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Malignant Tumours and Chromosomal Aberrations

By

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Malignant tumour cells show a variety of chromosome patterns, and even one and the same tumour may vary in this respect at different points of time. It is not known whether these alterations are of a primary or of a secondary nature. Chromosomal aberrations and increased fragility of the chromosomes (a phenomenon promoting aberrations) raise the frequency of malignancies; this would suggest that chromosomes are primary pathogenic factors of certain malignant tumours.

Chromosomal fragility cannot always be detected by current experimental techniques, therefore a method consisting of the examination of chromosomal breakages induced by alkylating agents has been devised; its results are presented.

Introduction

It has long been known that the chromatin of tumour cells displays changes but whether the chromosomal aberrations observed in tumour cells should be regarded as a primary or as a secondary phenomenon, has remained an unsettled issue. The question of the role of chromosomal aberrations in the development of tumours has been raised recently.

The investigations concerning human karyotypes have brought these problems nearer to solution. The present paper will report on some of the pertaining data and our investigations into the correlations between structural chromosome aberrations and malignant tumours.

Method

Chromosome examinations were carried out in 72-hour peripheral blood cell cultures by Moorhead's [47] slightly modified method [62]. Chromosome breakage was diagnosed when the broken end or the broken ends were dislocated, while the presence of achromatic gaps was disregarded. As an inhibitor, tetramethane sulphonyl-d-mannitol (R—52) was added to the test tubes at the beginning of cultivation; its concentration was so adjusted as to inhibit 50% of the mitoses. The usual dose was between 0.75 and 1.0 μg per ml.

Results and Discussion

The chromosome pattern encountered in tumour cells shows great numerical and structural variations. This aneuploidy, a high characteristic phenomenon, seems to be independent of whether the chromosome number is roughly diploid, triploid or tetraploid. Although aneuploidy occurs practically in every malignant tumour; no correlation has been found between the number of chromosomes and the clinical course of the disease [42, 58].

One or more marker chromosomes, characteristic of the given tumour, are demonstrable in the majority of cases [1, 2, 36]. Marker chromosomes may appear early, and they have been described also from carcinoma *in situ* [13, 71] and attempts are being made to find marker chromosomes characteristic of the various kinds of neoplasms, such as the large extrachromosome A observed in Waldenström's hyperglobulinaemia or the chromosome Ph_1 invariably encountered in the typical form of chronic myelosis.

Tumours of identical types appearing in different individuals display a number of similar features, although these similarities are not convincing. The presence of a large extrachromosome A together with a small extrachromosome in 31.4% of multiple myelomas [66] or an abnormally large acrocentric chromosome and the secondary constriction of the 10th pair of chromosomes in Burkitt's lymphoma [8, 31, 35, 44] are examples of such similarities. Certain authors encountered a marker chromosome in different testicular tumours [17, 39].

The greatest number of common features was observed in the chromosome pattern of malignant lymphomas [41, 45, 46, 68]. Such supposedly characteristic marker chromosomes are, however, not invariably present in every case; a Melbourne-like chromosome, for instance, has been found to be associated with several disorders, e.g. with congenital malformation [54] and also with Pendred's syndrome [34].

Malignant tumours are, according to LEVAN [37] characterized by a multiplication of chromosome C and a decrement of chromosomes D and G. In 50% of 25 different malignant tumours (laryngeal carcinoma, melanoma and malignant schwannoglioma), BENEDIKT et al. [6] observed a characteristic marker in the D-group; it was a long acrocentric chromosome with a satellite. Other authors [70, 67, 75] found in solid tumours the number, shape and size of the marker chromosome to vary with each individual case. Moreover, the karyotype was reported to undergo wide variations even in one and the same case [43, 56, 58]. In further 47 cases of carcinoma, these authors failed to support LEVAN's above-mentioned statement that, by a fusion of the chromosomes D and G, their number becomes frequently less, while that of the chromosomes C increases.

The variability of the chromosome pattern is further enhanced by the fact that cells with altered chromosomes are labile and susceptible to further mitotic disorders such as non-disjunction or endoreduplication of a single chromosome [27], phenomena which increase aneuploidy and lead to an increase in the number of aberrant cell lines.

It is evident from the foregoing that examination of the chromosomes in solid tumours does not solve the problem of the phenomenon being primary or secondary. The fact that malignancy is more frequent in connection with chromosomal aberrations argues in favour of the alter-

native. There are for instance observations that malignant tumours as well as aneuploidy are more frequent in aged persons [33, 57, 59]. PERGAMENT et al. [52] examined 39 married couples with a history of repeated spontaneous abortions: A chromosomal aberration — D/D dislocation — was displayed by one single woman who then died of mammary carcinoma at the age of 31 years.

HOLLAND et al. [30] studied 2030 subjects with Down's syndrome and found 2.6 times more malignant tumours than in the general population: most of the persons afflicted with a tumour were less than 20 years old. The incidence of malignancy was found to be higher, and chromosomal aberrations 33 times more frequent, in cases of hemihyperthrophy than in healthy individuals [21].

Certain chromosomal aberrations induce no perceptible change in the phenotype and are usually considered a family trait or a normal variant; they are mostly structural changes such as the Ch^1 chromosome, a chromosome 21 which has a smaller short arm [18, 28]. Lymphocytic leukaemia is known to be frequent in families displaying such anomalies.

MERZ et al. [40] found abnormally elongated chromosomes 2 in the healthy tissues of subjects with a malignant tumour, further in the healthy daughter of one such patient; they ascribed the phenomenon to translocation. In a case of hemihypertrophy a similarly long chromosome A was observed [20]; up to now the child has developed no tumour.

