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ARTÍCULO DE REVISIÓN

Genetic abnormalities in leukemia secondary to treatment in patients with Hodgkin's disease

Consuelo Salas,*,** Patricia Pérez-Vera,* Sara Frías*,***

* Departamento de Investigación en Genética Humana, Laboratorio de Cultivo de Tejidos y Laboratorio de Citogenética, Instituto Nacional de Pediatría.
** Facultad de Medicina, Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México.
*** Departamento de Medicina Genómica y Toxicología Ambiental, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México.

ABSTRACT

Hodgkin's disease has been treated mainly with two chemotherapy schedules, MOPP (nitrogen mustard, Oncovin, procarbazine and prednisone), which includes alkylating agents, and ABVD (adriamycin, bleomycin, vinblastine and dacarbazine), which includes topoisomerase II inhibitors, either with or without radiation therapy. Due to the types of agents used, patients with Hodgkin's disease often develop secondary leukemias. The alkylating agents included in the MOPP scheme were the first drugs associated with the development of therapy-related myelodysplastic syndrome (t-MDS) and acute myeloid leukemia (t-AML); both entities are the result of the clonal selection of cells with accumulated genomic lesions induced by antineoplastic therapy. In patients who developed t-MDS and t-AML, eight alternative routes with specific cytogenetic and molecular changes have been identified, and the routes are related to the type of therapy, alkylating agents or DNA topoisomerase II inhibitors. At the cytogenetic level, patients treated with alkylating agents show deletion 5q/monosomy 5 and deletion 7q/monosomy 7; in contrast, those who were treated with topoisomerase II inhibitors show 11q23 translocations involving the *MLL* gene. At the molecular level, there are two types of mutations: Class I, which alter the RAS-BRAF signal transduction pathways and increase cell proliferation; Class II, which disrupt genes that encode transcription factors and *NPM1* that are involved in cell differentiation, and the inactivation of p53 tumor suppressor gene. Knowledge of the genetic alterations in these conditions is important for the classification, treatment and prognosis of patients as well as essential for increasing the knowledge of the biology of these diseases, which leads to identifying potential therapeutic targets.

Anormalidades genéticas en leucemia secundaria a tratamiento en pacientes con enfermedad de Hodgkin

RESUMEN

La enfemedad de Hodgkin (EH) se ha tratado principalmente con dos esquemas quimioterapéuticos: el MOPP (mostaza nitrogenada, Oncovin, procarbazina y prednisona) con alto contenido de agentes alquilantes y actualmente un esquema que incluye inhibidores de la topoisomerasa II, el ABVD (adriamicina, bleomicina, vinblastina y dacarbazina) ambos con o sin radioterapia. Por el tipo de agentes utilizados, los pacientes con EH desarrollan frecuentemente leucemias secundarias. Los agentes alquilantes incluidos en el esquema MOPP fueron las primeras drogas asociadas al desarrollo de síndrome mielodisplásico y leucemia mieloide aguda secundarias a terapia (t-SMD y t-LAM); ambas entidades resultan de la selección clonal de células, con acumulación de lesiones genómicas transformantes, inducidas por el tratamiento antineoplásico. En los pacientes con EH que desarrollan t-SMD y t-LAM se han identificado cambios específicos tanto citogenéticos como moleculares, a partir de los cuales se distinguen ocho vías alternativas de alteraciones citogenéticas y moleculares. La ruta de alteraciones se relaciona con el tipo de terapia al que fue sometido el paciente: agentes alquilantes o inhibidores de la DNA topoisomerasa II. A nivel citogenético los pacientes tratados con agentes alquilantes muestran alteraciones no balanceadas como deleción 5q/monosomía 5, así como deleción 7q/monosomía 7; en contraste, en aquéllos que se trataron con inhibidores de la topoisomerasa II se observan translocaciones en la banda 11q23 involucrando al gen MLL. A nivel molecular se distinguen dos tipos de mutaciones: Clase I, que alteran las vías de transducción de señales RAS-BRAF e incrementan la proliferación celular; Clase II, en genes que codifican factores de transcripción y en NPM1, que alteran la diferenciación celular y en el gen supresor de tumor p53. El conocimiento de las alteraciones genéticas en estos padecimientos es relevante porque ayuda a su clasificación, sugiere el tratamiento a seguir, informa sobre el pronóstico de los pacientes y permite

Key words. Hodgkin's disease. Secondary leukemia. Cancer survivors. Chemotherapy genotoxicity. Alkylating agents and DNA topoisomerase II inhibitors.

abundar en el conocimiento de su biología, lo cual conlleva a la identificación de posibles blancos terapéuticos.

