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In Vivo Tumour × Host Cell Fusion in Spontaneous Syrian Hamster Metastasis

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Abstract—*The HSV-2 tumour system was originally derived from an* in vitro *transformation of HEF by inactivated HSV-2. When injected s.c. these cells produce spindle-cell sarcomas which are metastatic at a low level. Detailed cytogenetic studies have provided evidence of tumour × normal host cell fusion in two of seven cell lines derived from metastatic lung deposits (Met D and Met G). This is the first report, in an unselected, intraspecific system, of* in vivo *cell fusion in spontaneous metastatis. The cells of Met D consisted of a heterogeneous population of fused and unfused tumour cells, whereas those of Met G were a homogeneous population of hybrid cells. Fusion, therefore, is likely to have occurred after metastasis in Met D and prior to, or at, metastasis in Met G. The fused cells of Met D showed comparatively little chromosome loss, while in Met G there was loss of approaching one haploid set of chromosomes. The generation of metastatic variants by cell fusion contributes to genetic diversity and emphasizes the importance of tumour heterogeneity in malignancy.*

INTRODUCTION

IT HAS BECOME increasingly apparent that tumour heterogeneity is a major factor in malignant disease, although the mechanisms for this variation are poorly understood. Hybridization of somatic cells and tumour cells has been used in many elegant studies on the control of malignancy [1–3] but more recently attention has focused on the role of cell fusion in the generation of tumour heterogeneity and the formation of metastatic variants. The first such case was that of Goldenberg *et al.* [4] who reported *in vivo* hybridization of a human lymphoma line GW478 with a normal hamster cell. Further studies have demonstrated cell fusion in a variety of experimental systems both *in vivo* and *in vitro* (for review see [5]), and some of the factors relevant to fusion have been identified. Thus, the nature of the participating host cell, the immunological state of the hybrid cell, and the degree of chromosome segregation that follows fusion are all thought to be important in determining the malignancy of the hybrid cell [5–7].

Despite a considerable amount of experimental work, only two reports have provided evidence of intraspecific *in vivo* cell fusion in metastasis [6, 8]. Both of these systems, however, were designed to promote cell fusion. In Kerbel's case [6] the tumour line used (MDW4) had been selected for a membrane mutant which may have 'artificially amplified' the level of cell fusion. Similarly De Baestelier *et al.* [8] chose a tumour line (BW514) because it was fusogenic *in vitro*, the cells were then injected intravenously to increase the probability of organ tumour development.

The HSV-2-333-26 cell line (Parent) has not been experimentally manipulated other than for its ability to grow *in vitro* as a cell line and *in vivo* as a transplantable tumour. When Parent tumour cells are injected subcutaneously into hamsters they form spindle cell sarcomas which are spontaneously metastatic at a low level [9]. In the present study we provide evidence of *in vivo* cell fusion in two out of seven cell lines derived from spontaneous lung metastases. Analysis of the two cell lines revealed differences which may have important implications for the biological nature of *in vivo* cell fusion. Our results are discussed in relation to other experimental work on cell fusion and its relevance to human malignancy.

MATERIALS AND METHODS

Animals

Male Syrian golden hamsters aged 6–10 weeks, taken from a closed randomly bred colony at the University of Sheffield, were used in all experiments.

Tumours

The HSV-2-333-2-26 cell line was originally obtained by an *in vitro* transformation of hamster

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embryo fibroblasts (HEF) with inactivated HSV-2 and was kindly provided by Dr F. Rapp (Department of Microbiology, Penn. State University, Hershey, PA, U.S.A.). Following s.c. inoculation a transplantable tumour line was established and maintained by trocar implantation for six passages. After removal of necrotic tissue, tumour fragments were digested with 0.25% trypsin, the cell suspension was then seeded into 4 oz medical flats, and grown on Hams F10 (Gibco, Paisley, U.K.) supplemented with 5% (v/v) newborn calf serum (Flow, Irvine, U.K.). This was termed the Parent line. The seven sublines (Met A to Met G) were derived, in a similar manner to the Parent line, from lung metastases in hamsters whose primary Parent load had previously been resected [10].

