Mutation Research 793 (2015) 48–54

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Influence of radiation quality on mouse chromosome 2 deletions in radiation-induced acute myeloid leukaemia

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ARTICLE INFO

Article history:
Received 22 July 2015
Accepted 23 July 2015
Available online 28 July 2015

Keywords:
Radiation
Chromosome deletion
aCGH
Sfpi1/PU.1
Myeloid leukemia
Mouse model

ABSTRACT

Leukaemia is the prevailing neoplastic disorder of the hematopoietic system. Epidemiological analyses of the survivors of the Japanese atomic bombings show that exposure to ionising radiation (IR) can cause leukaemia. Although a clear association between radiation exposure and leukaemia development is acknowledged, the underlying mechanisms remain incompletely understood. A hemizygous deletion on mouse chromosome 2 (del2) is a common feature in several mouse strains susceptible to radiation-induced acute myeloid leukaemia (rAML). The deletion is an early event detectable 24 h after exposure in bone marrow cells. Ultimately, 15–25% of exposed animals develop AML with 80–90% of cases carrying del2. Molecular mapping of leukaemic cell genomes identified a minimal deleted region (MDR) on chromosome 2 (chr2) in which a tumour suppressor gene, *Sfpi1* is located, encoding the transcription factor PU.1, essential in haematopoiesis. The remaining copy of *Sfpi1* has a point mutation in the coding sequence for the DNA-binding domain of the protein in 70% of rAML, which alters a single CpG sequence in the codon for arginine residue R235. In order to identify chr2 deletions and *Sfpi.1*/PU.1 loss, we performed array comparative genomic hybridization (aCGH) on a unique panel of 79rAMLs. Using a custom made CGH array specifically designed for mouse chr2, we analysed at unprecedentedly high resolution (1.4 M array- 148 bp resolution) the size of the MDR in low LET and high-LET induced rAMLs (32 X-ray- and 47 neutron-induced). Sequencing of *Sfpi1*/PU.1DNA binding domain identified the presence of R235 point mutations, showing no influence of radiation quality on R235 type or frequency. We identified for the first time rAML cases with complex del2 in a subset of neutron-induced AMLs. This study allowed us to re-define the MDR to a much smaller 5.5 Mb region (still including *Sfpi1*/PU.1), identical regardless of radiation quality.

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1. Introduction

Acute myeloid leukaemia (AML) is one of the most common cancers occurring in humans following exposure to ionising radiation (IR) and epidemiological studies ofsurvivors of the Hiroshima and Nagasaki atomic bombings showa dose-dependent increase in AML [1,2]. It can also be induced by radiotherapy treatment, so called therapy-related or secondary AML, and recently paediatric exposures to low doses of IR from CT scans have been shown to increase the risk of leukaemia [3]. However, although a clear association between radiation exposure and leukaemogenesis has been established, the underlying mechanisms remain unidentified.

Radiation-induced leukaemogenesis is a complex multi-step process that can be difficult to study in humans due to the scarcity, and genetic as well as etiological complexity, of appropriate samples, therefore mouse models of rAML are invaluable in providing a better understanding of the molecular mechanisms of tumour initiation and development. A number of murine models are available, which develop AML with a close histopathological similarity to human AMLs defined by a combination of cell morphology, immunocytochemistry, immunophenotyping and clinical features. The best characterized and most widely used model is the inbred strain CBA. The background rate of AML in CBA mice is very low (less than 1 in 1000) and there is a consistent induction rate of 15–20% following exposure to an optimal leukaemic dose of 3 Gy whole body X-rays [4,5]. In addition to X-ray studies, rAMLs have been induced with a range of different radiation types, including heavy ions and alpha particles [6–8].

Deletion and/or rearrangement of one copy of chromosome 2 (del2) is a common feature of murine rAML in CBA, with 80–90%

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http://dx.doi.org/10.1016/j.mrgentox.2015.07.012
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of cases carrying del2. Hemizygous deletion can be detected 24 h after exposure to IR, with a clonal expansion of bone marrow cells detectable 9–12 months later in approximately 50% of mice [9–11]. Chr2 deletions have been accurately mapped at the molecular level using loss of heterozygosity (LOH) analysis in rAMLs induced in F1(CBA × C57BL/6) mice [12]. A minimally deleted region (MDR) on chr2 was defined and mapped to identify candidate tumour suppressor genes within the region. The haematopoietic transcription factor Sfpi1/PU.1was identified as a potential candidate tumour suppressor in this region. Sfpi1/PU.1 is an essential regulator of haematopoiesis that is known to act as a proto-oncogene in murine erythroleukaemias [13,14]. Recent studies have shown that approximately 70% rAMLs and cell lines have hemizygous loss of one copy of Sfpi-1/PU.1 and a point mutation within exon 5, the DNA binding domain of the remaining copy and it is therefore safe to conclude that biallelic PU.1 mutations are common in low-LET rAML [15]. This mutation affects the arginine 235 (known as R235) residue of the protein, resulting in amino-acid substitutions of cysteine, serine or histidine (R235C, R235S and R235H, respectively) [16–18]. It is currently not known at what time after irradiation this point mutation occurs as the origin is likely to be spontaneous [19]. Deletion and/or point mutation of PU.1 in human AML has been less frequently reported [20], although heterozygous point mutations in PU.1have been found in 7% of cases. In these cases [21], it was found that the mutation leads to a reduced or aberrant function of the transcription factor [20]. PU.1 has further been shown to be of importance in certain types of AML for example those carrying the translocation t (15;17) [22].