Palabras clave. *Enfermedad de Hodgkin. Leucemia secundaria. Sobrevivientes de cáncer. Genotoxicidad por quimioterapia. Agentes alquilantes e inhibidores de la DNA topoisomerasa II.*

INTRODUCTION

Advances in cancer treatment have increased patient survival; currently, there are 12 million cancer survivors in the US, representing 4% of the population.[1,2] More than 80% of children who have been diagnosed with Hodgkin's disease (HD) will survive for ten years or more; however, antineoplastic therapy for HD is not specific to tumor cells and affects normal cells. Thus, the majority of these survivors are at risk for developing one or more long-term sequelae of their therapy.[3] The development of secondary cancer is a complication that is often associated with cancer therapy in HD survivors.[4] The risk of developing a secondary cancer may be related to several factors such as lifestyle, environmental exposure and their interaction with the genetic background of the patient. In particular, the risk related to the specific treatment for the first cancer is one of the most important factors.[5]

Therapy-related myeloid neoplasms (t-MN) are recognized by the World Health Organization (WHO) as a distinct entity that includes myelodysplastic syndrome (t-MDS) and acute myeloid leukemia (t-AML).[6] From the mid-1960s to early 2000s, the most widely used chemotherapy regimen for the treatment of HD was the MOPP scheme (nitrogen mustard, Oncovin, procarbazine and prednisone), which included alkylating substances such as nitrogen mustard and procarbazine that are recognized as potent mutagenic and clastogenic agents.[7] In the early 70s, another combination of chemotherapy drugs, ABVD (adriamycin, bleomycin, vinblastine and dacarbazine) was developed with increased potency and less toxicity in comparison to MOPP. While it contains fewer alkylating agents, this combination includes other mutagens and an inhibitor of topoisomerase II, adriamycin.[4,8-10]

After treatment regimens of chemotherapy and radiotherapy, it has been observed that 20% of HD survivors develop three types of secondary tumors: different solid tumors (58%), acute leukemia (25%) and non-Hodgkin lymphoma (17%) (Figure 1). In this work, we will refer only to acute leukemia since represent alone the most frequent group.[11]

The alkylating agents included in the MOPP scheme were the first drugs found to be related to the development of secondary malignancies, especially t-MDS and t-AML, with a risk of occurrence of 2% that increases to 12.4% when radiotherapy is also used.[4,8,9] Patients may develop t-MDS 2 to 5 years after treatment with these agents, and t-MDS can often progress into t-AML. ABVD, which includes topoisomerase II inhibitors, has also been linked with the development of t-AML without a prior t-MDS state.[12,13]

The cytogenetic and morphological traits of t-MDS/t-AML are related to the type of therapy received for the primary tumor, and both occur within 5 to 7 years after chemotherapy and radiotherapy and confer a poor prognosis.[14] Both entities are well-recognized clinical syndromes[12] that may arise as a result of the clonal selection of cells with accumulated genomic lesions due to defects in DNA repair mechanisms, defects in detoxification systems designed to limit oxidative damage to DNA[15] and changes in factors for chromatin assembly, resulting in the accumulation of double-strand breaks in DNA.

In addition, increased frequencies of the following gene polymorphisms in t-AML patients have been observed compared with healthy individuals: human

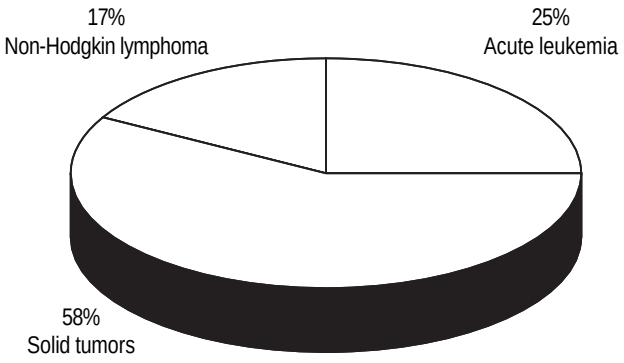

Figure 1. *Percentages of treatment-related secondary cancers in HD patients. Approximately 20% of HD patients developed secondary cancer, including different solid tumors (58%), acute leukemia (25%) and non-Hodgkin lymphoma (17%).*

homeobox *HLX1* gene (HLX1-C/T 3 'UTR), which is essential for hematopoietic development; the promoter region of the DNA repair gene *RAD51* (-135 G/C); and the DNA repair gene *XRCC3*(18067 C/T).[16,17] These findings suggest a direct relationship between these polymorphisms and the risk of developing t-AML,[16,18] as all of these alterations are assumed to increase susceptibility for developing secondary cancer.[15-19]

The risk of developing t-MDS and t-AML is up to 100-fold higher in HD patients after treatment than in the general population, indicating that at least 99% of cases of secondary cancer should be considered as induced by treatment and changes dependent on the type and dose of drug administered, in addition to patient age at the time of the therapy.[20] Previous studies have found that the risk of t-MDS and/or t-AML increases with respect to the square of the patient's age at the time of receiving treatment for primary cancer and directly with the cumulative dose of alkylating agents used.[21,22]

There are few studies on genetic risk factors for developing secondary cancers in HD, but it has been observed that 75% of patients with HD who are treated with alkylating agents such as cyclophosphamide or procarbazine and have developed t-AML have a G>A polymorphism in position -93 of the promoter of the *MLH1* repair gene. Thus, it follows that this polymorphism confers a high risk of developing t-AML.[4,23] Because of this, it is important to associate the genetic characteristics of individuals with secondary leukemia and specific treatment to identify individuals at high risk of t-AML and monitoring to detect the emergence of a second cancer.

