Dose, LET, time and strain dependence of radiation-induced 53BP1 foci in 15 mouse strains *ex vivo* and associations to *in vivo* radiation susceptibility

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We present a comparative analysis on the repair of radiation-induced DNA damage *ex vivo* in 15 strains of mice, including 5 inbred reference strains and 10 collaborative-cross strains, of both sexes. Non-immortalized primary skin fibroblasts derived from 76 mice were subjected to both low- and high-LET radiation (0.1, 1 and 4 Gy of X rays; 1.1 and 3 particles/100µm^2 of 350 MeV/n ^{40}Ar and 600 MeV/n ^{56}Fe). Automated image quantification of 53BP1 radiation-induced foci (RIF) during the first 4-48 h post-irradiation was performed as a function of dose and LET. Similarly to what we had previously reported for immortalized human cell lines [1], we observed a saturation of RIF number with dose at 4h post-irradiation, with more RIF/Gy for lower LET (X rays and ^{40}Ar) compared to ^{56}Fe. However at later time points (24h and above), the trend was inverted with more RIF/Gy for higher LET. Our data suggest that multiple DSBs cluster into RIF: as the linear density of DSBs increases with LET, so does the probability of having more DSBs per RIF, which makes it more difficult for cells to fully resolve high-LET-induced RIF, explaining the hypersensitivity to high-LET radiation despite a low number of RIF. Taking into account the amount of clustering at a given dose and LET, but also the kinetics of DNA damage repair, we introduced a novel mathematical formalism to evaluate the number of remaining RIF over time. We showed that the newly introduced kinetic metrics can be used as surrogate biomarkers for *in vivo* radiation toxicity, with potential applications in radiotherapy and human space exploration. In particular, we observed an association between the repairable fraction of RIF measured *in vitro* and survival levels of immune cells collected from irradiated mice. Moreover, the speed of DNA damage repair correlated with spontaneous cancer incidence data collected from the Mouse Tumor Biology database, suggesting a relationship between the efficiency of DSB repair after irradiation and cancer risk. In addition to the efficacy of repair and persistent RIF levels, even the amount of spontaneous foci without irradiation was shown to be strain dependent, indicating that these phenotypes are at least partially driven by genetics, and supporting their potential as indicators of individual radiation sensitivity.

[1] Neumaier, T., et al., PNAS, 2012 (8) 109:443