Chromosome preparations

Subconfluent cultures were incubated for 2–4 h with 0.1 ml/ml v/v colcemid (Gibco Diagnostics, Grand Island, NY). Cells were detached with trypsin/versene (0.25% trypsin; 0.5 g/l versene) and centrifuged. Cells were suspended in hypotonic solution (0.075 M KCl) centrifuged and fixed in methanol/acetic acid (3:1 v/v). Slides were prepared by drop-wise addition of the cell suspension, in fresh fixative, onto grease free, cold wet slides which were allowed to air dry after addition of a few drops of fixative. G banding was performed (0.1% trypsin in Sorensen's buffer) and the slides stained (Leishman's stain diluted 3:1 v/v Gurr's buffer pH 6.8).

Chromosome analysis

Cytogenetic studies were performed on all Met lines but only Met D and Met G showed evidence of fusion [the five remaining Met lines all had modal numbers (MN) of chromosomes lower than that of the Parent line]. One hundred cells of Met D were scored, 79 were unfused tumour cells and 21 hybrid (tumour × host) cells. Twenty unfused and six fused cells were fully analysed and photographed. One hundred cells of Met G were scored and 11 fully analysed and photographed. Non-clonal chromosome abnormalities were not annotated on the karyotypes. In excess of 500 cells from the Parent line were scored for evidence of cell fusion.

RESULTS

The karyotype of the Parent line has been reported elsewhere [11]. Briefly, it has a MN of 74 chromosomes, with considerable variation in both numerical and structural abnormalities. Despite this cytogenetic instability all cells of the Parent line contained a common theme of marker chromosomes.

Most of the cells of Met D (Fig. 1) had a MN (67) similar to the Parent line and contained many of the same marker chromosomes, but could be readily distinguished from the Parent line by the presence of an isochromosome 3q unique to Met D. Further detailed cytogenetic analysis revealed a subpopulation of cells (20%) which contained the marker chromosomes of Met D but which had many more chromosomes (MN105). The unexpected discovery of cells with an extra set of normal chromosomes (without duplication of marker chromosomes) suggested that they represented hybridization of a Met D cell with a host cell (Fig. 2). Convincing evidence that this gain in chromosomes had occurred by cell fusion was provided by the presence of two normal chromosome 3's and 15's which were not found in the unfused tumour cells of Met D. It is most unlikely that these normal chromosomes could be aquired by a mechanism other than hybridization with a host cell.

Evidence of cell fusion in Met D led us to study another cell line Met G (Fig. 3) which had a MN of 104 chromosomes. As with the fused cells of Met D the cells of Met G had a similar marker chromosome profile to that of the Parent but again contained a large number of extra normal chromosomes. Further evidence that Met G was also the result of tumour × host fusion was provided by the presence of two normal chromsome 15's in the cells of Met G which were not present in the cells of the Parent line.

It could be argued that the cells of Met G and the higher MN cells of Met D were not derived by cell fusion but pre-existed as a sub-population within the parent line. Although this possibility cannot be excluded there was no evidence of such cells in over 500 cells scored from the Parent line, furthermore FACS analysis and *in vitro* cloning failed to demonstrate any cells of this type in the Parent line [10].

The unfused cells of Met D were derived from a clonal metastasis and showed relatively little variation in chromosome number (63–71). It was therefore possible to estimate the amount of chromosome segregation that had occurred in the hybrid cells by subtracting the MN of the unfused Met D cells from the MN of the fused cells (105 − 67 = 38). Since a normal hamster has 44 chromosomes this indicates an average loss of six chromosomes (44 − 38) in the hybrid cells of Met D.