SECONDARY LEUKEMIAS ACCORDING TO THE TYPE OF CHEMOTHERAPY

Secondary leukemias are classified in two types according to the chemotherapy used to treat the primary cancer:

- Classical t-MDS/t-AML, which is secondary to exposure to alkylating agents and
- t-AML, which is secondary to the use of topoisomerase II inhibitors.

Classical t-MDS/t-AML

Treatment with alkylating agents has been directly linked with t-MDS/t-AML. It is known that alkylating agents can induce various types of alterations in the cells such as the following:

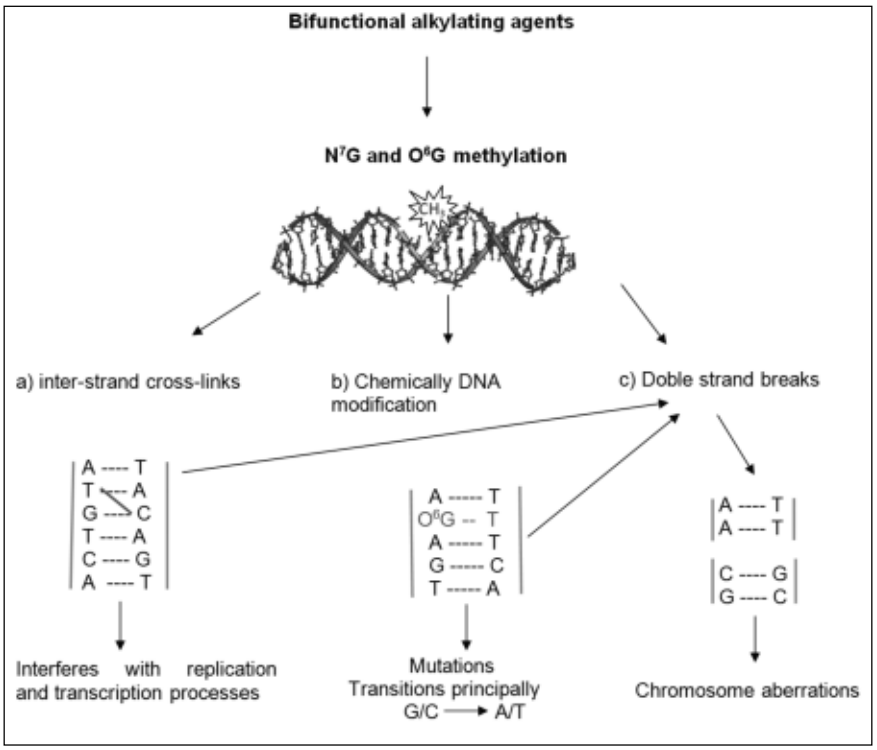

Figure 2. Alterations induced by alkylating agents. The chemotherapeutic agent results in methylation of DNA bases, thus activated repair mechanisms can produce different alterations such as: a) interstrand cross-linking of DNA that prevents replication and transcription; b) chemical modification of bases, such as the generation of methylguanine N^7 or 0^6, which tends to pair with thymine instead of cytosine and generates transition-type mutations; and c) double-strand breaks, which are extremely dangerous lesions because they can induce chromosome alterations such as deletions and translocations that are directly involved with progression toward cancer.

- DNA inter-strand cross-links, which interfere with replication and transcription.
- Chemically modified DNA bases, which generate point mutations; and
- Double-stranded breaks, which lead directly to chromosome aberrations[3,20,24] (Figure 2).

In most patients with the classical form of t-AML, total or partial deletion of chromosomes 5 and 7 are identified together with hypodiploid karyotypes and complex structural chromosomal abnormalities such as unbalanced chromosomal rearrangements. In general, these alterations are not restricted to specific genes or chromosomal bands, therefore the predominance of cases with alterations in chromosomes 5 and 7, probably reflects a selection of cells, due to a proliferative advantage.[12-14,20,25,26] In t-MDS, balanced aberrations are rare, while unbalanced aberrations, such as the deletion of the long arm of chromosome 5 [del(5q)] or loss of chromosome 5 (monosomy 5), and the deletion of the long arm of chromosome 7 [del(7q)] or loss of chromosome 7 (monosomy 7) (Figure 3A), were observed in 50 to 70% of patients, and the presence of normal karyotypes were observed in 5 to 10% of cases. In t-AML, balanced aberrations (translocations or inversions) (Figure 3B) occur in 15 to 20% of the cases, unbalanced changes are common (70%) and normal karyotypes were found in 10 to 15% cases.[27]

