

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

P.F. Wilson^{1,2}, H. Nagasawa³, A.C. Kohlgruber², S.S. Urbin², F.A. Bourguet², J.R. Brogan³, J.S. Bedford³, M.A. Coleman², J.M. Hinz⁴, and J.B. Little⁵

¹ Biology Department/NASA Space Radiation Laboratory, Brookhaven National Laboratory, Upton, NY 11733

² Biosciences and Biotechnology Division, Lawrence Livermore National Laboratory, Livermore, CA 94551

³ Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO 80523

⁴ School of Molecular Biosciences, Washington State University, Pullman, WA 99164

⁵ Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, MA 02115

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, including IR [1]. The disease is transmitted either as an autosomal-recessive or X-linked trait, and hypomorphic biallelic mutations in 15 FA and homologous recombinational repair (HRR) genes have been identified in FA patients (with more likely to be discovered). We have previously reported hypersensitivity of the isogenic *fancg* Chinese hamster ovary (CHO) mutant KO40 for sister chromatid exchange (SCE) induction following low-dose (<3 mGy) 3.86-MeV plutonium-238 α -particle irradiation where <1% of cell nuclei are hit. Compared to wild-type CHO AA8 and *CgFANCG*-complemented KO40 (40BP6) cells in which SCE frequencies increased ~30% above background levels and HRR-deficient *rad51D* 51D1 cells which showed no SCE induction [2,3], SCE frequencies in KO40 cells increased ~50% over background levels in α -irradiated cultures – a somewhat surprising result given the direct overlap and interplay between the FA and HRR pathways. This hypersensitivity for SCE induction was likewise observed when KO40 cells were grown for two cell cycles with 25 μ M BrdU (for SCE detection) in medium transferred either from 2 Gy γ -irradiated AA8 cultures or fresh medium to which a 1:10 or 1:50 dilution of Hank's balanced salt solution (HBSS) from 2 Gy γ -irradiated AA8 cultures was added (indicating the bystander molecule can be released when cells are irradiated in simple isotonic buffers).

More recently, we employed the γ -irradiation medium transfer protocol using filtered donor medium from 2 Gy-irradiated AG05965/MRC-5 apparently normal primary human fibroblasts transferred to recipient FA primary fibroblast strains from the FA-A, C, D2, and G complementation groups and their human gene-complemented derivatives. Marked hypersensitivity for SCE induction was observed for the FA-G strain GM02361, followed in severity by the FA-A strain GM16631 and FA-C strain GM16754. SCE induction in the FA-D2 strain GM16633 was identical in magnitude to the normal strain AG05965/MRC-5 and the complemented FA-A and FA-C strains (GM16632, GM16755 respectively). This differential SCE induction among the FA strains likely reflects specific roles of the various FA proteins – FANCA, FANCC, and FANCG are part of the FA core complex, which functions as a ubiquitin ligase, while FANCD2 functions downstream alongside FANCI, BRCA2, BRIP1, and PALB2 to initiate HRR following FANCD2/FANCI ubiquitylation and activation by the FA core complex. Significantly higher levels of DSB-associated γ -H2AX pS139 nuclear foci were observed in FA-C cells and less so in FA-A cells, but not in their gene-complemented derivatives (FA-D2/G strains are being evaluated), and *only* in G2-phase cells (identified by CENP-F nuclear staining) and not in G1-

phase cells (which showed no differences between sham-irradiated and 2 Gy-irradiated medium exposures). Panomics cytokine arrays and R&D Systems Quantikine quantitative ELISAs showed significant elevation of IL-6 and IL-8 cytokines in irradiated AG05965/MRC-5 donor medium. Transcriptional responses were measured following 48 h culture of normal, FA-D2 and FA-G cells in sham-irradiated or 2 Gy-irradiated medium using Affymetrix U133A 2.0 microarrays. Transcripts significantly up-regulated in AG05965/MRC-5 cells cultured in bystander medium included proteins associated with TGF- β signaling, MAPK signaling, Jak-STAT signaling and cytokine receptor interaction pathways. Transcripts significantly up-regulated in FA-G GM02361 and FA-D2 GM16633 cells included proteins associated with cell cycle control, Tp53 signaling, DNA replication, chromosome organization, spliceosome, HRR, and the base excision and mismatch repair pathways. Transcripts significantly down-regulated in the FA cells included proteins associated with focal adhesion, ECM-receptor interaction, TGF- β signaling, MAPK signaling, actin cytoskeleton, and “pathways in cancer” pathways (per GO nomenclature). Of great interest from these microarray studies is the finding that several stress signaling pathways up-regulated in the normal cells were down-regulated in FA cells, and normal cells showed no induction of DNA damage response (DDR) pathways, unlike the strong induction of multiple DDR pathways in FA cells cultured in bystander medium.

Collectively, these data support our model that DNA damage induced in non-irradiated bystander cells consists primarily of ROS/RNS-mediated single-stranded lesions (*i.e.*, not direct “frank” DSBs) that can interfere with replicative DNA polymerases and require the FA proteins to direct the resolution of stalled/collapsed replication forks and the repair of one-sided DSBs using either specialized translesion (TLS) polymerases or HRR. The hypersensitivity of both rodent and human FA cells to bystander effect-mediated DNA damage using both irradiation protocols and the marked induction of multiple DDR pathways following treatment with bystander medium makes FA cells attractive candidates for identifying additional bystander effect signaling molecules and associated regulatory mechanisms that are likely important factors modulating low dose IR cancer risk following both low and high LET IR exposures.

References

- [1] L.H. Thompson and J.M. Hinz, Cellular and molecular consequences of defective Fanconi anemia proteins in replication-coupled DNA repair: Mechanistic insights, *Mutat. Res.* **668** (2009) 54-72.
- [2] H. Nagasawa, Y. Peng, P.F. Wilson, Y.C. Lio, D.J. Chen, J.S. Bedford and J.B. Little, Role of homologous recombination in the alpha-particle-induced bystander effect for sister chromatid exchanges and chromosomal aberrations, *Radiat. Res.* **164** (2005) 141-147.
- [3] H. Nagasawa, P.F. Wilson, D.J. Chen, L.H. Thompson, J.S. Bedford and J.B. Little, Low doses of alpha particles do not induce sister chromatid exchanges in bystander Chinese hamster cells defective in homologous recombination, *DNA Repair* **7** (2008) 515-522.

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 Brookhaven National Laboratory under contract DE-AC02-98CH10886 and was supported by grant DE-FG02-07ER64350 and FWP SCW-0543 from the U.S. Department of Energy Low Dose Radiation Research Program.