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Correlation of Chromosome Patterns in Human Leukemic Cells with Exposure to Chemicals and/or Radiation

FINAL REPORT
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DISCLAIMER

NOTICE

I. COMPREHENSIVE PROGRESS REPORT for the DEPARTMENT OF ENERGY: 1980-1997

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A. Major Research Accomplishments

1. Introduction

This project began in 1962 when I became a member of the staff of the Argonne Cancer Research Hospital at the University of Chicago, which was fully funded by the Atomic Energy Commission. I was studying chromosome abnormalities with the use of autoradiography. In about 1965, I began to study the chromosome pattern in marrow cells from patients with leukemia and preleukemia. After a sabbatical 1970-71, I used chromosome banding techniques and discovered a number of recurring chromosome translocations in the cells of these patients. I showed that there was a non-random pattern of chromosome gains and losses in leukemia cells and that specific translocations were relatively consistently associated with particular morphologic subtypes of acute myeloid leukemia (AML) de novo. Moreover, I showed that these aberrations had some interesting variations in frequency that were related to the age of the patient. Thus recurring translocations tended to be more frequent in children whereas loss of chromosomes 5 and 7 tended to occur in older patients.

In 1977, my colleagues and I reported on the karyotype of leukemia cells in the first large (10 patients) series of Hodgkin's disease patients with a history of prior mutagenic therapy. I noted that 9 of these patients had loss of all or part of the long arm of chromosome 5 and that 5 patients also had loss of chromosome 7. This observation has been confirmed by many other investigators as well as by our continuing analysis of more patients.

I then embarked on an ongoing investigation as to the nature of the chromosome aberrations in t-AML/t-MDS because I felt that a careful analysis of these patients' cells would provide unique insights into the location of genes that were important in leukemogenesis and that this information might help us to determine which patients who appeared to have AML de novo, might, in fact, have mutagen related leukemia. This hope is still just that; however we are getting progressively closer to the gene or genes involved as we use ever more sophisticated molecular and cytogenetic techniques. Beginning in 1984, I embarked on a project to clone the genes at translocation breakpoints; in fact, we have cloned some of the genes involved in balanced translocations in t-AML and in AML de novo. My colleague, Dr. Michelle Le Beau has narowed the location of some of the genes involved in deletions of the long arm of chromosomes 5 and 7 in t-AML as well as in AML de novo and she hopes to clone some of them in the next few years.

Since 1974, we have examined the karyotype of 260 patients with AML who have a history of prior treatment with radiation and chemotherapy usually for a malignant disease. We have sufficient clinical data on all patients so that we can correlate the type of prior treatment with the chromosome abnormalities in the patient's leukemic cells (Tables 1, 2, 3). As can be seen in Table 1, 38 (14%) of these patients had received only radiation therapy. In the past, we have concentrated on the deletions involving chromosomes 5 and/or 7 because these were the most common changes.

In the course of our analysis of these 260 t-AML patients, it became apparent that there was another group of patients who lacked aberrations of chromosomes 5 and/or 7 and whose leukemic cells had certain specific changes that were quite unusual for t-AML (Table 2). These patients have balanced translocations usually involving chromosome bands 11q23 (9 patients) that are associated with specific subsets of AML de novo and are common in younger patients. Not only is there an increased frequency of aberrations of chromosome 11, there is also an increase in 21q22 translocations involving either chromosome 8[t(8;21)(q22;q22) or chromosome 3[t(3;21)(q26;q22)] (8 patients). We were the first to describe the 3;21 translocation that with rare exceptions is only seen in t-AML or CML in blast crisis. Finally other recurring translocations, such as the t(15;17)(q21;q12) (4 patients) in acute promyelocytic leukemia (APL) or inv(16)(p13q22) (4 patients) in acute myelomonocytic leukemia with abnormal eosinophils (M4Eo) are also occasionally seen in the t-AML. Ironically, we used to comment emphatically that these aberrations were very rarely seen in t-AML. This new form of t-AML also differs from the usual pattern, namely losses of chromosome 5 and/or 7, in that it has a much shorter interval between treatment and leukemia, rarely has a preleukemic phase, and appears to have a higher response rate to treatment. Of special interest is the fact that these patients have usually received high doses of topoisomerase II inhibitors, especially the epipodophyllotoxins, etoposide (VP16) or teniposide (VM26). These latter drugs were first used with any frequency in the 1980s. Just as we were the first to show that losses of chromosomes 5 and/or 7 were a common feature of leukemia resulting from prior treatment with alkylating agents, we were also the first to suggest that high doses of etoposide (VP16), one of the epidophyllotoxins, was associated with translocations involving chromosome 11 band q23. Our group at the University called attention to this association in 1987, when we reported on the greatly increased risk of t-AML in a series of lung cancer patients treated with very high doses of etoposide (Ratain, et al., *Blood*, 70:1412-1417, 1987). Our re-evaluation of the basis for balanced translocations in t-AML began in 1986 after we analyzed our cytogenetic data on cancer patients treated with epipodophyllotoxins. Thus in our series of 119 patients with non-small cell lung cancer who received aggressive chemotherapy with cisplatin and other drugs, 24 (including 21 who received etoposide, VP16) survived more than one year.(14) Nineteen of these patients died within another 14 months with no evidence of AML, one patient is still alive, and the remaining four patients developed AML. It was noteworthy that two of these four patients had a translocation involving 11q23 and M4 or M5 leukemia, the third had a balanced translocation involving chromosomes X and 10, and the fourth patient had a translocation involving chromosomes 5 and 7 leading to loss of both 5q and 7q with

