CMLS, Cell. Mol. Life Sci. 61 (2004) 2125–2131
1420-682X/04/162125-07
DOI 10.1007/s00018-004-4200-2

CMLS Cellular and Molecular Life Sciences

Research Article

Spontaneous fusion between cancer cells and endothelial cells

K. Mortensen [a], J. Lichtenberg [b], P. D. Thomsen [a] and L.-I. Larsson [a,*]

[a] Department of Anatomy and Physiology, KVL, Gronnegaardsvej 7, 1870 Frederiksberg C, Copenhagen (Denmark), Fax: +45 35 28 25 47, e-mail: lail@kvl.dk
[b] Neurosearch, Ballerup (Denmark)

Received 10 May 2004; received after revision 21 June 2004; accepted 2 July 2004

Abstract. Endothelial cells line the inside of blood and lymphatic vessels, and cancer cells must cross this barrier, first to gain access to the circulation, and, second, to exit and metastasize. How this occurs is incompletely understood. We now demonstrate that human cancer cells are able to fuse with endothelial cells to form hybrid cells displaying proteins and chromosomal markers characteristic of both parent cells. The hybrid cells are viable and capable of undergoing mitosis. Fusions between cancer cells and endothelial cells were shown to occur both in vitro, in co-cultures of human breast cancer cells and endothelial cells, and in vivo, following intravascular dissemination of human breast cancer cells in nude mice. These observations demonstrate a new type of cancer-endothelial cell interaction that may be of fundamental importance to the process of metastasis.

Key words. Cancer; endothelial cell; fusion; FISH; metastasis.

Most deaths from cancer are caused by the spread of cancer cells to vital organs through the blood or the lymph [1]. Endothelial cells line the inside of blood and lymphatic vessels, and cancer cells must penetrate this layer in order to gain access to the circulation. Once in the circulation, cancer cells must again traverse the endothelial barrier in order to extravasate and form metastases. A number of factors that are important to cancer cell intra- and extravasation have been detected, but complete understanding of these mechanisms has not yet been achieved [reviewed in ref. 2]. One essential factor to cancer cell intravasation is tumor vascularization, which is important not only to tumor cell growth [3] but also to tumor cell dissemination [4]. Interestingly, recent studies have demonstrated that cancer cells may partly replace ordinary endothelial cells in the lining of intratumoral blood vessels [5, 6].

Our own research in cancer-endothelial cell interactions was stimulated by the observation that high levels of endothelial cell nitric oxide (NO) synthase in peritumoral microvessels was associated with significantly improved overall survival and disease-free survival in breast cancer patients [7]. NO has been shown to regulate apoptosis of human breast cancer cells [8] and to reduce tumor cell adhesion to endothelial cells [9]. Moreover, inhibition of NO generation in liver sinusoids has been found to result in an increased number of liver metastases in an animal model [10]. To follow up on these observations we decided to study interactions between co-cultured endothelial cells and breast cancer cells. In such co-cultures we observed that cells simultaneously expressing markers for both endothelial cells and cancer cells appeared, while no such cells were detected in single cultures. Using genetic and protein markers, we now document that spontaneous fusion between cancer cells and endothelial cells occurs in vitro and in vivo. The hybrid cells formed are viable and capable of undergoing mitosis. This is the first observation of a novel type of tumor-endothelial cell interaction through which fused cancer cells may gain characteristics that enhance their capability to penetrate the endothelial barrier.

* Corresponding author.

Materials and methods

Cell culture

MCF-7 and MDA-MB-231 cells were cultured in Eagle's minimum essential medium (MEM) as described elsewhere [8]; human umbilical vein endothelial cells (HUVECs; ATCC) were cultured in F-12 K medium (Kaighn's modification; Gibco, Grand Island, N. Y.) supplemented with 10% FCS and 0.4% endothelial cell growth supplement/heparin (ECGS/H-2; PromoCell, Heidelberg, Germany); calf pulmonary artery endothelial (CPAE) and bovine adrenal medulla endothelial (EJG) cells were cultured in Eagle's MEM supplemented with non-essential amino acids, sodium pyruvate and 10% (EJG) or 20% (CPAE) FCS. Cells were harvested by trypsinization and permitted to recover for 1 h in medium before mixing or plating. For co-cultures, equal numbers of cancer and endothelial cells were mixed. In addition, endothelial cells were grown to near confluence, where-after breast cancer cells were seeded on top of them. Cells were grown on sterile glass slides or on chamber slides (Nalge Nunc, Naperville, Ill.) and were fixed in 3.7% paraformaldehyde in phosphate-buffered saline and permeabilized in 0.1% triton X-100 for immunocytochemistry or in methanol-acetic acid for fluorescence in situ hybridization (FISH). When appropriate, metaphase arrest was induced by adding colcemide to a final concentration of 0.1 µg/ml medium for 4 h prior to fixation.