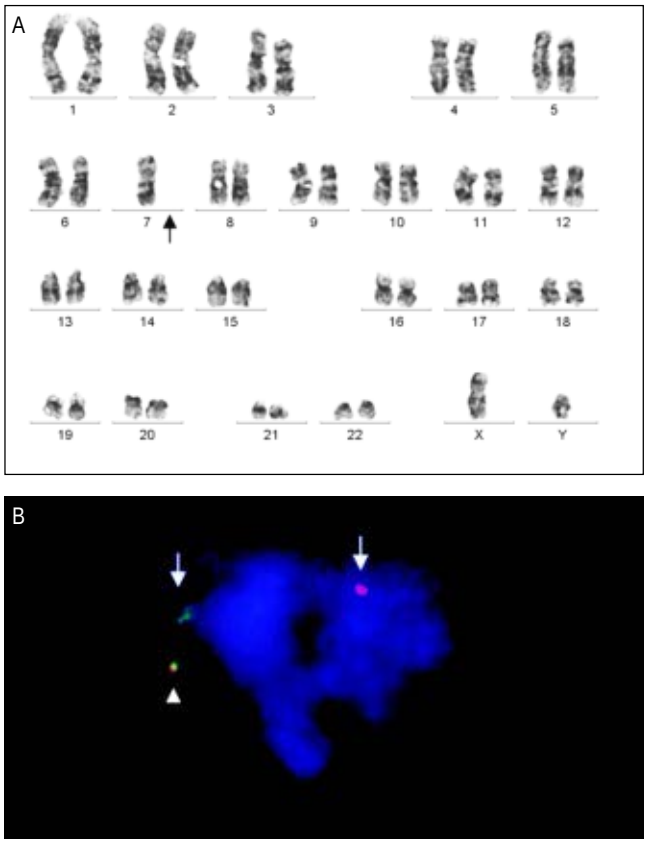

Figure 3. *Chromosome alterations in t-MDS/t-AML.* ***A.*** *Karyotype with monosomy 7.* ***B.*** *FISH in Interphase nucleus using* MLL *unique.sequence probe (orange/green), showing normal* MLL *with two contiguous signals (arrow head) and* MLL *with separated orange and green signals (arrows) indicating the breakage of the gene.*

In order of frequency, the most common chromosomal abnormality is monosomy 7 followed by del(5q) and monosomy 5.[28,29] The same alterations were observed in *de novo* MDS and AML, especially in adult patients and in those occupationally exposed to carcinogens such as benzene.[12]

t-AML secondary to topoisomerase II inhibitor exposure

Mitoxantrone, etoposide and adriamycin, among others, are related to the induction of t-AML and are characterized by blocking the rejoining of double-stranded breaks in DNA and induce illegitimate mitotic recombination between the broken ends of two different chromosomes, resulting in translocation[3] (Figure 4). t-*AML* can occur as early as 2 years after initial treatment for primary cancer and is associated with balanced chromosomal aberrations as the only abnormality, mainly 11q23 translocations resulting in *MLL* gene rearrangements that generate fusion proteins and prevent methylation of the histone H3 lys4, which is related to the transformation of hematopoietic cells into leukemic cells.[13,19,25-27,30,31]

Less commonly, there may be alterations in 21q22, 16q22, 11p15.5, 17q21 and less frequently in 3q26. Unlike classic t-AML, these secondary leukemias have a short latency period from the beginning of the use of chemotherapy for primary cancer to their development and are rarely preceded by t-MDS.[12,14,20]

IMPACT OF CYTOGENETIC ABNORMALITIES ON THE PROGNOSIS OF t-AML

In follow-up studies of patients with HD that covered the start of treatment with alkylating agents and/or topoisomerase II inhibitors through the development of t-MDS and t-AML,[11] it has been observed that the presence of particular chromosomal aberrations are important factors for leukemic transformation and are associated directly with clinical features and response to treatment (Table 1).

Table 1. Cytogenetic characteristics in EH patients with t-MDS/t-AML.

