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Use of G Banding Technique in the Cytogenetic Study of Metastatic Breast Cancer Effusion. A Case Report

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Abstract—*Cells from cancer effusion (breast cancer) were analyzed by G banding techniques. The following observations were made: the frequent losses of chromosomes: A1, D16 and G22; the origin of three marker chromosomes (a ring chromosome, a long acrocentric, a long subtelocentric) could be traced accurately by this banding technique; the fragment 1 q was involved in these 3 markers.*

INTRODUCTION

THE CHROMOSOMES of cells in malignant effusions have been studied for many years, but only recently techniques became available for the detailed identification of the abnormal chromosomes [1–4]. We have used the G banding technique to identify chromosome abnormalities in cells from the pleural effusion of a patient with breast cancer.

MATERIALS AND METHODS

Fresh pleural liquid was delivered to the laboratory within 1 hr of therapeutic tapping and the cells therein sedimented by gentle centrifugation (500 ***g***). These cells were resuspended in 199 medium containing 30% human serum (blood group AB) and 10 μg colchicine/ml medium was immediately added to block the dividing cells in metaphase. After 1 hr, at 37°C, the cells were recovered by centrifugation at 500 ***g*** for 8 min, they were then resuspended in a hypotonic KCl solution (0.075 M) for 8 min, at 37°C. After a further centrifugation, the cells were fixed twice in cold methanol/acetic acid (3/1), then, spread onto cold slides and dried at room temperature.

The G banding was developed by the use of Seabright's technique [2] with some modifications: briefly, the samples were treated 24 hr after fixation with a 0.01% (w/v) solution of trypsin in phosphate buffered saline (PBS) for 13–17 sec; the trypsin was then removed by rinsing in PBS and tap water, and the samples were stained with Giemsa for 4 min. After drying the preparations were rinsed in Xylol and mounted under a neutral mounting medium.

G banded preparations of chromosomes from lymphocytes after stimulation with PHA were prepared exactly as described by Seabright [2].

Case report

A 51-yr-old woman with an undifferentiated adenocarcinoma of the right breast was treated in September 1969 by radical mastectomy and radiotherapy. Axillary nodes were involved. Her general condition was good with no clinical evidence of disease until March 1975, when she developed a right pleural effusion with chest wall nodes. She was initially treated with an anti-estrogen (Tamoxifen) for 2 months, then an ovariectomy was performed on 18 June 1975, with no evidence of regression. A chemotherapy treatment with vincristine, cyclophosphamide, and fluoro-uracil was started in July 1975, and was pursued on a diminishing regimen, up to now. She remained in good condition with no evidence of pleural effusion until May 1977, but, at present, she gives evidence of cerebral metastases.

Four samples of pleural effusion were obtained, the first on 19 March 1975 before the start of the treatment by hormonal or cytotoxic drugs, and the 3 others during the

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course of the treatment on 23 March 1975, 9 April 1975 and 5 August 1975. Cytological examinations of these samples were performed at the same time.*

RESULTS

A comparison between the findings of chromosome analysis and cytological examination of the 4 samples is shown in Table 1.

The modal number of chromosomes remained constant at 43–46 for the 4 samples, but the range varied from 32 to 92 in the first sample down to 43–46 in the last one (Table 2).

Of a total of 137 mitoses observed in the 4 samples 21 could not be counted, however it could be determined that, among these, 12 contained a ring chromosome, 2 had 2 ring chromosomes, and 1 had both a ring chromosome and an abnormal long acrocentric chromosome. The chromosomes in 37 of the 116 countable mitoses were sufficiently well separated for a detailed caryotypic analysis. The results are summarized in Table 3. It can be seen that there were frequent losses of chromosomes: A1, D16 and G22, with gains of 3 abnormal chromosomes: a ring (R), a long acrocentric (LA) and a long subtelocentric (LS). The ring chromosome appears to possess 2 centromeres (Figs. 1 and 2). The G banding pattern of the abnormal chromosomes suggests that LS is derived from a translocation of 1 q into D16, and LA from 1 q into G22. The ring chromosome also appears to involve the 1 q segment.

Chromosome abnormalities were also frequently observed in the 79 countable mitoses whose chromosomes were not sufficiently well separated for a caryotypic analysis. We identified 13 mitoses where all 3 abnormal chromosomes, R, LA and LS, were distinctly present, 20 with R and either LA or LS, 22 with R alone and containing LA alone, LS alone, 2 ring chromosomes, a ring with 2 LA and 2 rings with LA.

The caryotype and G banding of peripheral blood leucocytes taken on 25 March 1975 was in all respects perfectly normal.

DISCUSSION

We have observed a very high frequency of caryotypic abnormalities involving the loss of 3 normal chromosomes (A1, D16 and G22) and the acquisition of 3 atypical chromosomes (R, LA and LS) in cells from the pleural effusion of a patient previously treated for carcinoma of the breast. The presence of these marker chromosomes correlated well with the clinical state of the patient; they were already present in the first sample taken on 19 March 1975 when the cytology of the cells did not suggest malignancy, and they were absent from the cells taken on 5 August 1975 when the patient was responding well to the treatment with cytotoxic drugs. The cells in this last sample still had a malignant cytology and some other chromosomal abnormalities. We believe that the cells containing these markers are of malignant origin as the patients lymphocytes gave a perfectly normal caryotype and G banding pattern, and such markers have not been seen in cases of benign effusions (eg., cardiac effusion) even when treated by radiotherapy.