For Met G, which is a homogeneous population of fused cells, fusion could have occurred prior to, or immediately after metastasis. In either case the precise karyotype of the tumour cell involved in fusion is not known. Cells of the Parent line vary considerably in both numerical and structural abnormalities and it was therefore more difficult to evaluate how much chromosome segregation had taken place in Met G. If it is assumed that fusion occurred with a tumour cell of around the parental MN (74) then there appears to be loss of about 14

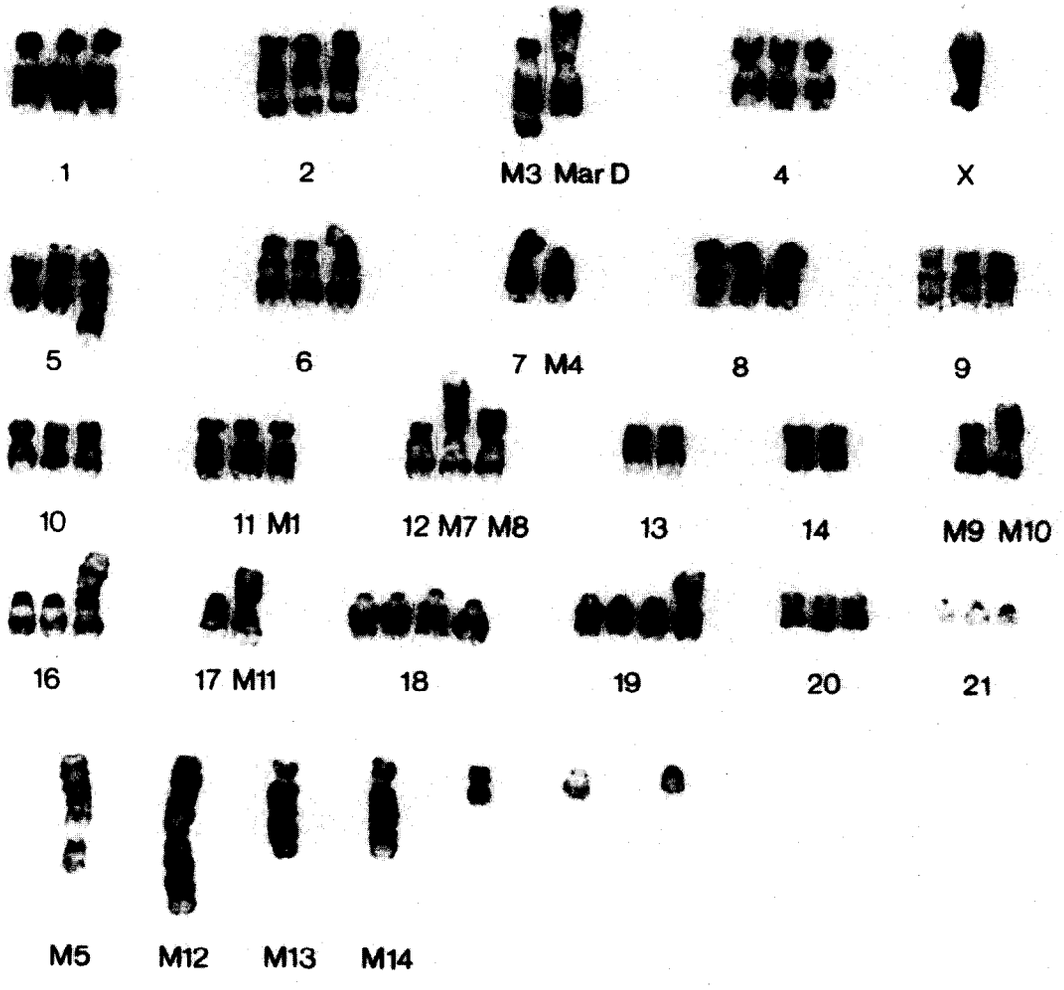

Fig. 1. G banded karyotype of an unfused cell of Met D. Note: (i) the absence of normal chromosome 3's and 15's; (ii) the presence of Mar D.

