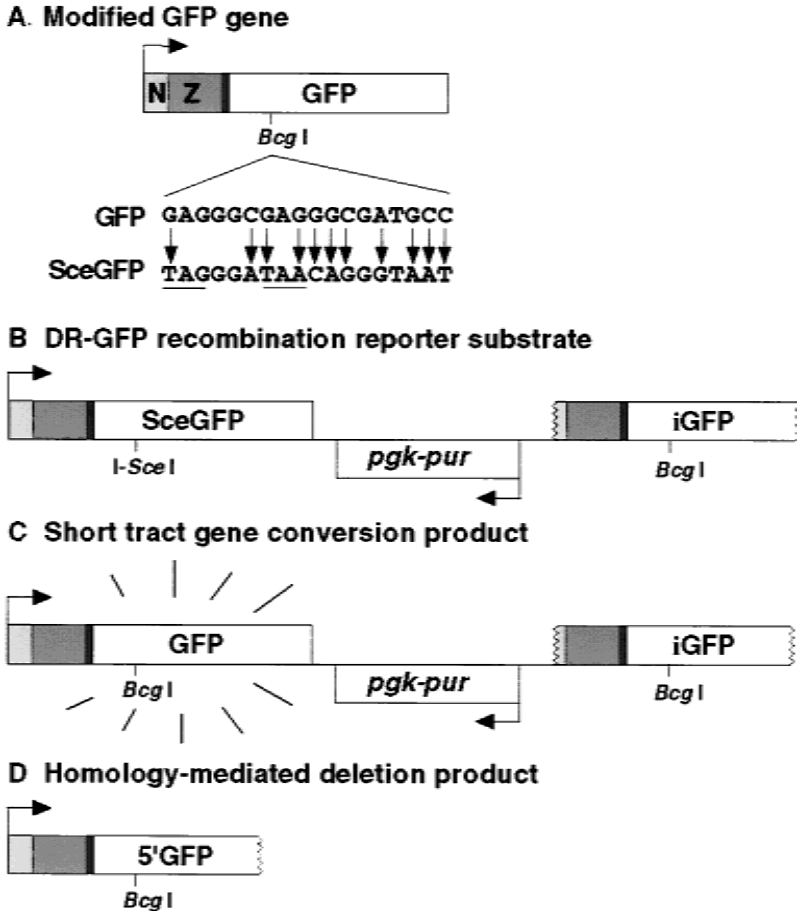

Figure 1. GFP expression plasmids. (*A*) Modified *GFP* gene. The modified *GFP* gene encodes the EGFP protein fused to a nuclear localization signal (N) and zinc finger domain (Z). It is expressed from a hCMV enhancer/chicken b-actin promoter (arrow) on a spliced message (not shown). ***GFP* is modified 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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