Animals

Five 5 to 6-week old nude Balb/c mice weighing approximately 25 g were obtained from the M&B Breeding Centre (Ejby, Denmark). The mice were housed with a 12-h light-dark cycle. Room temperature and relative humidity showed values between 22–23.5ºC and 45–63%, respectively. The animals were fed a pelleted rodent diet ad libitum and had free access to tap water, autoclaved hay and wood biting sticks. The mice were injected intravenously in the tail vein with 7.5–15 million MDA-MB-231 cells in a volume of 0.2 ml and were sacrificed by cervical dislocation 24 or 48 h after injection. Specimens from the lungs were frozen in crushed carbon dioxide and stored at –80ºC.

Immunocytochemistry

Cultured cells were immunocytochemically double-stained with monoclonal antibodies reacting with vimentin (3B4, IgG2a) or cytokeratin (pancytokeratin, IgG1; cytokeratin-8, IgM) or with rabbit antibodies to the endothelial marker von Willebrand factor (vWF; Dakocytomation, Glostrup, Denmark). The site of antigen-antibody interaction was detected with species- or subclass-specific secondary antibodies labeled with fluorescein isothiocyanate (FITC), aminomethyl coumarin (AMCA) or Texas red (Southern Biotechnology Associates, Birmingham, Ala.). Specimens were examined in a Molecular Dynamics (Eugene, Ore.) multiprobe confocal laser scanning microscope or in a Leica (Wetzlar, Germany) DMRXA microscope. Nuclear morphology was assessed by staining with bisbenzimide (Hoechst 33258; Sigma, St. Louis, Mo.). In counting experiments, at least 500 cells were counted in random fields of three to four specimens using selective filters. Controls included use of conventional staining controls [11]; type-matched monoclonal antibodies and staining of parental cell lines cultured alone and were uniformly negative.

Fluorescent in situ hybridization

The mouse-specific probe was obtained by labeling genomic DNA isolated from liver tissue of B6D2F1 mice with digoxigenin (Dig) or biotin using nick translation. The bovine-specific probe was a Dig-labeled cosmid clone JAB 8 (cJAB8) that produces a strong signal at the subcentromeric region of bovine chromosome 7 [12]. The probe detecting all human chromosomes was an Alu sequence (BLUR8) probe [13]. DNA from pUC3Alu (containing three copies of BLUR8) was labeled with biotin or Dig, and DNA from cJAB8 was labeled with Dig using nick translation. Additionally, a biotin-labeled probe detecting the alpha satellite sequence of human chromosome 17 was purchased from Oncor (Gaithersburg, Md.). Cells and sections were fixed in methanol-acetic acid, denatured in 70% formamide at 71 °C for at least 2 min and hybridized with different mixtures of Dig- and biotin-labeled probes as described elsewhere [12, 14]. Hybridization sites of biotinylated probes were visualized by a three-layer method using, in sequence, Cy3-labeled streptavidin, biotinylated anti-avidin D and, finally, Cy3-labeled streptavidin again (Vector Laboratories, Burlingame, Calif.). Dig-labeled probes were detected by a three-layer method using, in sequence, FITC-labeled anti-Dig, Dig-labeled anti-mouse Ig and, finally, FITC-labeled anti-Dig again (Boehringer Mannheim, Germany). Specimens were counterstained with DAPI and examined in a Molecular Dynamics multiprobe confocal laser-scanning microscope or in a DMRXA microscope equipped with a Cohu CCD camera and Q-FISH software (Leica). Controls consisted of hybridizations to parental cells cultured alone.

Results

Fusions between cancer cells and endothelial cells in vitro

We co-cultured equal numbers of human breast cancer (MCF-7) cells and HUVECs. Double immunofluorescence for cell-type-specific intermediate filaments (cy-

tokeratin and vimentin) showed that expression of cytokeratin never occurred in HUVECs, cultured alone, and that expression of vimentin never occurred in MCF-7 cells, cultured alone. However, in co-cultures, 3.1 ± 0.4% of all cytokeratin-positive cells were also vimentin positive and 3.7 ± 0.4% of all vimentin-positive cells were also cytokeratin positive (fig. 1a). Hybrid cells were detected irrespective of whether the cells were co-cultured in HUVEC or MCF-7 cell medium. Plating of MCF-7 cells on top of preformed HUVEC monolayers also resulted in the formation of hybrid cells in a time-dependent fashion. Thus, hybrid cells were not detected by 30 min after plating but were regularly observed by 2 h after plating and reached a frequency of 3–5% by 24 h. In addition, co-cultures of MCF-7 and bovine endothelial (CPAE) cells resulted in hybrid cells in similar proportions. Triple staining for cytokeratin, vimentin and DNA showed that most hybrid cells were binucleate and that the cytokeratin and vimentin filaments were closely interwoven in the hybrids. Additionally, some hybrid cells contained only one nucleus (fig. 1b), while a few contained three nuclei. The vast majority of hybrid cells appeared healthy and showed no signs of apoptosis or nuclear condensation. Co-cultures of MCF-7 cells and another bovine endothelial cell line (EJG) revealed only a very few fused cells (far below 0.1%). These data show that cancer cells and endothelial cells are able to fuse to form hybrid cells and that some endothelial cell types are more prone to fusion than others.