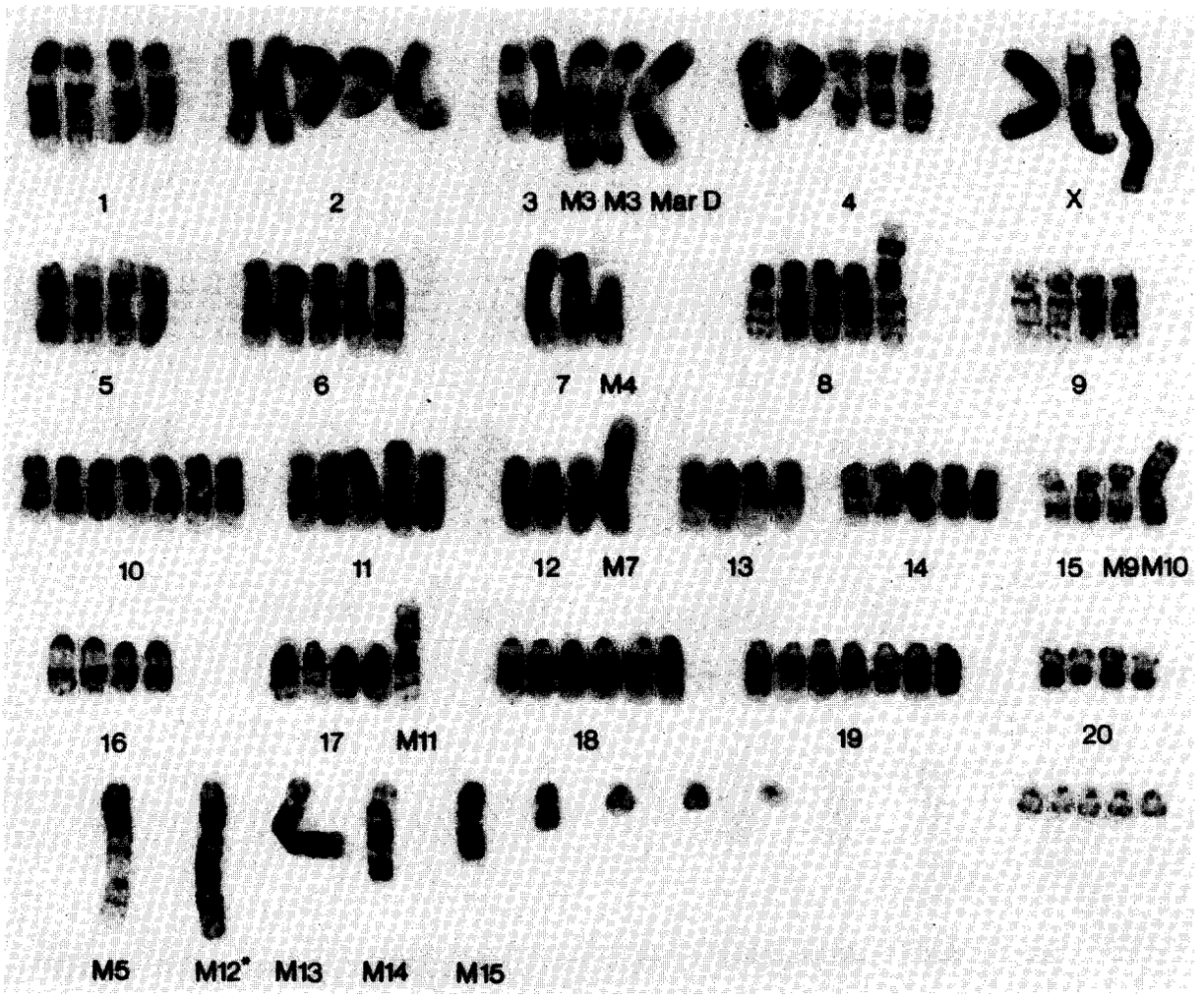

*Fig. 2. G banded karyotype of a fused cell of Met D. Note: (i) normal chromosome 3's and 15's and the large number of chromosomes; (ii) the presence of Mar D. (*One off delection of the p-arm of M12.)*

