Abstract

Radiation Leukaemogenesis at Low Doses
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Introduction

The complete and quantitative understanding of radiation leukaemogenesis at low doses and dose rates will require an improved understanding of the mechanisms by which radiation causes leukaemia. This mechanistic understanding is most readily obtained through the study of animal model systems as suitable human materials are not available. The CBA mouse model of radiation-induced acute myeloid leukaemogenesis has been used extensively for quantitative and mechanistic studies. The CBA model is being used in this project and a linked DoE funded study (DE-FG02-05 ER 63946) headed by Prof Ullrich at Colorado State University (CSU). CSU are irradiating CBA mice with gamma rays at low dose rates (10 cGy/day) to examine leukaemogenic effectiveness. The AML materials arising will be analysed in both CSU and HPA labs when available. Present efforts focus on the development of methods for analysis of AMLs.

Mechanisms of mouse leukaemogenesis

It is known that chromosome (chr)2 deletions associate strongly (~95% of cases) with AMLs in the mouse induced by high dose/dose rate radiation. More recently a candidate AML-suppressor gene locating within the commonly deleted region of chr2 has been identified (1,2). This gene, *Sfpi1/PU.1*, suffers hemizygous loss and specific point mutations in exon 5 in the majority of radiation-induced AMLs studied. These mutations are within the DNA binding Ets domain of this key haemopoietic system transcription factor. The aim of this project is to determine whether similar mechanisms operate in leukaemogenesis at low dose rates.

Development of tools for AML analysis

We have developed fluorescence *in situ* hybridisation assays to assess *Sfpi1/PU.1* loss. The standard assay includes controls for hybridisation efficiency and chr2 identification. The assay can reliably identify *Sfpi1/PU.1* deletions in AMLs and in bone marrow cells at very early times post-*in vivo* irradiation. The latter approach confirms that *Sfpi1/PU.1* loss is a very early event in leukaemogenesis and will facilitate a long term follow up study. Currently a dose-response study of early *Sfpi1/PU.1* losses is being carried out initially down to 0.1 Gy. The timing of the point mutational events is not currently known but previous G-band cytogenetic analyses suggest that a second significant event in AML development occurs between 9 and 12 months post 3 Gy acute irradiation. Tracking of point mutations will require the development of new tools.

Low dose-rate radiation-induced AMLs will be examined for *Sfpi1/PU.1* loss by FISH and point mutations in exon 5 will be determined by DNA sequencing. Thus it will be established if AMLs induced by 10 cGy/day irradiation develop by the same mechanism that predominates at dose rates of ~0.5 - 1 Gy/minute.

Understanding the process of leukaemogenesis will also require an appreciation of the effect of *Sfpi1/PU.1* loss/mutation on cell physiology. To start to address this issue, quantitative PCR assays have been developed for *Sfpi1/PU.1* and some downstream target genes of this haemopoietic system transcription factor. To date these have been applied to a panel of 6 AML cell lines. Differing expression levels of *Sfpi1/PU.1* have been detected. Correlations are now

being sought with the expression of potential transcriptional targets. Assays have been developed for 4 genes. Additional downstream targets are being sought by cDNA microarray comparisons of gene expression in a *Sfpi1/PU.1* null cell line with an AML cell line with high level expression of a mutant *Sfpi1/PU.1*. Data from gene expression studies will be presented at the workshop.

Summary

The CBA mouse model of radiation leukaemogenesis is well understood mechanistically. A range of tools suitable for the analysis of both high dose/dose rate and low dose/dose rate AMLs is available and these continue to be developed. The linked projects should provide data to assess the similarities/differences in myeloid leukaemia development at high and low doses.

References

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