Cancer Biology 1; Exercises week 4

1) A typical tumor contains a couple of so-called “driver-gene” mutations that can promote tumorigenesis. The remaining mutations are “passenger mutations” that confer no selective growth advantage.
a) How can you distinguish between the two?
b) Can you also distinguish between tumor suppressor and oncogenes when analyzing cancer genome sequences?
c) You have identified frequent mutations in YFG (your favorite gene) in melanoma patients. How would you test if mutant YFG has oncogene versus suppressor gene function?

2) You have identified a mutant cell line that is hypersensitive to UV radiation.
a) How would you determine if the mutation that gives rise to UV-hypersensitivity defines a new gene or if the gene is already known (i.e. corresponds to XP-A to XP-G, XP-V).
b) How would you test if the mutant gene product is responsible for recognition of damaged DNA ((6-4) PP and CPD).
c) The experiments in a) indicate that you have identified a mutation in a novel gene. How would you identify the corresponding gene?

3) You identified a novel gene (NOVEL1) whose loss of function causes sensitivity to agents that induce DNA double strand breaks (such as X-rays). You suspect that the gene product is either involved in NHEJ collaborating with NHEJ-factors (Ku, DNA-PKcs, Artemis, DNA ligase 4, XRCC4 etc), in HR collaborating with HR-factors (RAD51, BRCA1, BRCA2, and others) or that it may be involved in ATM-dependent checkpoint signaling.
a) What genetic experiments can you carry out to determine if it functions in the DNA ligase 4 or the BRCA2-dependent pathways?
b) How would you determine if it is involved in checkpoint signaling elicited by DNA double strand breaks?

4) a) It turns out that NOVEL1 is not involved in DNA double strand break repair. However, it is frequently mutated in prostate cancer with a mutation spectrum suggesting a loss of function. How would you identify compounds that specifically kill prostate cancer cells carrying NOVEL1 mutations.
b) Homology searches with the NOVEL1 poylpeptide identify a domain with similarity to a MYB-type DNA binding domain. How would you identify the putative DNA binding sites of NOVEL1.
c) How would you identify proteins that physically interact with NOVEL1. Please describe your experimental approach and all control experiments you consider of doing.

5) a) Describe how a DNA damage response is triggered by long stretches of single stranded DNA. Mention three proteins you know which are involved in this DNA damage response and describe their molecular function.
b) What are the consequences if this pathway is activated during S phase of the cell cycle?

6) For the proteins listed below, state whether they act as a tumor suppressor or oncoprotein when mutant. What kind of mutations do you expect in cancer? Briefly justify your conclusion. Also state, when appropriate, how the G1/S control is affected by mutations of these proteins.

ARF:

P53:

ATRIP:

BCL2:

APAF1:

BAX:

7) During your studies on p53 mutations in cancer you identify novel mutations that have not been characterized in the literature.

a) You wonder if the mutations alter the DNA recognition sequence of p53. How can you test this hypothesis?

b) You also want to test if the newly identified p53 mutations have a dominant negative effect on wild type p53. How can you do this? Please consult Figure 3A-C of Science 365, 599 (2019).