Structural chromosomal aberrations are due to chromosome breakage. It would follow that factors causing breakage are tumorogenic as well [26]. An increase in the number of breakages elevates the frequency of "faulty" healings, and thus leads to the appearance of more mutant cells which may result in the development of a tumour. Exposure to X rays and radioactive substances [10, 11, 23, 60, 74], further treatment with benzene [73] induce the breakage of chromosomes and are cancerogenic.

Opinions are divided as regards chromosome breakage caused by viruses. In vivo breakage was registered in cases of measles, after measles vaccination, in German measles, epidemic hepatitis, and chickenpox [24, 49, 16, 50, 3, 4, 5, 72, 51]. Vaccinia virus does not, according to our observations, promote chromosome breakage in vitro, and even in vivo it was only in two cases that we registered breakages whose number exceeded the normal limit but slightly [63]. While the significance in embryonic growth of virally induced breakages, cell destruction and inhibited cell division is well-known, their oncological role is obscure. Of the oncogenic viruses SV-40 and the virus of Rous' sarcoma have been studied in human tissue cultures [15, 48]; the observed chromosomal anomalies were like those seen in the tumours caused by these viruses [69].

In addition to infections and other exogenous factors that may induce chromosome breakage there are inherited diseases in which the number of

"spontaneous breakages" is above the normal limit.

In cases of Fanconi's anaemia, a recessively inheritable disease accompanied by pancytopenia and congenital malformations, the familial occurrence of leukaemia is about 40 times more frequent than otherwise [19]. In our own patients with Fanconi's anaemia, chromosome breakages were some 20 times more frequent than among healthy individuals; translocation figures have also been observed in such cases [12, 61, 64] (Table I).

TABLE I

Chromosome breakages in Fanconi's anaemia

Name	Age (yrs)	Number of cells examined	Number of breakages
Z. T.	6	150	60
T. T.	11	20	13
Control		300	5

Lymphoreticular tumour and leukaemia are frequent in ataxia telangiectasia, a syndrome displaying ocular telangiectasis, cerebellar alterations and a complete absence of immune globulin A. GROPP and FLATZ [25] observed an increased rate of chromosome breakages in this syndrome. We examined four such cases, but it was only in one patient (who was to develop gastric carcinoma) that the number of breakages was above the normal value [65] (Table II).

Literary data point to the existence of a correlation between chromosome breakages and malignancy in Bloom's syndrome [22]. Lymphoreticular tumours were registered in 12% of patients suffering from Wiskott–Aldrich's syndrome, a disease accompanied by isolated thrombopenia, eczema and susceptibility to infection [7]. We had one such case, a two-year-old child, in whom 15% of the cells exhibited chromosome breakages as against 0.4% in the control. The patient died of malignant reticulosis two months later (Table III).

TABLE II

Chromosome breakages in ataxia telangiectasia

Name	Age (yrs)	Number of cells examined	Percentage of breakages
Z. S.	14	38	10
L. D.	9	30	3
J. P.	11	27	—
L. H.	11	15	6
Control		300	1.4

TABLE III

Chromosome breakages in Wiskott—Aldrich syndrome

Name	Age (yrs)	Number of cells examined	Percentage of breakages
K. K.	1	20	15
Control		300	1.4

In our studies of the in vitro action of certain compounds on the chromosomes of peripheral human leucocytes in Fanconi's anaemia, a disease often

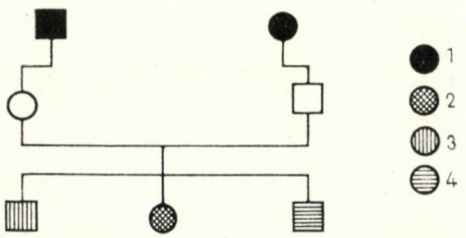
leading to leukaemia, the chromosomes were abnormally sensitive to the breakage-promoting agent R-52 [64].

Chromosome breakages were frequent (12 and 55% in cultures containing 0.1 and 0.5 μg/ml R-52 against a control value of 9 and 18%) also in a child suffering from agammaglobulinaemia combined with lymphoreticular hyperplasia [29]. Agammaglobulinaemia is known to be accompanied by increased tumorous disposition [53] and increased chromosomal fragility [32].

We assumed that if these chromosomes are less stable and more sensitive to chemicals, they would respond to breakage-promoting agents with an increased number of breakages also in vivo. This would mean that increased chromosome breakage was to be found in the background of some tumours, especially in that of familial recurrent neoplasms. We examined, therefore, a family (Case 1) in which several malignancies had occurred, further one patient with tumour (Case 2) and one with leukaemia (Case 3), whose respective family history contained no reference to malignancy. As regards Case 1, all three children of a healthy couple belonging to this family died of malignant tumours or leukaemia, and two grandparents had succumbed to gastric cancer (Table IV). The number of spontaneous breakages in the two examined children and their mother

TABLE IV

Pedigree of patient with familial tumour



1) gastric cancer 2) sarcoma of lower limb 3) leucosis 4) malignant reticulosis

TABLE V

Chromosomal aberrations, spontaneous or induced by R—52

Name	Age (yrs)	Diagnosis	Number of cells examined		Number of breakages	
			Spontaneous	R—52	Spontaneous	R—52
I. S.	6	Malignant reticulosis	100	100	10	20
M. S.	4	Leucosis	100	100	17	35
M. S.	33	Healthy mother	100	100	12	44
Control			300	100	5	19

did hardly exceed the normal value [38] but the R-52 test revealed increased chromosomal fragility (Table V). The number of spontaneous breakages as also the R-52 tests were normal in Cases 2 and 3.

Our experiments with R-52 and other compounds showed that their effect on the chromosome varies from individual to individual. To establish the usefulness and the value of the method, numerous further examinations in normal persons, tumorous patients, familial tumorous diseases and cases of tumorous disposition will have to elucidate the significance of individual and familial differences.

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