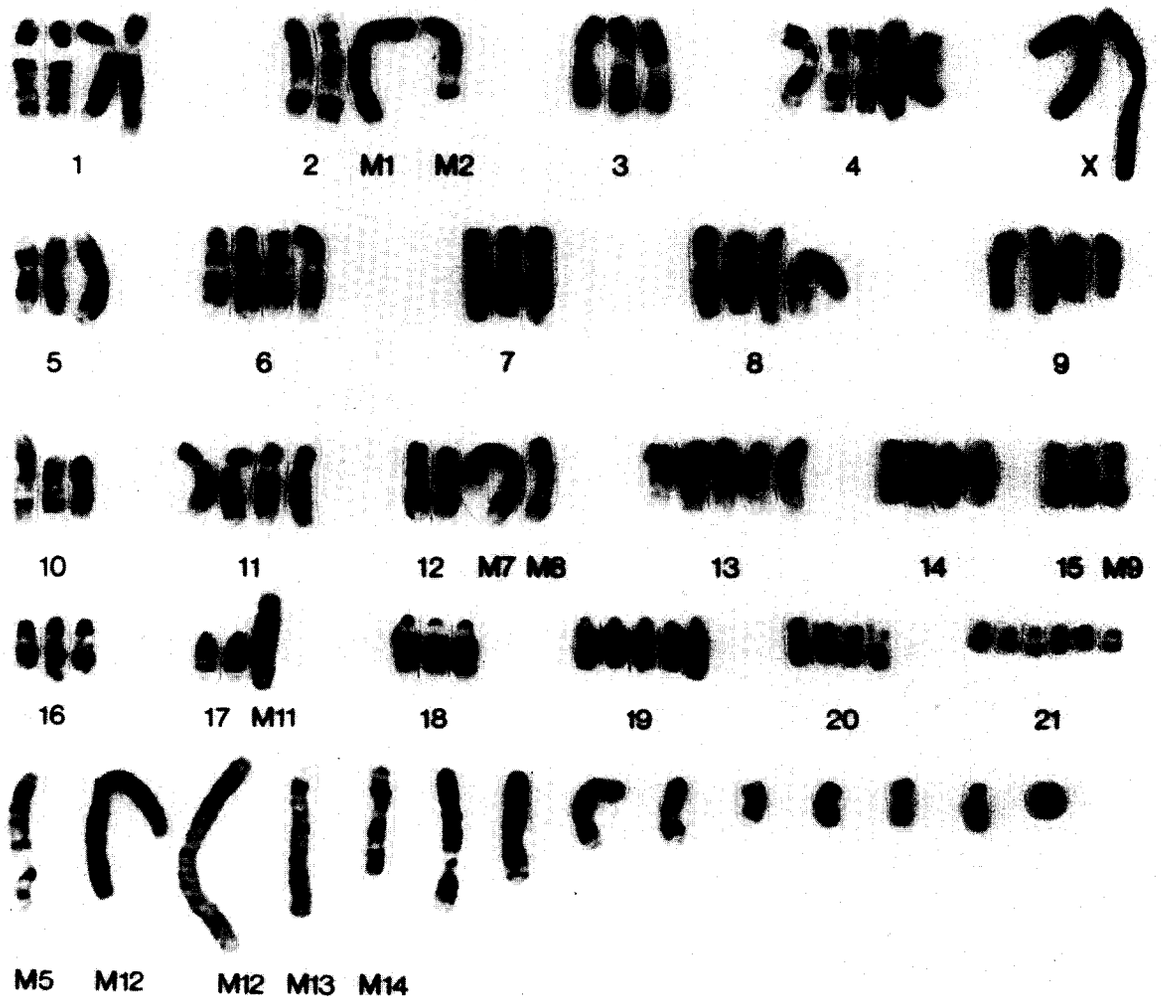

Fig. 3. G banded karyotype of a Met G cell. Note: the presence of normal chromosome 15's and the large number of normal chromosomes.