The use of G banding allowed us to study the chromosomal re-arrangement with precision, and to identify the 1 q segment in the altered chromosomes. It was, however, much more difficult to obtain a sharp banding pattern when we used pleural effusion cells rather than lymphocytes. This may be due either to the malignant character of these cells, or to their rather unfavorable environment in the pleural liquid. In order to obtain interpretable banding we were obliged to modify the times of treatment of the samples without changing the chemicals used.

The cells examined might represent a clone which had lost 3 normal, and gained 3 abnormal chromosomes, leaving the total chromo-

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Table 1. Cytology and chromosomal analysis of four examinations of pleural liquid

Date	Cytology			Abnormal chromosomes		
	Malignant	Suspicious	Negative	Present	Suspicious	Absent
19.3.75			+	+		
23.3.75	+			+		
9.4.75	+			+		
5.8.75	+				+	

Fig. 1. *Caryotype of pleural tiquid showing three abnormal chromosomes* (*R, LA, LS*) *and losses of A*1, *D*16 *and G*22 (*G banding*).

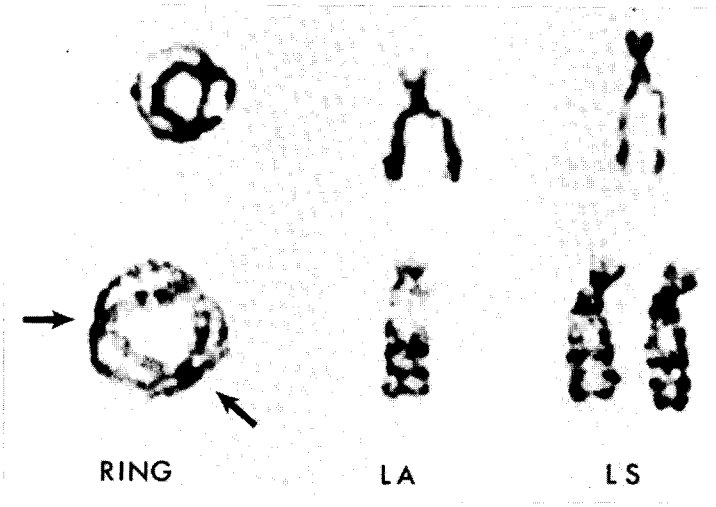

Fig. 2. Abnormal chromosomes: ring (R), long acrocentric (LA) long subtelocentric (LS). (G banding). Arrows indicate two centromeres.

Table 2. Chromosome counts and stemline number in the four examinations

Date	Number of countable mitoses	Modal chromosome number	Chromosome numbers																																				
			32	34	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	53	75	77	81	82	84	85	86	87	88	89	90	92	94	104						
19.3.	74	45–46	1	1		1	1	1	2	1	3	7	16	17	6	3	1	1	2	1	1	1	1			2		1	1	1		1							
25.3.	18	46			1				1		1		3	4	2									1	1			1		1	1			1					
9.4.	20	46								1	3	1	3	10													1					1							
5.8.	4	46									1			3																									

Table 3. Distribution of marker chromosomes (R, LA, LS) and loss of chromosomes (1, 16 and 22) in 37 cells R: ring; LA: long acrocentric; LS: long subtelocentric

Loss of normal chromosomes \ Markers	R+LA+LS	R+LA	R+LS	No marker
−1−16−22	14			
−1−2	6	1		
−1−16	6			
−16−22	1			
−1	3		1	
−16	1			1
−22	1			1
No loss	1			

some number unchanged at 46. One of the new chromosomes, however, was a ring form with 2 centromeres, so that there were 47 centromeres per cell (Fig. 2). Some cells were seen containing 2 ring chromosomes or 2 LA suggesting that these abnormal forms can in fact replicate, and this particular pattern of abnormal chromosomes persisted in 3 samples taken over a period of 3 weeks suggesting that viable cell divisions did occur.

The segment 1 q appears to be involved in the formation of all 3 of our abnormal chromosomes. The participation of 1 q in translocations has been called the common denominator for breast cancer by Cruciger [5], who studied 7 metastatic breast carcinomas. Ayraud [6, 7] using R banding, and Kakati [8] have also shown the translocation of 1 q into different fragments in cases of breast cancer.

Rare aberrations of ring chromosomes in chromosomal structure have been reported in some cases of malignant disease, for example Alimena [10] observed two cases of acute leukaemia with ring chromosomes; Falor [11] described a ring chromosome in non-invasive bladder carcinoma; Katayama [12] observed a ring chromosome in breast cancer; Spriggs [13] described ring chromosomes in carcinoma *in situ* of the cervix; Mark [14] observed ring chromosomes in a human recurrent meningioma.

Involvement of the 1 q fragment in translocation in other forms of cancer seems to be frequent; Castoldi [15] observed the fusion of a whole A chromosome with a fragment resembling the long arm of C10 in a case of histiocytic sarcoma; Oshimura [16] described a trisomy of part of, or the whole of, the 1 q in 4 patients with leukaemias. Kakati [17] observed that "chromosome No. 1 was more frequently involved in aberrations than any other chromosome" in melanoma. Recently Atkin [18] published a review on the structural changes involving the chromosomes 1 in ovarian cancers.

Mitelman and Levan [19] reviewed 287 cases of human neoplasia representing 10 different groups of neoplastic conditions. They indicated that only a few of the human chromosomes types were engaged regularly in aberrations, viz. No. 7, 8, 9, 14, 17, 20, 21 and 22, and Kakati [17] "would like to add chromosome No. 1 to this list".

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