Recently, loss and point mutation of Sfpi1/PU.1 has been tracked in radiation exposed CBA mice using BAC-FISH techniques [23]. Whilst this allows loss events at early time points post radiation exposure to be viewed, over a wide range of doses, it is limited to only showing the presence or absence of a deleted region which can be considerably large. With the advances in array-based CGH (aCGH) technology, we have used a custom-made mouse chr2 array at unprecedentedly high resolution (1.4 M array, 148 bp resolution), to analyse a panel of murine rAML samples. These samples were generated following exposure to two different radiation qualities, X-rays and neutrons. Neutrons were delivered as single and split doses over a dose range. Our aim was to compare the chr2 deletion structures generated by these different radiation types, and to re-visit and re-define the MDR in murine AML.

2. Materials and methods

2.1. AML samples

79 radiation-induced AML samples were used in this study, 32 X-ray- and 47 neutron-induced. Of the X-ray-induced, 8 cases were on an F1CBA/H × C57BL Lia background and were induced and characterised at NRG, Petten, Netherlands as described previously [12]. 24 CBA/CaH AML were induced and characterised at Public Health England, Chilton, Oxfordshire as described previously [10]. Mice were irradiated at 10–12 weeks of age at MRC Harwell using a Pantak X-ray source 250 kVp, 11 mA at a dose rate of 0.887 Gy/min to give a single whole body dose of 3 gray. Neutron-induced AMLs were irradiated with fast fission neutrons from a ^{235}U converter in the Low Flux Reactor at NRG, Petten, Netherlands, see Fig. 1.

AMLs were diagnosed using the criteria described in the Bethesda Proposals for Classification of Non-lymphoid Hematopoietic Neoplasms in Mice [24]. Mice were examined daily for signs of illness and were euthanised with a rising concentration of CO_2 when moribund. Mice found to have increased white blood cell counts in the peripheral blood and to display splenomegaly upon dissection were treated as suspect AMLs. Diagnosis was confirmed

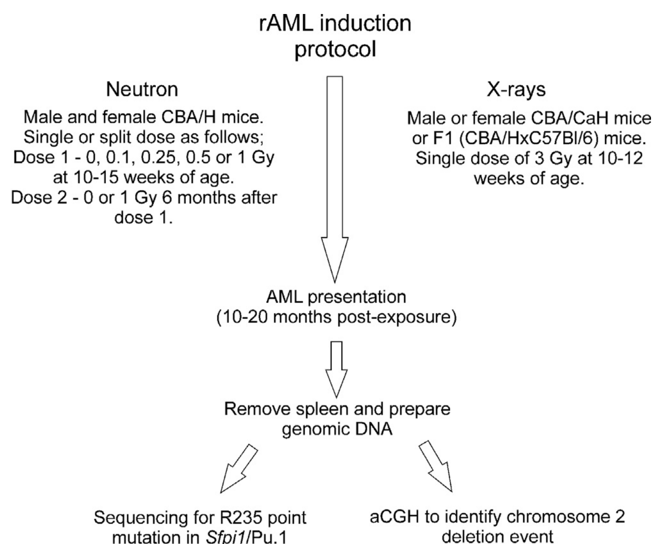

Fig. 1. Schematic diagram of rAML induction protocol for the panel of rAML samples used in this study (80 murine rAML samples in total (48 neutron- and 32 X-ray-induced)).

by examination of blood films, immunophenotyping and transplantation of tumours into recipient hosts. All animals were bred and handled according to UK Home Office Animals (Scientific Procedures) Act 1986 and with guidance from the local ethical review committee on animal experiments.

2.2. DNA preparation

For DNA 10 mg of spleen tissue was disrupted in a MagNALyser Green Beads tube in 200 μl MagNA Pure DNA Tissue Lysis Buffer using a MagNALyser Instrument (Roche Diagnostics GmbH, Mannheim, Germany) according to manufacturer's instructions. DNA was prepared using a MagNA Pure Compact Instrument and MagNA Pure Compact Nucleic Acid Isolation Kit 1-Large Volume (Roche Diagnostics GmbH, Mannheim, Germany) according to manufacturer's instructions.

2.3. Polymerase chain reaction and DNA sequencing

PCR reactions were performed in a total volume of 10 μl containing 25 ng DNA, 0.33 μM each of forward and reverse primers (oligonucleotides synthesized by Sigma–Aldrich, Poole, UK), 200 μM dNTPs(Invitrogen, Paisley, UK), 1× PCR buffer with added Q solution and 1.25 U Taq polymerase (Qiagen). Reactions were carried out in a Hybaid PCR Express thermal cycler (Thermoquest Scientific, Basingstoke, UK) with the following temperature cycling conditions 94 °C for 4 min 1 cycle; 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, 35 cycles; followed by a final extension of 72 °C for 10 min. PCR products were analysed on 2.5% agarose gels (Biorad, Hercules, US) run for 2 h at 80 V, stained with ethidium bromide and visualised using UV with a BioRad Gel Doc 2000 system.

Exon 5 R235 mutations in *Sfpi1/PU.1* were determined by DNA sequencing as described [17] using primer sequences as follows—F: CGACATGAAGGACAGCATCT, R: TTTCTTCACCTCGCCTGTCT.

2.4. Array comparative genomic hybridisation (aCGH)

For the majority of the aCGH a custom made array was used consisting of 1.4 M features (loci) (NimbleGen Custom CGH 3 × 1.4 M, Roche NimbleGen, Madison, WI, USA), 70% of which are located on murine chromosome 2, the remainder spread through the rest

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