Nuclear protrusions in malignant tumours with large abnormal chromosomes: Observations on C-banded preparations

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Summary. The appearances are described in 4 human tumours having nuclear protrusions associated with large abnormal chromosomes. In C-banded preparations, chromocentres were seen in the protrusions only where interstitial C-bands were present on the long arm of the abnormal chromosome, providing evidence that the protrusions are indeed formed by the long arms.

In 1964 we described nuclear protrusions in malignant cells in ascitic fluid secondary to a carcinoma of the ovary; it was considered that the protrusions were caused by the long arm of a large abnormal chromosome which was seen in the metaphases[2]. Since then, we have observed this phenomenon in a large number of malignant tumours, the common feature of which is the presence of 1 or more abnormal chromosomes whose long arms are about as long as, or longer than, the whole of the No. 1 chromosomes. Similar findings have been described by other workers[3-6]. Besides a wide variety of human malignant tumours, nuclear protrusions have been seen in the normal cells (fibroblasts in culture) of a female carrier of a translocation which resulted in a large abnormal chromosome[5], and in a pig kidney[7] and a feline lymphosarcoma[8] cell line, both of which had a large abnormal chromosome.

Observations. The appearances will be described in 4 untreated primary tumours (table and figures 1–4). Direct preparations pretreated for chromosome studies were stained by the C-banding (B.S.G. technique[9]) and G-banding (saline-sodium citrate/trypsin) techniques.

In tumour No. 1 (figure 1), a very long subtelocentric chromosome was present whose long arm was about 1.24 times as long as the whole of chromosome 1. Particularly prominent interphase protrusions were seen in this tumour.

In tumour No. 2 (figure 2), the centromeric heterochromatin of the abnormal chromosome was unusually large; this chromosome was obviously derived from a 1qh+ chromosome which was identified in a blood culture from the patient. The large heterochromatic region formed a distinctive large chromocentre in interphase which was seen in both normal (e.g. plasma cells) and tumour cell nuclei. Its typical situation in relation to the protrusion in tumour cells was on the nuclear membrane a short distance from the base of the protrusion (figures 2b and c).

In tumour No. 3 (figure 3), the abnormal chromosome had 2 prominent interstitial C-bands on its long arm. The protrusions in this tumour were frequently seen to contain 1 or, less commonly, 2 chromocentres. (Where, as in many tumours with large abnormal chromosomes, there is no interstitial C-band on the long arm, the protrusions are free from chromocentres in C-banded preparations (e.g. tumours Nos 1 and 2, figures 1d, 2b and c).)

In tumour No. 4 (figure 4), 2–4 large chromosomes were seen. Most constantly present was a chromosome derived from chromosome 1 which had an additional C-band near the end of the long arm. A chromocentre was present in many of the protrusions in C-banded preparations.

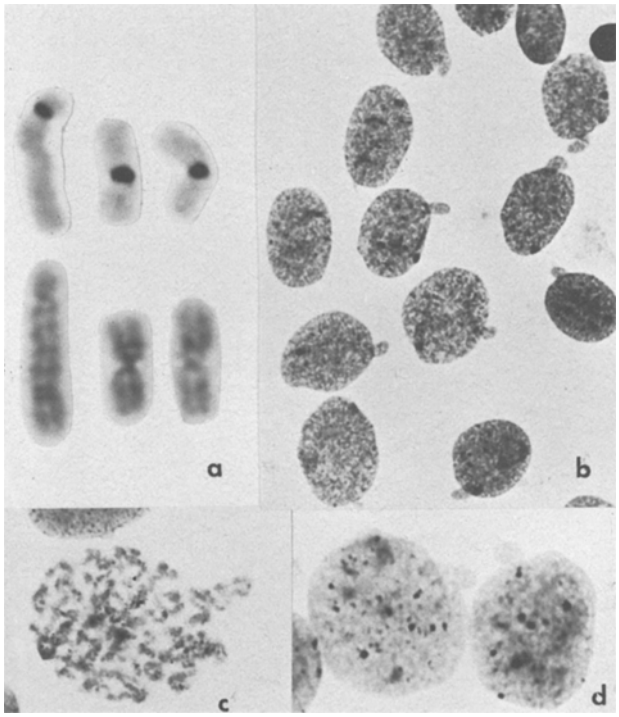

Fig. 1. Tumour No. 1. *a* Abnormal chromosome (left) and 2 chromosomes 1 from C-banded (top) and G-banded metaphase; protrusions in *b* nuclei, G-banded, *c* prophase, G-banded and *d* nuclei, C-banded. *a* × 2760; *b*, *d* × 560; *c* × 840.

