Hypersensitivity of human and rodent Fanconi anemia (FA) cells to bystander effect-induced DNA damage

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Fanconi anemia (FA) is a chromosomal instability and cancer predisposition syndrome characterized by developmental defects, progressive bone marrow failure, and cellular hypersensitivity to agents that induce DNA interstrand crosslinks and oxidative stress [1]. The disease is transmitted either as an autosomal-recessive or X-linked trait, and mutations in 13 FA genes have been identified in FA patients (with more likely to be discovered). Previously, we reported on the hypersensitivity of the isogenic *Fancg*-deficient Chinese hamster ovary (CHO) mutant KO40 for sister chromatid exchange (SCE) induction following low dose 3.86-MeV plutonium-238 α-particle irradiation where <1% of cell nuclei are hit. Compared to wild-type AA8 and *Fancg*-complemented KO40 cells (40BP6) in which SCE frequencies increased ~30% over background levels in irradiated cultures [2,3], SCE frequencies in KO40 *fancg* cells increased ~50% over background levels in irradiated cultures. More recently, this hypersensitivity for SCE induction was also observed when KO40 cells were grown for two cell cycles (required for SCE detection) in medium transferred from 2 Gy γ-irradiated AA8 cultures. Together, this data supports our hypothesis that DNA damage induced in non-irradiated bystander cells consists primarily of single-stranded oxidative lesions that interfere with replicative DNA polymerases and require the FA proteins to direct repair using either specialized translesion polymerases or homologous recombinational repair (HRR). We are currently investigating bystander effect-mediated SCE induction in other CHO mutants, including UV40 and NM3 *fancg* cells and base-excision repair-defective EM9 *xrcc1* cells, and *fanca*, *fancc*, *fancd2*, and *fancg* primary human fibroblasts and their complemented controls using both the low dose α-particle irradiation and high dose γ-irradiation medium transfer protocols. Bystander effect-mediated transcriptional responses in the human FA fibroblasts grown in bystander medium are being assessed by quantitative RT-

PCR and microarray analyses following a recent report from our group demonstrating significantly increased induction of *Tp53*-responsive genes and chromosomal aberrations in *fanca* lymphoblastoid cell lines compared to normal controls following treatment with mitomycin C and hydroxyurea, agents which likewise inhibit DNA replication [4]. The hypersensitivity of FA cells to bystander effect-mediated DNA damage makes them attractive candidates for identifying potential bystander effect molecules and associated signaling mechanisms that are likely important factors modulating low dose IR cancer risk following both low and high LET exposures. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract DE-AC52-07NA27344 and supported by grant DE-FG02-07ER64350 and FWP SCW-0543 from the U.S. Department of Energy Low Dose Radiation Research Program.

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