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Transmission of clonal chromosomal abnormalities in human hematopoietic stem and progenitor cells surviving radiation exposure

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ABSTRACT

In radiation-induced acute myeloid leukemia (rAML), clonal chromosomal abnormalities are often observed in bone marrow cells of patients, suggesting that their formation is crucial in the development of the disease. Since rAML is considered to originate from hematopoietic stem and progenitor cells (HSPC), we investigated the frequency and spectrum of radiation-induced chromosomal abnormalities in human $CD34^+$ cells. We then measured stable chromosomal abnormalities, a possible biomarker of leukemia risk, in clonally expanded cell populations which were grown for 14 days in a 3D-matrix (CFU-assay). We compared two radiation qualities used in radiotherapy, sparsely ionizing X-rays and densely ionizing carbon ions (29 and 60–85 keV/μm, doses between 0.5 and 4 Gy). Only a negligible number of *de novo* arising, unstable aberrations (≤0.05 aberrations/cell, 97% breaks) were measured in the descendants of irradiated HSPC. However, stable aberrations were detected in colonies formed by irradiated HSPC. All cells of the affected colonies exhibited one or more identical aberrations, indicating their clonal origin. The majority of the clonal rearrangements (92%) were simple exchanges such as translocations (77%) and pericentric inversions (15%), which are known to contribute to the development of rAML. Carbon ions were more efficient in inducing cell killing (maximum of ~30–35% apoptotic cells for 2 Gy carbon ions compared to ~25% for X-rays) and chromosomal aberrations in the first cell-cycle after exposure (~70% and ~40% for 1 Gy of carbon ions and X-rays, respectively), with a higher fraction of non-transmissible aberrations. In contrast, for both radiation qualities the percentage of clones with chromosomal abnormalities was similar (40%). Using the frequency of colonies with clonal aberrations as a surrogate marker for the leukemia risk following radiotherapy of solid tumors, charged particle therapy is not expected to lead to an increased risk of leukemia in patients.

1. Introduction

Acute myeloid leukemia (AML) is one of the malignancies which can be induced by ionizing radiation, for example after radiotherapy of solid cancers [1]. A confounding factor in tracing back the origin of AML is the multiple drug treatment of cancer patients, but a higher risk for AML following radiation exposure has also been observed in atomic bomb survivors [19], occupational radiation workers [35] and children receiving repeated CT scans [32]. The risk for secondary malignant neoplasias is high in cancer survivors, especially for pediatric patients. Approximately one third of pediatric medullobastoma patients are affected by a secondary cancer up to 30 years after radiation treatment, which includes the exposure of brain and spinal cord to radiation [14,38]. Evidence has been provided that the risk for radiation-induced AML (rAML) depends on the radiation dose that reaches active bone marrow, where hematopoietic stem and progenitor cells (HSPC) are located [5,42].

Radiation-induced DNA damage, especially misrepaired DNA double-strand breaks (DSBs), can result in the formation of chromosomal aberrations. If the aberrations are non-transmissible, they

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can lead to cell death. In contrast, non-lethal, transmissible chromosomal abnormalities are those that can instead be passed on to the following cell generations [7]. Clonal aberrations in mature hematopoietic cells have been detected up to 30 years after exposure in blood samples of accidently exposed persons or atomic bomb survivors [2,16,26], suggesting transmission of radiation-induced non-lethal rearrangements.

The clonal expansion of cells with chromosomal aberrations is likely to be a general feature in the development of AML, and cells sharing the same chromosomal abnormalities are often detected in bone marrow cells of leukemia patients, treated previously with chemotherapy, radiotherapy or a combination of both [33]. Multiple types of aberrations have been found in the so called "leukemic clones" which can potentially prime the cells toward the leukemic phenotype: Translocations can lead to the formation of fusion genes resulting in an uncontrolled expression of oncogenes; chromosomal deletions can lead to the inactivation of one allele of a putative tumor suppressor gene [9,28,50].

As AML results from more than one genetic aberration, genomic instability is discussed as an additional trigger for the progression of the disease [37,41]. Genomic instability in cells surviving radiation exposure may accelerate the accumulation of abnormalities in crucial transforming genes [1,15,20]. The occurrence of radiation-induced genomic instability is a debatable issue. Some studies analyzing bone marrow cells of rAML patients at the molecular level report that radiation exposure triggers mutations in repetitive DNA sequences (so called microsatellite instability) [10,47] or mutations in genes important for the regulation of hematopoiesis like *AML1* gene [18,53].

At the chromosomal level, radiation induced instability is considered as a "delayed" phenomenon with respect to the time of irradiation, because *de novo* arising aberrations, such as dicentrics and other non-transmissible aberrations, have been observed in the clonal progeny of the irradiated cells many cell divisions after radiation exposure. Chromosomal instability has been reported for the first time after *in vivo* exposure of CBA/H mice [23] and, since then, has clearly been established in mice sensitive to rAML [30,43]. Radiation induced chromosomal instability in human HSPC as a direct effect of radiation exposure is still controversial. The corresponding results were obtained in mature blood cells of exposed individuals or in bone marrow cells exposed *in vitro* [2,16,24,26,52].