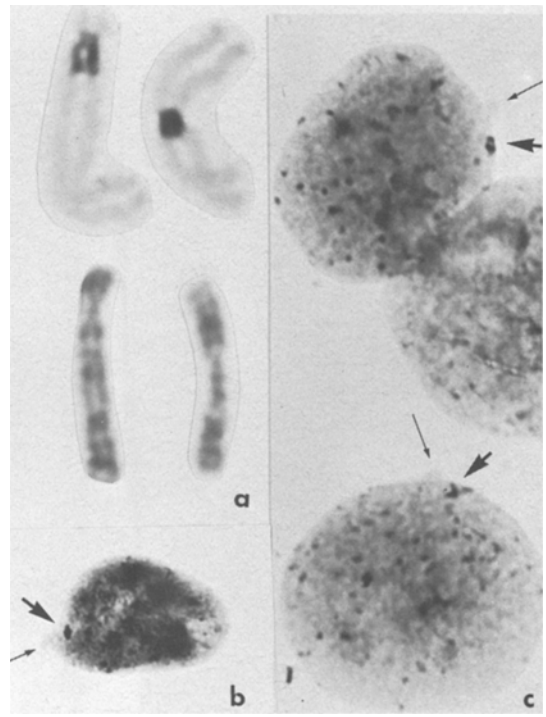

Fig. 2. Tumour No. 2. *a* Abnormal chromosome (left) and chromosome 1 from C-banded (top) and G-banded metaphase; *b*, *c* nuclei showing small protrusions (thin arrows) and associated chromocentres (thick arrows), C-banded. *a* × 2760; *b*, *c* × 1720.

Abnormal chromosomes in 4 tumours associated with interphase protrusions

Tumour		Approximate modal chromosome number	Mean lengths of arms of abnormal chromosome relative to length of whole of chromosomes 1 (based on measurements on 10 markers and 10–20 chromosomes 1)		Comments
			Short arm	Long arm	
1	Poorly-differentiated adenocarcinoma of ovary (aged 64)	58	0.21	1.24	Protrusions lacking chromocentres in C-banded preparations
2	Grade 3 transitional cell carcinoma of bladder (male, aged 65)	64	0.11	0.98	Very large centromeric heterochromatic region (derived from chromosome 1qh+ present in normal cells), seen as chromocentre near base of protrusion
3	Grade 2 transitional cell carcinoma of bladder (male, aged 44)	75	0.46	1.20	2 interstitial C-bands on long arm; frequently, 1 or 2 chromocentres in protrusions
4	Poorly-differentiated solid carcinoma of ovary (aged 77)	71	0.41	1.06	C-band near end of long arm. Other long markers without additional C-bands less constantly present

In general, the length of the protrusions seems to bear a relationship to the length of the abnormal chromosome's long arm which, together with other factors, may also influence the incidence of protrusions. Although protrusions were sometimes seen in over half the nuclei (figure 1b), their incidence was usually somewhat lower than this. Possibly, factors such as the position in the cell cycle (e.g. whether in early or late G_1 phase) and the metabolic activity of the cell influence the appearance of protrusions and may result in different incidences in different regions of the tumour. The chromosome number of the tumour does not appear to be of significance; although the 4 tumours described here all have high modes, we have also seen protrusions in hypodiploid tumours.

Conclusions. Nuclear protrusions tend to be formed wherever a chromosome is present whose long arm approaches or exceeds in length the whole of chromosome 1. The constitution of the abnormal chromosome varies from case to case and is apparently not of itself significant except in relation to the size of the chromosome. In tumours Nos 2–4 the centromeric region of the chromosome was derived from chromosome 1; in tumour No. 1 its origin was uncertain.

The findings described here leave little doubt that the protrusions are formed by part (excluding the juxta-centromeric heterochromatin and probably some of the adjacent euchromatic region) of the long arm of the abnormal chromosome.

Although little is known about the organisation of the chromosomes in interphase nuclei, there is evidence that the centromeric regions[10] and perhaps the telomeres[11] are

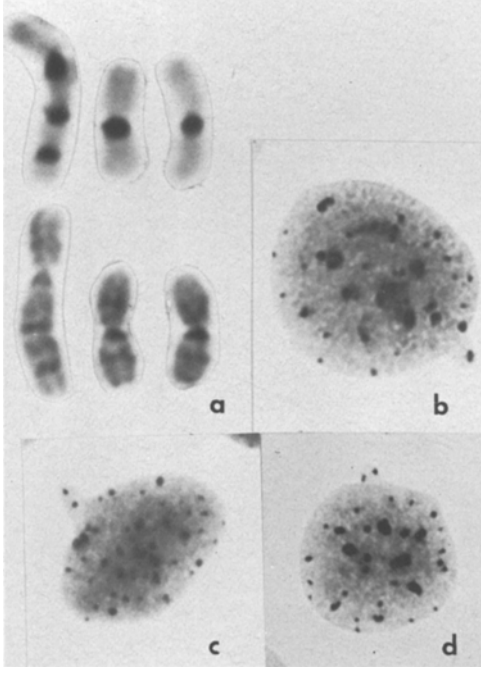

Fig. 3. Tumour No. 3. *a* Abnormal chromosome (left) and 2 chromosomes 1 from C-banded (top) and G-banded metaphase; *b, c, d* nuclei showing protrusions with 1 (b) or 2 (c, d) chromocentres, C-banded. *a* × 2760; *b, c, d* × 1720.