Reference	Number of EH patients	Treatment (Cycles)	Number of patients with t-AML	Time to develop t-AML (months)	Type of secondary leukemia	Cytogenetic alterations
8	761 (CR)	9 RT+MOPP (3-6) 4 ABVD (3)	13	12-123	11 ANLL 2 ALL	 t(1;11) (p32;q23) t(4;11) (q12;q23).
25	6	1 RT 1 RT +MOPVCtx 1 RT+ VPCCNU, 1 RT+OVAEtopPClb, 1 RT+MOVPClb, 1 RT+MOVP	6	41- 254	2 ANLL 1 RA 3 RAEB	46, XY 46,XY,t(3;21)(q26;q22) 46, XY, del(5)(q13q33), del(20)(q11) 43-44,XY,-3,-5,-7,der(12)t(12;13)(p13;q13), -13,+3-4mar 44,XY,del(5)(q13q33),-7,-9,-10,+mar 46, XY
15	6	2 MOPP/ABVD (8/8) 1 RT+MOPP/VBM (3/6) 3 ABVD (6)	3	25-76	t-AML M1 M0-M1 M3	Normal karyotype Normal karyotype t(15;17)(q22;q12) del(7)(q22q32) del(9)(q13q31) Genomic imbalances: 44 amplifications (MYCN, HRAS, CDK6) y 28 deletions (EGR2, MLL, CDKN2A, FGR, TOP1)
13	4	1 RT +MOPP(6) 1 MOPP + CtxAVP 2 MOPP(6)	4	24 -540	t- AML M4 t- AML M2 t- AML t- AML M2	46,XY 43,XX,-4,-5,add(13)(p13),-17 18,+mar[3]/42,idem,-11[12]/42,idem, add(13),+add(13)(p13)[2] 46,XX,add(3)(q24),del(12)(q21q24))[26]/ 46,XX[5] 43,XY,-5,-7,t(12;20)(p13;q11),- 18,add(21)(q22)[25]/45,idem,del(1)(q41),-6, -11,+18,add(11)(p14),+3mar[3]

C: Complete remission. RT: Radiotherapy. MOPVCtx: (Nitrogen mustard, vincristine, procarbazine, vinblastine, cyclophosphamide). VPCCNU: (Vinblastine, procarbazine, iomustine). OVAE topPClb: (Vincristine, vinblastine, doxorubicin, etoposide, chlorambucil). MOVPClb: (Nitrogen mustard, vincristine, vinblastine, procarbazine, chlorambucil). MOVP: (Nitrogen mustard, vincristine, vinblastine, procarbazine). VBM: (Vinblastine, bleomiycin, nitrogen mustard). CtxAVP: (Cyclophosphamide, doxorubicin, vinblastine, procarbazine). ANLL: Acute non-lymphoblastic leucemia. ALL: Acute lymphoblastic leucemia. RA: Refractory anemia. RAEB: Refractory anemia with excess of blasts. AML M1: Acute myeloid leukemia subtype M0,MI,M2, M3 o M4.