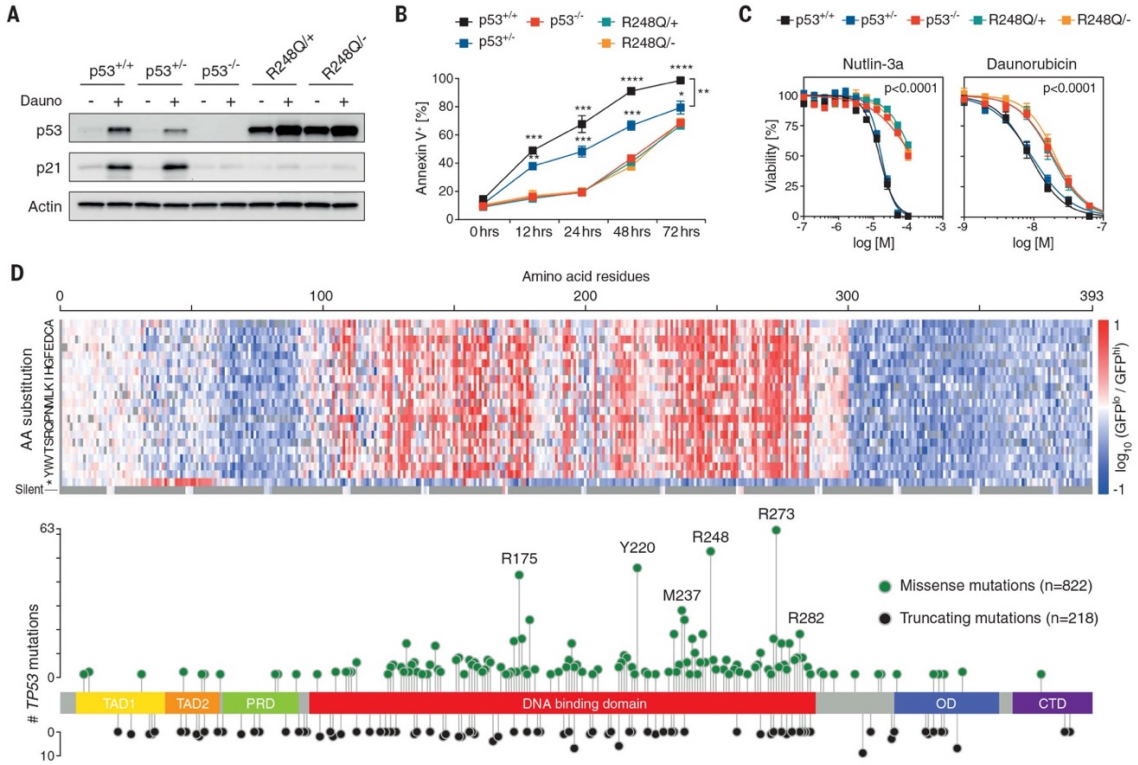

Fig. 3. *TP53* missense mutations in the DNA-binding domain confer a DNE. (**A**) MOLM13-*TP53* isogenic AML cell lines with $p53^{+/+}$, $p53^{+/-}$, and $p53^{-/-}$ as well as $p53^{R248Q/+}$ and $p53^{R248Q/-}$ were treated with DMSO (−) or 100 nM daunorubicin (+) for 6 hours, after which whole-cell protein lysates were collected, run on a polyacrylamide gel, and immunoblotted for p53, p21, and actin (replicates, $n = 3$; representative images are shown). (**B**) MOLM13-*TP53* isogenic AML cell lines were treated with 100 nM daunorubicin for up to 72 hours. At the indicated time points, cells were stained with Annexin V and analyzed by flow cytometry to assess total apoptotic cells (replicates, $n = 3$; symbols represent averages of experimental replicates; error bars indicate SEM; $*P = 0.05$, $**P = 0.01$, $***P < 0.001$, $****P < 0.0001$, two-tailed Student's t test). (**C**) MOLM13-*TP53* isogenic AML cell lines were treated with DMSO, nutlin-3a, or daunorubicin at increasing concentrations for 72 hours, after which cell viability was assessed by using CellTiter-Glo luminescent assay (replicates, $n = 3$; symbols represent averages of experimental replicates; error bars indicate SEM). (**D**) Heatmap depicting the *TP53* saturation mutagenesis screen results after nutlin-3a treatment, shown as $\log_{10}$ of the ratio of normalized read counts in GFP^{lo} over GFP^{hi} cells per *TP53* variant (top panel) overlaid on a lollipop plot demonstrating *TP53* mutational data from 1040 patients with MDS, myeloproliferative neoplasms, and AML (bottom panel). Missense mutations (green circles) and truncating mutations (black circles) comprising frame-shift, nonsense, and splice mutations are shown. AA, amino acid; CTD, C-terminal domain; OD, oligomerization domain; PRD, proline-rich domain; TAD, transactivation domain.