The pKZ1 transgene as a sensitive passive gene expression reporter for low dose radiation responses.

Pamela J Sykes and Benjamin J Blyth.

Haematology and Genetic Pathology, Flinders University and Medical Centre, Flinders Centre for Cancer Prevention and Control, Bedford Park, Adelaide, South Australia 5042 Australia. (pam.sykes@flinders.edu.au).

After high radiation dose exposures, changes in a number of biological endpoints *in vivo* can be readily observed above the normal background variation that exists in any biological system. Where these responding biological endpoints can be tied to carcinogenic risk, for example, apoptosis, proliferation or chromosomal aberrations, the net effect of the radiation exposure on cancer risk can be surmised. If the biological changes are proportional to radiation dose, then at much lower doses, the biological responses will eventually fall within the range of normal inter-individual and temporal variation, making statistical proof of any effect difficult or unfeasible (Staudacher et al. 2010, *Radiat. Res* 174: 727-731.). Other end-points such as gene expression changes at loci may provide greater sensitivity after low dose radiation but are subject to dynamic regulation, feedback loops, redundant signalling pathways and overcompensation responses which can vary in different cell types leading to considerable variation in normal levels, and hence the extent to which any one marker is informative of cancer risk is severely diminished.

One strategy to circumvent these limitations is to monitor a passive reporter gene that is not part of an existing regulatory network. In such a system radiation-induced changes that perturb the cell's regulatory environment can potentially 'switch on' a unique exogenous gene that can be detected with great sensitivity. In our experiments, we measure the expression of the *E. coli* β-galactosidase transgene in pKZ1 transgenic mice. In unirradiated and sham-irradiated mice, the overwhelming majority of cells in the spleen and prostate do not show the presence of the transgene-derived protein product. The small fraction of cells where the β-galactosidase protein is present, has presumably either undergone a physical change in the DNA sequence (a DNA inversion mediated by the flanking recombination signal sequences) which places the reporter gene downstream of the transgene promoter, or aberrant transcription of the transgene has occurred. Our previous published work demonstrates that low (1 mGy) and very low doses (5 μGy) of X-radiation *in vivo*, are able to alter the fraction of cells which stain for transgene-derived β-galactosidase enzyme activity. Furthermore, adaptive response experiments demonstrate that conditioning radiation doses in this low and very low dose range are able to modify the response to subsequent challenge radiation doses.

The reason for the ability of the pKZ1 system to detect radiation-induced changes at such low radiation doses, may be explained by: the lack of endogenous regulation of the locus which might ordinarily counter and subdue any radiation-induced changes; the binary nature of the signal which is easier to quantitate than looking for large changes above a pre-set threshold; and, the potential for a short term change in the gene regulation environment to result in a longer-term signal that widens the temporal window of analysis. We are currently undertaking detailed molecular analysis of the pKZ1 locus in order to tie the radiation-induced changes we observe *in vivo* in the tissues of pKZ1 mice and *in vitro* in pKZ1 hybridoma cell lines, to specific low dose radiation induced biological pathways. We have now confirmed by real-time quantitative PCR that DNA inversions do occur in pKZ1 tissues,

and by reverse-transcription PCR that inverted copies of the transgene do produce β-galactosidase mRNA initiated from the transgene promoter. Intriguingly, we have also discovered that alongside the inversion-dependent β-galactosidase transcripts, inversion-independent transcripts are also present in pKZ1 cells and tissues. We have shown using our pKZ1 hybridoma line that changes in β-galactosidase expression in response to the chemical mutagen etoposide, correlate with changes in the abundance of both the inversion-dependent and inversion-independent transcripts. Experiments are now underway to measure the levels of the two pKZ1 transcript types in response to low dose radiation. Our preliminary results on the regulation of the pKZ1 transgene suggest that our previously observed radiation-induced changes in pKZ1 cells and tissues could represent a sensitive detection of de-regulation of the pKZ1 transgene locus which appears to be silenced in the majority of cells. A potential cryptic promoter region has been identified in the pKZ1 transgene. The molecules which interact with and activate this normally silenced promoter region could represent effectors of radiation-induced gene expression changes.

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