131. Cytologic Studies of Tumors. LI

*Notes on Some Abnormal Chromosome Features in a Case of Mycosis Fungoides**

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A bulk of evidence has been gathered lately on cytogenetic features of various hematopoietic disorders such as leukemias, lymphomas, reticulosarcomas and others, providing data important for elucidation of the cytogenetic nature of malignant cells: differential response to therapeutic agents or cytogenetic evolution of malignant cells following therapeutic treatments, cytogenetic variability or invariability of malignant cells in different environments, and the mono- or polyclonal origin of malignant cell-lines (c.f. Spiers and Baikie 1968, Gartler *et al.* 1969, Obara *et al.* 1970b).

The present study deals with chromosome abnormalities and the occurrence of two cell lines with different proliferative ability in a case of mycosis fungoides. So far as we are aware, cytogenetic information has not been available for this disorder.

Materials and methods. Samples for chromosome studies were taken from the patient four times in peripheral blood, twice in bone marrow, and once each in skin tumor and lymph node during a period from November 28, 1969 to March 9, 1970.

One third of the samples from the above four tissues were directly prepared for chromosome slides following Tjio and Whang (1962), and the remaining samples were cultured according to Moorhead's method (1960) (see Obara *et al.* 1968). In lymph node, bone marrow and peripheral blood, two types of cultures were set up: one with the addition of phytohemagglutinin-M (PHA) at a final concentration of 0.02 ml/ml cell suspension and another without PHA. For skin cultures the plasma-clot technique was adopted.

Case history. Male, age 45. The patient had typhus at the age of 20 years, and received medical treatments for consumption for

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eleven years. On October 29, 1969, he was hospitalized complaining of epiphora and severe pruritus. On hospitalization, the eruptions were remarkable on trunk, especially on abdominal region. A number of enlarged superficial lymph nodes, 10–20 mm in diameter, were recognized on both bilateral axillae and inguinal regions.

On October 31, 1969, he was diagnosed as mycosis fungoides through skin biopsy. From November 11, 1969, he received bleomycin therapy (15 mg twice/week, 150 mg in total) in addition to SM and INA therapy for consumption. On and after December 16, 1969, he was treated with combined therapy with predonin (30 mg/day), endoxan (50 mg/day) and vincristine (2 mg/week) in lieu of bleomycin therapy. Then he relieved gradually, and the pruritic eruptions almost disappeared by March 9, 1970.

Hematologic data from peripheral blood were as follows (December 11, 1969): RBC, 384×10^4 ; hemoglobin content, 12.6 g/dl; hematocrit, 34%; platelet count, 22×10^4 ; WBC, 13,700 (small- and median-sized normal and atypical lymphocytes, 66%; monocyte, 2.0%; band form, 4.0%; segmented polymorph, 24%; eosinophil, 3.0%; basophil, 1.0%). Atypical lymphocytes were characterized by the slightly to severely curved transformation of the nucleus without prominent nucleolus, and by several vacuoles in the cytoplasm. They were not clearly distinguished from normal lymphocytes, because there were intermediate types between normal and atypical lymphocytes. Probably they may be lymphoreticular cells migrated from tumors of mycosis fungoides.

Cytologic findings and remarks. Two karyologically abnormal

Fig. 1. a: Karyotype characterized by an SM marker (arrow) directly prepared from lymph node. Note the loss of one A₂ chromosome. b: Karyotype characterized by two extra minute fragments directly prepared from peripheral blood.

cell lines were noted in samples from skin tumor, enlarged lymph node, bone marrow and peripheral blood. The one was predominant and characterized by the 46, XY chromosome constitution having a large submetacentric marker (SM) in lieu of one A₂ chromosome, and the other by the 48, XY constitution with two extra minute fragments (Fig. 1a and b). The SM marker was tentatively interpreted as being originated from one A₂ chromosome, probably through a reciprocal translocation between long arms of one A₂ chromosome and a part of some unknown element. The origin of two fragments remains unknown. A few cells with the 47, XY constitution having only one minute fragment occurred in addition to the above 48-chromosome line. As shown in Tables I and II, metaphases with the marker or fragments were in general more frequent in direct samples than in cultured cells, when examined at the rapid proliferation phase. The frequencies of cells with those abnormal chromosomes were as follows: 84.1% for lymph node, 69.8% for peripheral blood, 8.0%

Table I. Cytogenetic features in cells from cultured and directly prepared peripheral blood

Date of sampling	Days <i>in vitro</i>	PHA	No. of cells with							Total no. of cells obsvd.
			SM	1Fr.	2Fr.	N. chroms.	tetra	S.R	others	
	(direct)	—	29		1	15				45
Nov. 28, '69		+				74	1†	3		78
	3	—				2				2
	(direct)	—	31	3	3	12	1	1		51
Dec. 8, '69		+	1			47	1*	1		50
	2	—	2	1	1	4				8
		+		1		46		3		50
	3	—	3			10	1	1		15
	(direct)	—				5				5
Dec. 23, '69		+				50	5	5		60
	4	—	1			16	4**	1		22
	(direct)	—				35				35
March 9, '70		+				44	2†	1	3***	50
	3	—				6				6