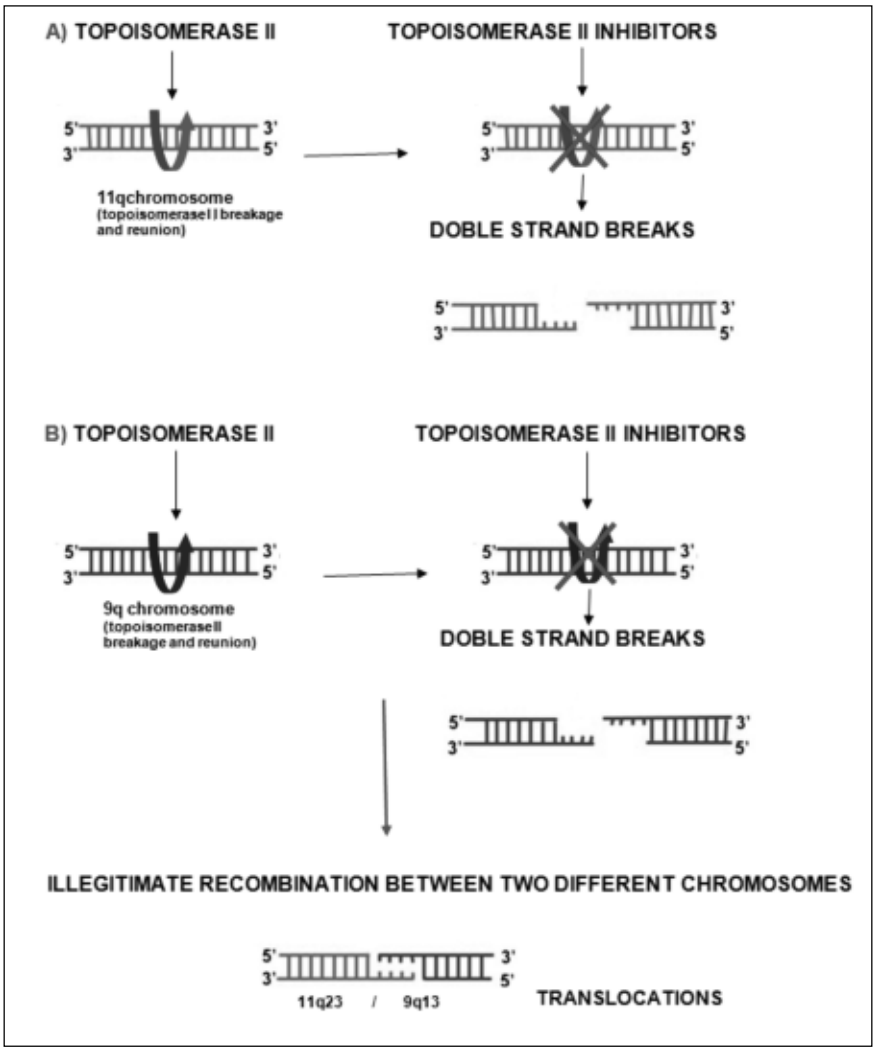

Figure 4. Chromosomal alterations induced by topoisomerase II inhibitors. Inhibitors of DNA topoisomerase II permit the enzyme to cut the covalent backbone of DNA, inhibit its rejoining and may facilitate illegitimate mitotic recombination between broken DNA strands of two different chromosomes that may result in the formation of chromosomal translocations.

In a study at the University of Chicago, 306 patients with t-AML were analyzed to determine the impact of cytogenetic findings on prognosis of the disease. Twenty-four patients (8%) with normal karyotypes survived for 3 years after diagnosis, and patients with abnormalities of chromosomes 5 and 7 had the shortest survival time (7-9 months), compared with 11 months for those with balanced translocations.[12,32] In another study by the German Cooperative Group (AMLCG) in 93 patients with t-AML and 1091 patients with *de novo* AML, it was found that overall survival (defined from the time of diagnosis of secondary leukemia to death) was 18 months for those with favorable karyotypes (normal karyotype and balanced chromosomal rearrangements) and 6 months for those with unfavorable karyotypes (alterations of chromosomes 5 and 7). In both t-AML and *de novo* AML, unfavorable karyotypes were associated with short survival (6 months in both entities).

In contrast, patients with balanced alterations such as t(15;17), t(8;21) or inversion of chromosome 16 [inv(16)] have a better prognosis, similar to that presented by patients with *de novo* AML and the same chromosome rearrangement.[12,33] In fact, in two series of patients with exposure to topoisomerase II inhibitors, it was observed that 33 of 39 patients (85%) with t-AML and inv(16) and 24 of 35 (69%) patients with t(15;17), achieved complete remission, and patients with t(8;21) had better prognosis compared with those who had other rearrangements involving the 21q22 region, such as t(3; 21) and t(16;21).[12,30,34]

In a study of 511 patients with t-MDS/t-AML, 32% had rearrangements in 11q23, and their survival time was 8 months, which was significantly lo-

wer than the survival times of patients with 21q22 abnormalities, inv(16) and t(15;17) of 14, 28 and 29 months, respectively.[12,33,35]

In conclusion, patients with chromosomal abnormalities such as unbalanced alterations on chromosomes 5 and 7 have a lower survival time than patients with balanced rearrangements; among these, patients with 21q22 abnormalities, inv(16), and t(15;17) have a better prognosis compared with those with rearrangements involving 11q23.

MOLECULAR CHARACTERISTICS OF t-MDS AND t-AML

The clinical and cytogenetic features observed in the two forms of t-AML may reflect the type of damage induced by the different therapies used to treat the primary cancer. Chromosomal deletions can cause that one normal allele of tumor suppressor genes may be inactivated; the evidence suggests haploinsufficiency of the suppressor gene *EGR1* or promoter methylation of the gene encoding α-catenin (*CTNNA1*), both located on 5q31, as the only alteration.[12,27]

However, loss of both alleles of a tumor suppressor gene may not be sufficient to confer a malignant phenotype; as described in a model of colorectal tumorigenesis, multiple genetic alterations may be required to transform a cell, and this series of changes require a long period of time, explaining the long latency between the initial antineoplastic therapy to alkylating agent-induced t-AML.[12] In contrast, balanced chromosome translocations that follow treatment with topoisomerase II inhibitors cause the activation of cellular oncogenes in a dominant fashion, and although the fusion gene alone may not be sufficient for the complete transformation of a hematopoietic progenitor cell, relatively few genetic events are required to progress to the leukemic phenotype.[12] At the molecular level, three classes of mutations have been proposed to be involved in t-MDS and t-AML (Table 2):

- Class I mutations alter RAS-BRAF signal transduction pathways, stimulating cell proliferation.
- Class II mutations occur in transcription factors genes and *NPM1*, causing altered cellular differentiation. Here are also included mutations that inactivate the p53 tumor suppressor gene (sometimes are classified in a third category Class III mutations).[14,26,36]

Table 2. Classification of mutations related with the involved genes.