In the current study, human HSPC were exposed to two radiation qualities used in radiotherapy, X-rays and carbon ions, the latter being particular suitable for the treatment of radioresistant tumors close to critical organs. Densely ionizing charged particles, such as protons and carbon ions, are different from sparsely ionizing photons (X-rays) both for the depth-dose distribution (increased energy deposition with penetration depth) and the enhanced biological effectiveness due to the high local density of ionizing events creating clustered DNA damage which is difficult to repair.

A high frequency of chromosomal aberrations is associated with increased cancer incidence [17] and specific chromosomal aberrations can be used as biomarkers of risk to compare different radiation qualities (see *e.g.* [39] for thyroid cancer, [40]). As stated above, clonal aberrations are found in leukemia patients, indicating their occurrence during the development of the disease. As chromosomal lesions are induced randomly in the genome upon irradiation, we made the assumption that the transmission of any chromosomal aberration scales with the probability of the propagation of rearrangements critical for leukemia development, allowing the use of transmission of chromosomal aberrations within the clonal descendants of irradiated cells as a surrogate marker of risk for induction of leukemia.

To achieve this we first analyzed in this study data on the induction of chromosomal aberrations in the first cell-cycle after exposure (see Appendix A) with respect to their transmissibility to daughter cells. Then we measured the frequency of clonal and *de novo* arising, unstable chromosomal aberrations in myeloid colonies derived from single irradiated human HSPC after exposure to X-rays or carbon ions.

2. Materials and methods

2.1. Cell separation

HSPC were isolated from peripheral blood of healthy G-CSF pre-treated donors as described in [6]. We have pooled for each experiment the CD34+ enriched HSPC from 2 to 5 donors, according to the availability of samples. The gender ratio in the pooled samples of CD34+ cells was not standardized, averaged over all samples (62% male and 38% female donors were used, with a mean age of 39 years, ranging from 27 to 51 years). The samples were provided by the Department of Cellular Therapeutics/Cell Processing of the German Red Cross Blood Centre (Frankfurt, Germany) with the donors' informed consent. The study was approved by the local advisory boards. HSPC were positively enriched for CD34+ (CD34 MicroBead-Kit, Miltenyi Biotech). For cytogenetic analysis of first cycle HSPC samples were available only from one donor ($N = 1$).

2.2. Irradiation

HSPC were exposed to 0.5, 1, 2, 3 and 4 Gy of photons (X-rays, 16 mA, 250 kV) or carbon ions at a dose-rate of 1 Gy/min. Carbon ion exposure was performed at the Heavy Ion Synchrotron SIS18 of the GSI Helmholtz Center for Heavy Ion Research (Darmstadt, Germany). For carbon ion exposure either a monoenergetic beam or a spread-out Bragg peak (SOBP) were used to simulate the exposure of normal and tumor tissue during radiotherapy, respectively. This resulted in a different linear energy transfer (LET) of 29 keV/μm and 60–85 keV/μm, respectively. The LET is measured in keV/μm, which refers to energy depositions per μm-distance along the track of an accelerated ion. Further information on technical details of the irradiation procedure and dosimetry are available in [6]. The relative biological effectiveness (RBE) of ions compared to X-rays was determined. This factor is defined as the dose which is needed to achieve the same biological effect for a test radiation (carbon ions) and reference radiation (X-rays).

2.3. Expansion culture

HSPC were expanded in serum-free medium supplemented with 100 ng/ml Flt-3 ligand, 100 ng/ml Stem Cell Factor, 20 ng/ml Interleukin-3 and 20 ng/ml Interleukin-6 (StemSpan SFEM plus Cytokine Cocktail CC100, StemCell Technologies Inc.) at 37 °C in a humidified atmosphere (95%).

2.4. Colony forming unit (CFU-) assay

HSPC were differentiated within 14 days in a 3D matrix along the myeloid lineage to colonies at 37 °C in a humidified atmosphere (95%). The matrix consisted of semisolid methylcellulose (4000 cps) supplemented with 30% Fetal Bovine Serum, 1% Bovine Serum Albumin, 10^{-4} M 2-Mercaptoethanol, 2 mM L-Glutamine, 50 ng/ml Stem Cell Factor, 10 ng/ml rh Granulocyte–Monocyte–Colony Stimulating Factor, 10 ng/ml rh Interleukin-3 and 3 U/ml rh Erythropoietin (Methocult GFH4434, StemCell Technologies Inc). The colonies were counted and classified according to morphological features using light microscopy as exemplarily shown in Appendix A (Fig. A.1). The multi-potential and immature CFU-GEMM progenitors were recognized due to the presence of erythroid and other cells (granulocytes, macrophages, megakaryocytes) within one colony (>500 cells). Colonies with clusters of predominantly