Non-linear DNA damage response to low-dose ionizing radiation suggests clustering of DNA breaks in normal human cells

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Goal

One of the goals in Project 3 of the Berkeley Lab SFA is to create accurate quantitative tools and develop mathematical formalisms to interpret in an unbiased manner the early response of normal human mammary epithelial cells (HMEC) to radiation-induced DNA double-strand breaks (DSBs). Using these tools we have observed non-linear DNA damage responses to low doses of Xrays suggesting clustering of the DNA breaks in HMEC that changes with increasing dose. Image analysis tools developed for this purpose are also used by other members of the LBNL SFA to quantify radiation-induced foci (RIF). This permits a unified quantification of DNA damage for the whole LBNL SFA, and enables us to compare results between *in vivo* and *in vitro* data from the different projects.

Background and Significance

American nuclear regulatory agencies consider that risk is proportional to dose of ionizing radiation, and thus any doses of radiation is harmful. This is referred to as the linear no threshold model (LNT), and it assumes that the biological response to DNA damage is also linear. Such model has been questioned by the radiation biology community and is the subject of an ongoing controversy.

The earliest measureable event in the DNA damage response is the relocalization of DNA damage sensing proteins, such as 53BP1 and γH2AX, to sites of DSB. This occurs within seconds to minutes following ionizing radiation (IR) exposure, and it results in the formation of immunofluorescently stainable nuclear domains referred to as radiation-induced foci (RIF). By fully characterizing the spatiotemporal properties of 53BP1 and γH2AX RIF in non-malignant human breast and skin cells exposed to different doses and radiation qualities, we plan to test the linearity assumption of the DNA damage response.

Approach

The number of RIF measured at any time-point following exposure to IR only reflects a net number of RIF: this number does not account neither for RIF that have already been resolved, nor for RIF that have not yet appeared. Novel image-analysis algorithms for automatic RIF detection coupled with a mathematical model allow us to deduce from the measured RIF kinetic the total number of RIF produced for a given dose of IR. Our fits suggest that the absolute RIF yield normalized to dose (α) is not constant, as the total yield has been assumed to scale with dose, but rather is 2 to 3 fold higher at a low dose of 5 cGy compared to what is measured at a higher dose of 200 cGy of X-rays. In addition, we show that RIF are produced faster and removed more slowly with increasing doses. Similar trends are also observed when depositing very high local doses of IR in the nucleus by using high

LET ions: for example, RIF induction is 25 times faster after exposure to 1 GeV/amu Fe ions. Interestingly, damages outside the ion tracks, which are estimated to be equivalent to 17 cGy of X-rays, elicit a RIF kinetic comparable to that of X-rays.

Live cell imaging of 53BP1-GFP in irradiated human fibrosarcoma cells HT1080 and non-malignant human breast cells allow us to simultaneously measure the total number of RIF produced by IR, and the kinetic of the number of RIF at any time point in a cell. Comparing time lapse between doses is extremely instructive. At high dose, the majority of RIF appear within the first 30 min following 100 cGy of X-rays and they stay unresolved for at least 2 hours (i.e. 12 RIF/Gy 30 min post-IR versus a total of 17 RIF/Gy in cumulated count over 4 hours). At low doses, RIF appear more sporadically across the first hour following 10 cGy and they are resolved fast, leading to a net number of 15 RIF/Gy 30 min post-IR versus a total cumulated count of 40 RIF/Gy. Thus, even though RIF/Gy measured at a given time point seem to be unchanged across doses, the cumulated number of RIF/Gy is much smaller at higher doses. Note that fitting the kinetic data with our mathematical model leads to values similar to the cumulated counts, validating our model and suggesting that the apparent linear dose dependence of RIF at a given time is the result of two competing non-linear events: faster induction and slower resolution with higher doses.

Assuming the number of DSB is proportional to dose (i.e. for human cells, we assume 35 DSB/Gy randomly distributed in the nucleus and 1.3 DSB/μm along 150 keV/μm Fe ion tracks), we show with Monte Carlo simulations that our experimental results can be interpreted as the existence of an attractive force between DSBs. We find that an interaction range of 1 μm leads to a dose dependence of RIF/Gy that is similar to what we measure after X-rays. This interaction range is confirmed experimentally by high-LET ion data as we observe no further increase of RIF/μm for LET larger than 200 keV/μm, with a constant linear frequency of ~1 RIF/μm. Monte Carlo simulations using such interaction range indicate that the probability of DSB clustering increases as a quadratic function of dose. Consequently, as dose increases, the percentage of RIF made of clustered DSBs increases. Overall, this leads to faster RIF induction but slower RIF resolution, as all DSBs within one RIF must be repaired before it can be resolved.

Conclusion and Future Plans

Based on the above data, we would argue that the relocalization of DSB into common nuclear regions is a repair process optimized by the evolution of the human species living in an ambient level of very low doses of ionizing radiation. However, when a human cell is exposed to non-physiological higher doses of IR, we would predict that this very same repair process becomes detrimental to the cell as it enhances non-linearly the probability of mutations, misrejoinings, and chromosome aberrations, which are all precursors of cancer. Therefore, extrapolating cancer risks linearly from high to low doses seems to be an oversimplification, that may lead to overestimating risk. We plan to further characterize the spatiotemporal response in ATM- or DNApkcs-deficient cells or as a function of chromatin territory, to extend the work done by the Karpen Lab using the Drosophila model studies in Project 3 in the Berkeley SFA.

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