Class I	Class II
Tyrosine-kinase	Transcription factors
KRAS	AML1
NRAS	CEBPA
BRAF	NPM1
	p53
RAS/BRAF pathway	
PTPNII	
FLT3	
cKIT	
cFMS	
JAK2	

One of the most important works at the molecular level was the Copenhagen series, which was conducted in 140 patients, including 89 with t-MDS and 51 with t-AML,[23,24,26,27] on cryopreserved bone marrow cells upon the diagnosis of secondary cancer. They searched for Class I mutations in tyrosine kinase receptor genes such as *FLT3*, *cKIT, CFMs* and intracellular *JAK2* and in signal transduction genes involved in the RAS-BRAF-MEK-ERK pathway such as *KRAS*, *NRAS*, *BRAF* and *PTPN11*; Class II mutations in the transcription factors *AML1*, *CBFB*, *MLL*, *EVI1* and *CEBPA*, the transcription regulator *NPM1* and the retinoic acid receptor *RARA*; and mutations in the tumor suppressor gene *p53*. The results of this study showed that some mutations occur with high frequency. In particular, 34 and 22 patients had mutations in *p53*, and *AML1*, respectively. Seventeen patients had mutations in *FLT3*, *cKIT* and *JAK2*, and 20 patients had mutations in genes downstream of the RAS/BRAF signal transduction pathway. *RAS* mutations were associated with the progression from t-MDS to t-AML. Finally, 61 mutations in transcription and hematopoietic differentiation genes were observed in 59 patients with t-MDS or t-AML. The chimeric rearrangements of *AML1*, *CBFB*, *MLL*, *RARA* and *EVI1* were related to previous therapy with topoisomerase II inhibitors.[26,27]

Another finding observed in therapy-related leukemia are epigenetic changes, such as promoter methylation of *CTNNA1* and promoter methylation of $p15^{INK4B}$ on 9p21, which is involved in the G1 checkpoint,[23,24,26,27,29,37] were observed in 58-68% of t-MDS/t-AML patients.[38] Gene inactivation by homozygous deletion or hypermethylation of CpG sites in the promoter region of $p16^{INK4A}$ and $p15^{INK4B}$, both located in a small region (2.5 kb) on chromoso-

Table **3**. Genetic pathways identified in t-MDS and t-AML patients.

	GENETIC PATHWAYS							
	I	II	III	IV	V	VI	VII	VIII
Presentation	t-MDS	t-MDS/ t-AML	t-AML	t-AML	APL	t-MDS/ t-AML	t-MDS/ t-AML	
Type of previous therapy	Alkylating agents	Alkylating agents	Topo II inhibitors	Topo II inhibitors		Topo II inhibitors	Without specific therapy	Without specific therapy
Chromosome alterations	7q deletions and/or monosomy 7	5q deletion and/or monosomy 5 Complex karyotypes	11q23 balanced alterations	21q22 inv(16) balanced alterations	17q21 abnormalities	11p15 abnormalities	Normal karyotype	Some cases with trisomy 8
Genes involved			MLL	AML1 CBFB	RARA	NUP98		
Mutations	AML1 point mutations with t-AML development	p53	NRAS, KRAS or BRAF	C-kit and PTPN11			FLT3, RAS or AML1	
Other alterations				Deletion 7q and/or monosomy 7				
Epigenetic modifications	$p15^{INK4B}$ promotor methylation	CTNNA1 promotor methylation		$p15^{INK4B}$ promotor methylation			$p15^{INK4B}$ methylation with FLT3 tandem duplication	

APL: Acute promyelocytik leukemia. Topo II. Topisomerase II.

me 9p21, are common in acute lymphoblastic leukemia. In t-MDS or t-AML patients, studies have been conducted to examine the methylation status of the *p14*, *p15* and *p16* gene promoters and relate it with previous treatment, clinical and cytogenetic characteristics and prognosis of the disease.[39,40] Au, *et al.*,[32,40] in 17 patients with secondary leukemia, found that 58% had methylation of $p15^{INK4B}$ associated with del(7q), and in 3 of 6 patients with bone marrow samples obtained before the diagnosis of t-MDS/t-AML, methylation of *p15* was observed 2 years before leukemia development, demonstrating that in some patients, this disturbance is an early event in the development of leukemia.

In another study, in 81 patients with t-MDS or t-AML, Christiansen, *et al.*,[39] found that 68 and 6% had methylation of *p15* and *p16*, respectively, unrelated to age, previous therapy (alkylating agent, topoisomerase II inhibitors or radiotherapy) or the period from the start of therapy for primary cancer to the development of secondary leukemia; however, survival time from the diagnosis of secondary leukemia was significantly shorter in patients with *p15* methylation.[41]

In general, it has been observed that the development of t-AML after treatment of the primary tumor was significantly shorter in methylated than in unmethylated patients.[38] There was a strong association between *p15* methylation and chromosomal abnormalities, including monosomy 7, del(7q) and tandem duplication in 13q12 *(FLT3)* and 11q23 *(MLL)*, but there was no association with *p53* mutations.[37,42]

Finally, it is clear that there are combinations of genetic alterations at different levels (molecular, epigenetic and chromosomal) that lead to the appearance of genetic pathways directly related to the development of t-MDS and/or t-AML.