definite t-AML characterized by typical trilineage dysplasia in bone marrow cells. Patients who developed leukemia had received significantly more etoposide than the nonleukemic patients (6,795 mg/m1 compared with 3,025 mg/m2, respectively). Another group of 35 patients received cyclophosphamide, doxorubicin, methotrexate and procarbazine; none of five patients who survived more than one year developed AML. We proposed that this unusual karyotypic pattern was related to the high dose of etoposide received by these patients. This association has now been confirmed by many other laboratory groups. More recently, we and others have found a very close correlation between the presence of balanced translocations involving 11q23 or 21q22 and exposure to topoisomerase II inhibitors.

We correlated the type of treatment with the presence of balanced and unbalanced translocations involving chromosome bands 11q23 amd 21q22.(22) We had nine patients with balanced translocations of 11q23 and eight patients with balanced translocations of 21q22 (Table 4). All patients in each group had received a topo II inhibitor. This compares with 2 and 8 patients with unbalanced translocations involving these bands.

Dr. Pedersen-Bjergaard had previously reported on a similar analysis in his t-AML patients. The results are virtually identical. Of 91 patients, six had balanced 11q23 translocations and all of them had received topo II inhibitors. Similarly of five patients with 21q22 balanced translocations, all five had received topo II inhibitors. Of 80 other patients with unbalanced translocations involving these bands, only three had received topo II inhibitors, and all three had also received alkylating agents.

Beginning in 1989, my laboratory focused on the identification of the gene in 11q23 that was the target for these translocations in t-AML. We were successful in cloning the gene in 1991; we called it *MLL* for myeloid lymphoid leukemia. We have been heavily involved in studying its function. There are at least forty different translocations that involved *MLL* based in FISH and/or Southern blot analysis. Cloning these breakpoints, which has been completed for 16 translocations, has been very important. Most of the partner genes are new and thus we identify new targets for leukemogenesis. But some of the genes have been identified previously. In all of the translocations the challenge is to determine how the translocation alters the function of both *MLL* and the partner gene so that they lead to leukemia. We continued our functional analysis of the various domains of *MLL*, as well as of the partner genes to try to identify some of the changes in function of *MLL* as a consequence of the translocation. The involvement of *MLL* in acute leukemia following treatment with drugs which inhibit the function of topoisomerse II is being recognized in about 5-10% of *MLL* translocations.

More recently we have investigated the association of *MLL* rearrangements at 11q23 with the type of prior chemotherapy in t-AML patients. All patients had cytogenetically recognized 11q23 rearrangements. As can be seen in Table 5, every patient who had an

MLL rearrangement had received a topo II inhibitor, almost always an epipodophyllotoxin.

We have cloned the t(11;16)(q23;p13.3) and have shown that it involves *MLL* and *CBP* (CREB binding protein). We have studied eight t(11;16) patients with fluorescence in situ hybridization (FISH) using a probe for *MLL* and a cosmid contig covering the *CBP* gene. All patients had received treatment with topoisomerase II inhibitors for a prior malignant disease. Both probes were split in all eight patients and the two derivative (der) chromosomes were each labeled with both probes. Use of an approximately 100kb PAC cloned from the breakpoint of chromosome 16 from one patient revealed some variability in the breakpoint because it was on the der(16) in two patients, on the der(11) in two and split in four others. We assume that the critical fusion gene is 5'*MLL*/3'*CBP*. Our series of patients is unusual because two of them presented with a myelodysplastic syndrome (MDS) most similar to chronic myelomonocytic leukemia (CMMoL); MDS is rarely seen in 11q23 translocations either de novo or with t-AML. Using FISH and these same probes to analyze the lineage of bone marrow cells from one patient with CMMoL, we showed that the majority of mature monocytes contained the fusion genes as did some of the granulocytes and erythroblasts; none of the lymphocytes contained the fusion gene. The function of *MLL* is not well understood but many domains could target the MLL protein to particular chromatin configurations. *CBP* is an adapter protein that is involved in regulating transcription. It is also involved in histone acetylation which is thought to contribute to an increased level of gene expression. The fusion gene could alter the CBP protein such that it is constitutively active; alternatively it could modify the chromatin-association functions of MLL.

