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Predisposition to therapy-related acute leukemia with balanced chromosomal translocations does not result from a major constitutive defect in DNA double-strand break end joining

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Abstract

The frequency of acute myeloid leukemia (AML) with balanced chromosomal translocations arising after anticancer therapy with DNA-damaging agents such as DNA topoisomerase II inhibitors has increased over the last two decades. However, factors that predispose to these therapy-related disorders are still poorly defined. It has been reported that DNA double-strand break (DSB) repair by the non-homologous end-joining (NHEJ) pathway is impaired in myeloid leukemia cells. This led us to hypothesize that therapy-related AML (t-AML) may result from individual differences in the repair of DSBs generated by the treatment. We show here that DSB repair is accurate, *in vivo*, in non-tumoral cells derived from patients who developed t-AML with t(9;11) or t(15;17) translocation after treatment for a first cancer with DNA topoisomerase II inhibitors. These results indicate that a major constitutive defect in the NHEJ pathway is unlikely to predispose to t-AML with balanced chromosomal translocations.

Keywords: Therapy-related acute myeloid leukemia; DNA double-strand break repair; Topoisomerase II inhibitors

1. Introduction

Most cases of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) develop *de novo*. However, the frequency of AML and MDS arising after DNA-damaging therapy has increased over the last two decades, and they now account for 10% to 20% of all cases [1]. Therapy-related AML (t-AML) and MDS (t-MDS) are often clinically and genetically similar to *de novo* cases, suggesting that the molecular mechanisms underlying most of these diseases are similar. Two major types of t-MDS and t-AML are classically opposed [2–6]. The first type generally occurs 3–7 years after treatment with alkylating agents, presents an MDS with chromosomal deletions of chromosomes 7 and/or 5 and has become less frequent with reduction of the prolonged use of alkylating agents. The second one, of growing incidence, generally develops shortly (1–3 years) after treatment with topoisomerase II inhibitors (epipophyllotoxins, anthracyclines, or anthracenediones like mitoxantrone), are AML with no preleukemic phase, and have balanced translocations involving 11q23, or less often 21q22 or t(15;17) translocations. 11q23 rearrangements may especially occur after treatment with epipophyllotoxins, and t(15;17) after treatment with anthracenediones or mitoxantrone. Mechanisms underlying the development of chromosomal translocations are poorly understood [7]. However, recent studies support a

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central role for DNA double-strand break (DSB) repair pathways in both their prevention and their creation (for review, [8,9]).

DNA topoisomerase II is a homodimeric nuclear enzyme involved in many aspects of DNA metabolism. It catalyzes topological conversions of DNA by making transient DSBs in one segment of DNA allowing the passage of an intact duplex through the broken DNA, before re-ligating the break [10]. Topoisomerase II inhibitors prevent the enzyme from re-ligating cleaved DNA, thus generating DSBs [10]. DSBs constitute a major threat to genome integrity. In mammalian cells, DSBs can be repaired by two major pathways: the non-homologous DNA end-joining (NHEJ) pathway, which joins, precisely or not, broken DNA ends containing little or no homology, and the homologous recombination (HR) pathway, which requires a homologous sequence provided by either a sister chromatid or a homologous chromosome [11,12]. The NHEJ pathway requires the XRCC4/DNA ligase IV complex plus a functional DNA-dependent protein kinase complex (DNA–PK) composed of the Ku heterodimer Ku70/Ku80 and the catalytic subunit DNAPKcs [13]. The NHEJ pathway plays a predominant role from G1 to the early S phase of the cell cycle [14], and is predominantly involved in the repair of DSB induced by topoisomerase II inhibitors [15]. Some genomic loci that are involved in translocations in leukemias have been shown to co-localize before the event, and a significant association of PML with RARalpha (the genes involved in the translocation t(15;17)) was seen in hematopoietic precursors [16]. Thus, a partial defect in NHEJ could favor the association (by ligation or recombination) of broken genes that are in close proximity in the cell, instead of directly re-ligating DNA ends. Moreover, NHEJ has been reported to be impaired in myeloid leukemia cells [17]. To test whether constitutive differences in DSB repair by the NHEJ pathway predispose individuals to t-AML with balanced chromosomal translocations, we established non-tumoral lymphoblastoid cell lines from five patients treated for a first cancer with chemotherapy containing topoisomerase II inhibitors and who developed t-AML (three with a t(15;17) translocation and two with a t(9;11) translocation; Table 1). As controls, non-tumoral lymphoblastoid cell lines were established from two patients who had been treated for a first cancer with chemotherapy containing topoisomerase II inhibitors and who did not develop t-AML/MDS (Table 1). We used a plasmid-based host cell end-joining assay to analyze the *in vivo* capacity of these cells to repair DSBs by NHEJ [18].

2. Materials and methods

2.1. Cell lines

Peripheral blood mononuclear cells from patients treated in the Centre Hospitalier Universitaire of Lille (Service des Maladies du Sang), Institut Gustave Roussy, Villejuif (Service d'Hématologie Clinique,) and Hôpital Avicenne, Paris 13 University (Service d'Hématologie Clinique), were exposed to Epstein–Barr virus (EBV) to establish lymphoblastoid cell lines (Banque de Cellules et de l'ADN,

Table 1
Patients characteristics

Patient	Gender	First cancer	Age at first cancer	AML(FAB)	Karyotype	Interval from onset of chemotherapy to diagnosis of t-AML/MDS (months)	First cancer treatment
LILA	F	DLBCL	46	M3AML	t(15;17)	23	Adriamycin, novantrone, cyclophosphamide, ifosfamide, vindesine, bleomycine, etoposide, cytarabine, prednisone
LILB	M	DLBCL	51	M3AML	t(15;17)	15	Adriamycin, novantrone, cyclophosphamide, ifosfamide, vindesine, bleomycine, etoposide, cytarabine, prednisone
LIL C	F	MALT lymphoma	57	M3AML	t(15;17)	95	Cyclophosphamide, adriamycin, novantrone, vincristine, fludarabine, prednisone
IGRA	F	Breast carcinoma	57	M1 AML	t(9;11)	22	Cyclophosphamide, adriamycin, fluorouracil, radiotherapy
IGRC	F	Breast carcinoma	41	M1 AML	t(9;11)	26	Cyclophosphamide, epirubicin, fluorouracil, radiotherapy
LIL D	F	DLBCL	67	Control			Cyclophosphamide, adriamycin, vincristine, prednisone
LILE	M	Follicular lymphoma	50	Control			Cyclophosphamide, adriamycin, vincristine, prednisone

Cells lines LIL A to LIL C and IGR A and IGR C were collected from patients who developed *t*-AML with balanced chromosomal translocations after a first cancer treatment, LIL D and E were collected from control patients who did not develop t-AML after a first cancer treatment. DLBCL: diffuse large B-cell lymphoma. MALT: mucosa-associated lymphoid tissue lymphoma. AML: acute myeloid leukemia.