GENETIC PATHWAYS IN t-MDS AND t-AML

In 1995, Pedersen-Bjergaard, *et al.*, analyzed 140 patients with t-MDS or t-AML[13] and proposed eight alternative genetic pathways with specific genetic characteristics directly related to the pathogenesis of t-MDS and t-AML (Table 3), with important implications for the classification of patients:

- *Pathway I.* Comprises patients with 7q deletions and/or monosomy 7 and normal chromosome 5 without balanced aberrations. They presented with t-MDS following therapy with alkylating agents. These patients frequently have *AML1* point mutations that are associated with the progression of t-MDS to t-AML. Of 140 patients with t-MDS or t-AML, 39 were on pathway I, and 15 (38%) had mutations of *AML1*. A few patients had *p53* and *RAS* mutations, and none had mutations of *FLT3*.
- *Pathway II.* Patients with 5q deletions and/or monosomy 5 without balanced aberrations. They presented with t-MDS or t-AML. Most of these cases have *p53* mutations and complex unbalanced chromosome rearrangements. These alterations are generated by the use of alkylating agents and occasionally also involve 7q deletions and/or monosomy 7, deletion of 17p (loss of heterozygosity of *p53*), derivative chromosomes and complex karyotypes composed of material of at least three different chromosomes and amplifications or duplications in 11q23 and 21q22.
- *Pathway III.* t-AML patients. These patients do not have previous t-MDS do present unbalanced translocations involving band 11q23 (MLL) associated with topoisomerase II inhibitors (etoposide and cisplatin). In such patients, mutations of *NRAS*, *KRAS* or *BRAF* are common.
- *Pathway IV.* Patients with t-AML and balanced aberrations, frequently balanced translocations in chromosome band 21q22 (*AML1*) or inv(16) (16q22) with *CBFB* rearrangements. They are associated with the use of anthracyclines and presented t-AML, except for patients with t(3;21) with previous t-MDS. The second most common alteration in this group of patients is deletion of 7q/monosomy 7, and in some patients, mutations of *cKIT* and *PTPN11*.
- *Pathway V.* Comprises patients with acute promyelocytic leukemia and rearrangements of *RARA* gene at 17q21. Only one patient had a tandem duplication of *FLT3*.
- *Pathway VI.* Patients with t-MDS or t-AML and rearrangements of the *NUP98* gene on 11p15. They are found in cases treated with topoisomerase II inhibitors.
- *Pathway VII.* Patients with t-MDS and t-AML, normal karyotypes and no association with a specific type of therapy. In a study of 24 patients who had normal karyotypes, 50% had a mutation in at least one of the following genes: *FLT3*, *RAS* or *AML1*.
- *Pathway VIII.* Some cases only have one chromosome aberration (trisomy 8) that is not related to any specific type of therapy.[12,14,20,23,27]

CONCLUSIONS

In general, in patients with secondary leukemia, three cytogenetic subtypes are observed. The first subtype is represented by patients with unbalanced aberrations, mainly del(5q) and/or monosomy 5 or del(7q) and/or monosomy 7, which are associated with exposure to alkylating agents. Patients with recurrent balanced aberrations, such as translocations or inversions, are the second subtype, and in cases related to therapy, these aberrations are the result of the activity of topoisomerase II inhibitors that generate double-strand breaks and aberrant rejoining of chromosome segments. Finally, the third subtype includes patients with normal karyotypes. There are also two types of point mutations: Class I, mutations in the RAS-BRAF pathway leading to increased cell proliferation; Class II, inactivating mutations of genes encoding transcription factors leading to disturbed cell differentiation and mutations of *p53* that can occur in patients with any type of karyotype, unbalanced, balanced or normal.

When patients are studied from the point of view of the existence of cytogenetic alterations associated with molecular alterations, at least eight different genetic pathways can be defined that are characterized by specific alterations. Some abnormalities are associated with t-MDS and others with t-AML directly. t-MDS has great potential for transformation to t-AML, which is associated with point mutations of *AML1* and *RAS*.[27] Knowledge of the genetic alterations in these conditions is important because it can directly help with proper classification, treatment, and prognosis of patients and can increase the knowledge of the biology of the disease, which leads to identifying potential therapeutic targets.

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Correspondence and reprint request:

Sara Frías
Laboratorio de Citogenética
Instituto Nacional de Pediatría
Insurgentes Sur 3700-C, 6o. Piso
Col. Insurgentes Cuicuilco
04530, México, D.F.
Tel. and Fax: +52 55 1084-5533
E-mail: sarafrias@yahoo.com

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