In conclusion, it has been clear for the last 15 years that cloning translocation breakpoints in both AML de novo and t-AML would provide the DNA probes required to determine whether the breakpoints in cytogenetically apparently similar translocations were identical at the level of DNA. Therefore we have pursued an analysis of rearrangements in both types of leukemia simultaneously. We have also cloned and sequenced several translocations in acute lymphoblastic leukemia and in chronic lymphatic leukemia. Recently we cloned the breakpoint in a number of translocations involving chromosome bands 11q23 and 21q22. We have cloned the gene which we called *MLL*, that is located in 11q23 that is involved in the 6;11, 9;11, and 11;19 translocations that are seen in AML de novo as well as in t-AML.(15-17) We have evidence that the breakpoint in 11q23 and in the t(9;11) is relatively similar in de novo and secondary AML. In addition, we have cloned the gene at the breakpoint in chromosome 21 in the t(3;21). These studies have provided DNA probes that will be very important for diagnosis and for monitoring the patient's response to treatment.

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Table 1 **Primary Diagnosis and Primary Cytotoxic Therapy in 270 Patients with Therapy-Related Myeloid Leukemia**

No. of Patients (Row percentage)

Primary Diagnosis	N (%)	CT (%)	RT (%)	CMT (%)
Non-Malignant	14 (5)	11 (78)	1 (7)	2 (14)
Heme Malignancy	155 (57)			
HD	72 (27)	18 (25)	4 (6)	50 (69)
NHL	61 (23)	29 (48)	1 (2)	31 (51)
Myeloma	21 (8)	17 (77)	0	5 (23)
Other	1 (1)			
Solid Tumors	101 (37)	37 (37)	32 (32)	30 (30)
Breast	26 (10)			
Ovary	14 (5)			
Prostate	12 (4)			
Lung	7 (3)			
Cervix	7 (3)			
Other	35 (13)			
Totals	270 (100)	112 (42)	38 (14)	118 (44)

Table 2 **Cytogenetic Abnormalities in t-MDS/t-AML**

Number of Patients			270
Normal Number with clonal abnormalities			18 (6.6%) 252 (93.4%)
Number with abnormalities of chromosomes 5 and/or 7		191 (70.5%)	
Chromosome 5 only Chromosome 7 only Chromosomes 5 and 7	54 (19.9%) 76 (28.0%) 61 (22.5%)		
Other recurring abnormalities:		40* (14.8%)	
t(11;q23) t(3;21)/t(8;21)/t(21q22) t(15;17) +8 inv(16) -13/del(13q) del(20q) del(11q) +11 +21 -Y	9 8* 4 7 4 3 1 1 1 1 1		
Other abnormalities		22 (8.1%)	

*One patient had both a del(5q) and t(3;21); thus, these columns total 253, rather than 252 with abnormal karyotypes.

Table 3 **Primary Diagnosis, Primary Therapy and Clonal Cytogenetic Abnormalities in 270 Patients with Therapy-Related Myeloid Leukemia**

	Number of Patients (Row percentages)					
Primary Diagnosis	**Abn 5**	**Abn 7**	**Abn 5 & 7**	**Other Abn**	**Normal**	**Total**
Non-malignant	0	11 (79)	1 (7)	1 (7)	1 (7)	14
Heme Malignancy						
HD	14* (19)	23 (32)	15 (21)	18* (25)	3 (4)	72
NHL	10 (16)	13 (21)	23 (38)	11 (18)	4 (7)	61
Myeloma	7 (33)	8 (38)	4 (19)	1 (5)	1 (5)	21
Solid Tumor	23 (23)	21 (21)	18 (18)	30 (30)	9 (9)	101
Breast	2 (8)	10 (38)	3 (12)	10 (38)	1 (4)	26
Ovary	5 (36)	0	3 (21)	6 (43)	0	14
Prostate	3 (25)	0	2 (17)	5 (42)	2 (17)	12
Totals	53* (20)	76 (28)	62 (23)	62* (23)	18 (7)	270
Primary Therapy						
CT Only	22* (20)	33 (29)	23 (21)	26* (23)	9 (8)	112 (42%)
RT Only	11 (29)	6	6	11 (29)	4	38 (14%)
CMT	20 (17)	37 (31)	32 (27)	24 (20)	5 (4)	118 (44%)

*BG had both del(5q) and t(3;21)

Table 4 **Relationship Between Prior Chemotherapy and Clonal* Chromosomal Translocations in 260 Patients with t-MDS/t-AML**

	ATTop Only	ATTop + AA	AA Only	Other Treatment	Total
Number Studied	25	110	106	19	260
Balanced translocation band 11q23	3	6	0	0	9
Balanced translocation band 21q22	2	5	1	0	8
Unbalanced translocation band 11q23	3	1	2	0	6
Unbalanced translocation band 21q22	3	1	6	1	11

Abbreviations: ATTop, chemotherapy agents that target DNA topisomerase II; AA, alkylating agents. Balanced translocations involving band 11q23 or 21q22 were significantly associated with prior ATTop exposure (p=0.003, Fisher's exact test, two sided).

Table 5 **Association of *MLL* Rearrangements and Prior Therapy with Topoisomerase II Reactive Drugs**

		Prior treatment with topo II inhibitor	
		+	-
MLL rearrangement	+	9*	0
	-	1#	2†

The numbers of patients in each category is listed. The association of *MLL* rearrangements with prior topo II exposure was statistically significant (p=0.01, Fisher's exact test).23

* - All patients had balanced translocations
# - Patient had an unbalanced translocation
† - Both patients had del(11)(q23)