SM: large submetacentric marker, Fr.: minute fragment, N. chroms.: normal chromosomes (modal number, 46), S. R: Severely rearranged chromosome constitution involving tetra-, tri-, dicentrics, acentrics, fragments, rings, and other abnormalities. *: a cell with one SM marker. **: including a cell with one SM marker and three A₃ and a cell with one fragment. ***: 45, XY, B_q+, C-, 46, XY, G_q-, and 47, XY, B_p-, ?G+. †: an endoreduplicated cell, each.

Table II. Cytogenetic features in cells from cultured and directly prepared bone marrow, lymph node and skin tumor

Tissue	Date of sampling	Days <i>in vitro</i>	PHA	No of cells with						Total no. of cells obsvd.
				SM	1Fr.	2Fr.s.	N. chroms.	tetra	octo	
Bone	Nov. 28, '69	(direct)	—	2		2	46			50
		3	+	1			37	1	1	3
			—				19	1*		20
Marrow	March 9, '70	(direct)	—				6			6
		3	+				7	1		8
			—					few and no good mitosis		0
Lymph node	Dec. 8, '69	(direct)	—	61**	3	10	11	3		88
		3	+				56	6***	8	70
			—	4			43		3	50
Skin (tumor)	Dec. 8, '69	(direct)	—	5						5
		36	—		1		44	3	2	50

*: endoreduplication.

**: including one cell with loss of one A₂ and one extra C element.

***: including two tetraploid cells, one with an SM marker and a fragment and the other with two fragments.

for bone marrow and 100.0% for skin tumor. The situation was quite different in cultured samples. The frequencies of cells with SM marker or fragments were 2.9% and 8.0% in PHA-treated and non-PHA lymph node cultures, 1.1% and 24.0% in PHA-treated and non-treated blood cultures, 2.0% in skin tumor cultured for 36 days, and 0.0% in PHA-treated and non-treated marrow cultures (Tables I and II). In short, the two cell lines proliferate predominantly in the *in vivo* environment of blood and lymph node, while they show an apparently low proliferative ability in both non-PHA and PHA-treated cultures. The above data strongly point to the possibility that these two cell lines are those of malignant cells in this disorder.

In malignant hematopoietic disorders such as leukemias, lymphomas and reticulosarcomas, non-PHA cultures of peripheral blood, marrow and lymph node are usually rather reliable for examination of the chromosomes, since their malignant cells have higher potentiality of proliferation in the culture environment (Sandberg *et al.* 1964, Makino *et al.* 1969, Obara *et al.* 1970a, b). The malignant cell lines of mycosis fungoides here dealt with appeared, however, less suitable for the *in vitro* condition. Mycosis fungoides is clinically considered as a less malignant disease with longer expectancy in comparison with leukemias and lymphomas. The inactivity of malignant cells in cultures observed in this case is of interest in relation to malignancy.

There were cells having an apparently normal chromosome constitution in non-PHA blood cultures with a low frequency. On the basis of the fact that non-PHA blood cultures of normal human subjects show an extremely low mitotic frequency at least for the first three days, the chromosomally normal cells occurring in the present case may not always be of non-malignant nature.

Thirty tetraploid cells occurred in a total of 876 metaphases observed in the four tissues. Two of them had one SM marker, and one carried one SM and one fragment, while the remaining 27 cells showed no such ones. The union of abnormal and normal diploid cells is considered for the formation of the abnormal tetraploid cells, though there is no direct proof. A chromosomal translocation leading to the formation of the SM marker may also be conceivable following tetraploidization of normal diploid cells. There were also 30 abnormal cells containing tetra-, tri-, di- and acentrics, fragments, rings, and others, and showing near-diploid to near-tetraploid numbers (Tables I and II). They occurred frequently in PHA-treated peripheral blood, lymph node and bone marrow cultures, and less frequently in directly prepared blood samples and non-PHA cultures of peripheral blood and lymph node. Such chromosome abnormalities were

found to occur in several other bleomycin-treated hematopoietic disorders, as well as in bleomycin-treated normal human blood cultures. Most probably, their occurrence is associated with bleomycin therapy. Further statements on this respect may be made in near future, since cytogenetic effects of bleomycin on normal human lymphocytes have been studying in detail by the senior author.

Summary. Some cytogenetic features of mycosis fungoides are described. Two chromosomally abnormal malignant cell lines are noted in each sample from skin tumor, enlarged lymph node, bone marrow and peripheral blood of the patient: the one is characterized by a large submetacentric marker (SM), and the other by two minute fragments. These two lines proliferate predominantly in the *in vivo* environment of blood and lymph node, while they show an extremely low proliferative ability in non-PHA and PHA-treated cultures.

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