DEVELOPMENT OF A NEW IMPROVED BIODOSIMETRY METHOD FOR MEASURING PREVIOUS EXPOSURE TO HZE RADIATION

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Chromosome aberrations in blood lymphocytes provide a useful measure of past exposure to ionizing radiation. Despite the widespread and successful use of the dicentric assay for retrospective biodosimetry, the approach suffers substantial drawbacks, including the fact that dicentrics in circulating blood have a rather short half-life (1-2 years by most estimates). So-called symmetrical aberrations, such as translocations are far more stable in that regard, but their high background frequency, which increases with age, also makes them less than ideal for biodosimetry. We developed a cytogenetic assay for potential use in retrospective biodosimetry that is based on the detection of chromosomal inversions, another symmetrical aberration whose transmissibility (stability) is also ostensibly high. Many of the well-known difficulties associated with inversion detection were circumvented through the use of directional genomic hybridization (dGH^{TM}), a method of molecular cytogenetics that is less labor intensive and better able to detect small chromosomal inversions than other currently available approaches. Here we report the dose-dependent induction of inversions following exposure to radiations with vastly different ionization densities (i.e., linear energy transfer; LET). Using dGH probes that hybridize and provide coverage to about 6% of the human genome (human chromosome 3 chromatid paint) a dramatic dose-dependent increase in the yields of inversions induced by high LET charged particles was observed. These results were particulary striking when compared to the dose response generated using low LET gamma rays. As predicted in the literature, these results show induced inversion frequencies are particularly sensitive to the types of radiation encountered on deep space missions. While arguably an early study, the robust dose response of inversions after high HZE irradiation suggests that including the detection and enumeration of inversions in future studies will only strengthen the capabilities of biologically-relevant, retrospective biodosimetry.

In order to increase the sensitivity of the dGH assay even further it is necessary to increase the target size of DNA 'at risk' for inversions, i.e. develop more chromatid paints. The existing chromatid paints were developed using traditional oligonucleotide synthesis methods, which are both expensive and labor intensive. The current goal of developing probes for chromatid paints for the 4 largest human chromosomes (~30% of the genome) is predicted to increase the sensitivity of the assay by 4-5 fold. The approach is to use massively parallel synthesis of oligonucleotides, followed by a parallel amplification scheme and yet to preserve the directional aspect of the probe sequences. Progress towards this goal will be presented.

Development of first chromatid paint-NASA NNX09CE42P and NNX10CB05C
Development of massively parallel approach- NASA NNX12AM92G

REFERENCE

Ray, F.A., Zimmerman, E. Robinson, B., Cornforth, M.N., Bedford, J.S., Goodwin, E.H., and Bailey, S.M. (2013) *Chromosome Research* 21,165-174.