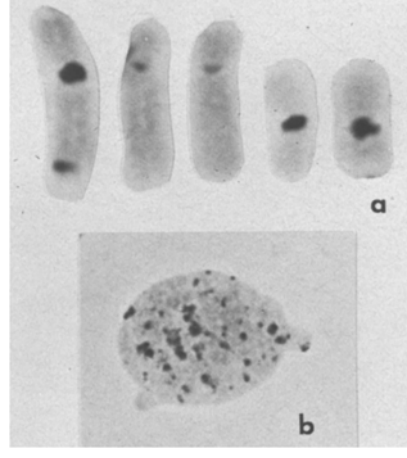

Fig. 4. Tumour No. 4. *a* Left to right: large abnormal chromosome with C-band near end of long arm, 2 other large abnormal chromosomes and 2 chromosomes 1, from a C-banded metaphase; *b* nucleus showing 2 protrusions, 1 with a chromocentre, C-banded. *a* × 2760; *b* × 1720.

attached to the nuclear membrane. Otherwise, apart from the occasionally observed alignment of homologues and the well-known association of acrocentrics and perhaps other chromosomes including the X and chromosome 1 with the nucleolus, it is not known whether the interphase chromosomes bear a consistent or nonrandom relationship to one another or to other nuclear structures. The important question of the extent to which aneuploidy, and in particular the presence of a large chromosome, results in a disorganisation of the nucleus cannot therefore be answered. The striking configuration of protrusions - projections in otherwise smoothly-outlined nuclei - signals a change in the normal pattern at least as far as the nuclear membrane is concerned, but whether this in itself is of any significance in relation to the function of the cell is unknown.

It is possible that lagging of the abnormal chromosome at anaphase is at least a contributory factor in the formation of protrusions; prominently protruding chromosome arms were seen in anaphases and telophases, as well as metaphases, in histological sections of a variety of malignant tumours[12] and a carcinoma in situ of the cervix uteri[13] known to have large abnormal chromosomes.

Protrusions serve as useful indicators of an aneuploid clone having a large abnormal chromosome and, although not per se indicative of malignancy, may thus (depending on the circumstances) provide evidence of a neoplastic condition.

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Hyperosmolar coma as etiological factor in the CNS radiation syndrome of rats

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Summary. Supralethal dose of whole-body or trunk but not head-irradiation in rats induced hyperosmolar coma accompanied by hypernatremia and hyperkalemia. This clinical entity has presented the symptoms of the CNS radiation syndrome with a characteristic short survival time.

The CNS radiation syndrome (CNS-S) is characterized by neuropathological symptoms such as ataxia, hyperirritability, convulsions, coma and by short survival time[2]. Although these symptoms appear indicative of CNS involvement, the pathophysiological sequence leading to lethality does not appear to be exclusively related to CNS injury. Observations on different species show that CNS-S can be induced after a supralethal dose delivered to the whole body but that the same dose is insufficient when only the head is irradiated[3]. Even when CNS symptoms are present after head-only irradiation, survival time is significantly longer than animals that received an equivalent dose to the whole body. The reason for this phenomenon remains to be elucidated and this was the object of our experiment.

Material and methods. Animals. Young, mature 12-week-old male albino rats weighing about 280–290 g were used in this study. Standard Purina rat chow and water was available ad libitum throughout the experiment. The experimental protocol contained the following studies: 1. Survival time of whole-body, head-only and trunk-only irradiated animals after 20,000 rad exposure (20 animals in each group). 2. Study of blood constituents (plasma osmolality, plasma electrolytes and hematological values) at 48 h after 20,000 rad irradiation in whole-body, head-only or trunk-only exposed rats. In the control group, the sham-irradiated animals were similarly handled but not exposed (20 animals in each group).

Irradiation. Rats were placed into individual Lucite restraining cages and were exposed to 30.5±0.5 MeV electrons from the AFRRI[4] high energy electron Linear Accelerator (LINAC). The primary reason for using the LINAC was the ability to obtain effective shielding. The significant parameters were 3.75 pulses/sec and 8.9 rad/pulse. The pulse width was about 0.9 μsec, giving an instantaneous dose rate of about 9.9 Mrad/sec. About 2000 pulses of

Table 1. Alterations of the hematological parameters of head-, trunk-, and total body-irradiated rats at 48 h after exposure of 20,000 rad

	No. of rats	Hematocrit	Red blood cells $\times 10^3$	White blood cells	Platelets $\times 10^3$
Control, sham-irradiated	20	44.5±2.5	6.360±1.056	6.413±952	1.012±215
Head-irradiated	20	46.9±1.3	5.687±730	5.274±1.298	1.239±174
Trunk-irradiated	20	42.4±1.6	5.388±472	948±413*	263±100*
Whole-body-irradiated	20	41.3±2.6	6.581±830	493±159*	244±73*

Asterisks are representing significant changes to the control group, $p<0.001$.