3 - 100 Novel Role for Fanconi Anemia Pathway in Clustered DNA Damage Repair

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Fanconi anemia (FA) is an autosomal recessive disorder that is characterized by bone marrow failure, developmental abnormalities and increased incidence of cancers[1]. At the cellular level, FA cells display increased chromosomal aberrations, cell cycle alteration and hypersensitivity to DNA interstrand cross-link (ICL) agents[2]. Fifteen "FANC" proteins (FANCA, FANCB, FANCD1/BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ/BRIP1, FANCL, FANCM, FANCN/PALB2, FANCO/RAD51C, and FANCP/SLX4) are identified to function in a common DNA repair signaling pathway, the FA pathway, which is required for resolving DNA ICLs during replication. The central event in the pathway is the monoubiquitination of FANCD2 and FANCI upon DNA damage, which is mediated by upstream FA core complex (FANCA, B, C, E, F, G, L, and M)[2]. Although the role of these proteins in cross-link repair has well studied, and some group report that FA pathway channel lesions in to accurate, as opposed to error-prone repair pathways[3]. However, it is not clear whether FA proteins participate in the processing of HZE (high charge and energy) particle induced clustered DNA lesions. The goal of my current research is to investigate how FA pathway factors co-ordinate the processing of clustered DNA lesions in response to HZE particles irradiation.

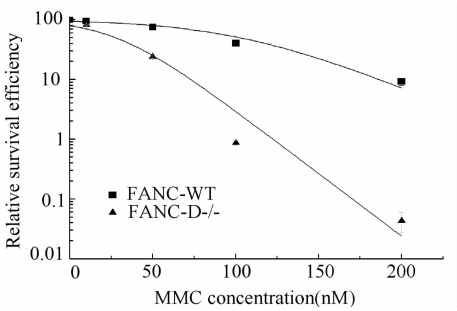

Fig. 1 FANCD2 defective cells are highly sensitive to Mitomycin C.

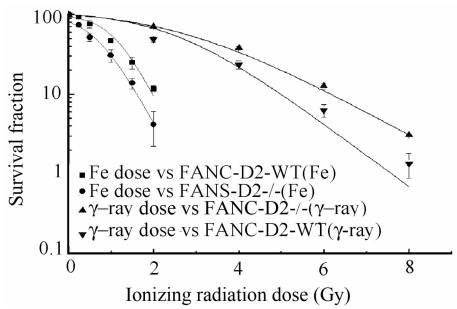

Fig. 2 FANCD2 defective cells are highly sensitive Fe particles irradiation.

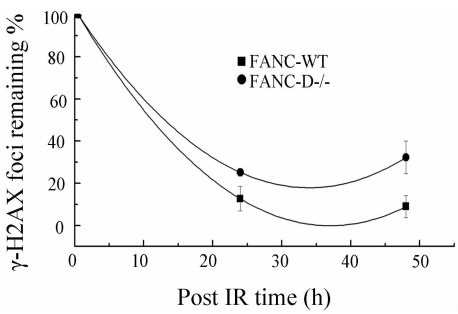

Fig. 3 FANCD2 deficient cells have more irreparable clustered DSBs in response to Fe particles irradiation.

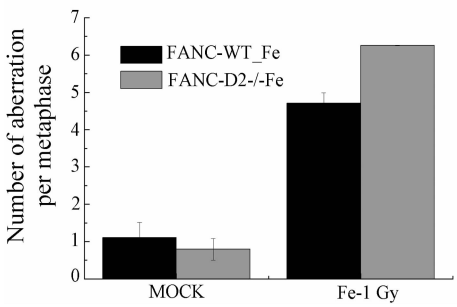

Fig. 4 FANCD2 deficient cells exhibit higher number of chromosomal aberrations in response to Fe particles irradiation.

For our study, we mainly used FANCD2 deficient (PD20) and FANCD2 cells complemented with wild type FANCD2 (PD20+WT) cells. Before we used these cells for our experiments, we first verified their sensitivity to mitomycin-C (MMC). For this purpose, we treated both WT and FANCD2 defective cells to varying concentrations of MMC for 8-10 days and examined their ability to form colonies. Colony survival efficiency data showed that, as previously shown by many groups, FANCD2 deficient cells are highly sen-

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sitive to MMC in comparison with wild type cell (Fig. 1). Next we examined the sensitivity of these cells to ? -ray and iron particles irradiation by colony formation assay. As shown in Fig. 2, compared with wild type FANCD2 deficient cells are slightly sensitive to γ-irradiation. In contract, FANCD2 cells are highly sensitive to Fe particle. This data clearly reveal a function for FANCD2 protein in cellular response to Fe particle irradiation. To determine whether the radiosensitivity of FANCD2 defective cells is due to defect in clustered DSBss repair, we irradiated both WT and FANCD2 defective cells to 1 Gy of Fe particles (1 GeV/n), immunostained with γH2AX antibody and quantified the number of γH2AX foci at different post-irradiation time. γH2AX foci dissolution kinetics showed that the number of γH2AX foci was maximum at 30 min after IR in both WT and FANCD2 defective cells. Interestingly, the number of unrepaired clustered DSBs was significantly higher in FANCD2 defective cells as compared with WT cells (Fig. 3). These data clearly demonstrate that the hypersensitivity of FANCD2 deficient cells is due to a defect in clustered DNA lesions repair. Next we examined how defect in clustered DNA lesions repair contributes to the stability of genome, we examined the yield of gross chromosomal aberrations in metaphase cells derived from WT and FANCD2 mutant cells at 16 h after 1 Gy of Fe irradiation. As shown in Fig. 4, the number of gross-chromosomal aberrations was significantly higher both in WT and FANCD2 defective cells as compared with mock treated cells. Importantly, the number of gross chromosomal aberrations was even higher in FANCD2 defective cells in compared with WT cells exposed to Fe particles. Thus, these results reveal that unrepaired clustered DNA lesions leads to the generation of cross-chromosomal aberrations and this might contribute to the initiation of carcinogenesis in response to HZE particles exposure.

The above data clearly indicate that FA pathway play a critical role in genome stability maintenance following Fe irradiation. The molecular mechanism by which FA factors coordinate with the factors of other DNA repair pathway during the processing of clustered DNA lesions is ongoing.

References

[1] A. D. D' Andrea, M. Grompe, Nature Reviews, 3(2003)23.
[2] Younghoon, D. D'Andrea Alan, Genes & Development, 24(2010)1680.
[3] A. Adele, et al., Molecular Cell, 39(2010)25.