These results were further corroborated by the use of chromosomal markers. We performed FISH of co-cultures of human breast cancer (either MCF-7 or MDA-MB-231) cells and bovine CPAE cells using centromeric probes specific for human chromosome 17 (hC17) and bovine chromosome 7 (bC7), labeled with biotin and Dig, respectively. This permitted detection of hC17 and bC7 in contrasting colors. While the bovine cells each possessed two copies of bC7, MCF-7 cells had four and MDA-MB-231 cells had two to four copies of hC17. Fused cells were frequently detected at the interface between clusters of cancer and endothelial cells (fig. 1c). Binuclear cells contained one nucleus positive for two copies of bC7 and one nucleus positive for two to four (MDA-MB-231) or four (MCF-7) copies of hC17. Additionally, cells with fused nuclei containing two copies of bC7 and four copies (MCF-7) or two to four copies (MDA-MB-231) of hC17 were regularly detected (fig. 1c). Some of the fused cells underwent mitotic division and mitotic figures containing both bovine and human chromosomes were regularly observed in colcemide-arrested cultures (fig. 2g).

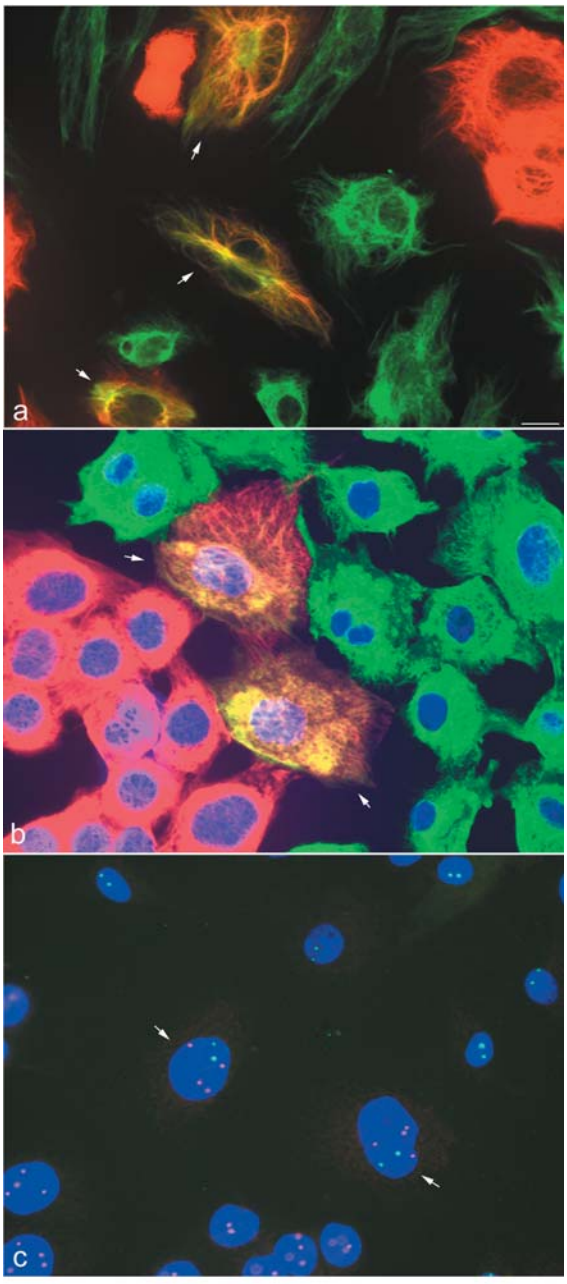

Figure 1. Spontaneous heterotypic cell fusion in vitro. (*a*) Co-culture of MCF-7 cells and HUVECS, double stained for vimentin (green) and cytokeratin (red). Note three fused cells (arrows), staining for both vimentin and cytokeratin (orange-yellow), while the remaining cells stain either for vimentin or cytokeratin. Scale bar, 20 µm. (*b*) Co-culture of MDA-MB-231 cells and CPAE cells, triple stained for vimentin (green), cytokeratin (red) and DNA (blue). Note a pair of fused cells (arrows) that stain for both vimentin and cytokeratin (orange-yellow), while the remaining cells stain either for vimentin or cytokeratin. (*c*) Co-culture of MDA-MB-231 cells and CPAE cells submitted to double FISH detecting human chromosome 17 (red) and bovine chromosome 7 (green). DNA is visualized by DAPI staining (blue). Note a pair of fused cells (arrows), each containing four copies of human chromosome 17 and two copies of bovine chromosome 7. Also note non-fused bovine endothelial cells (top right), which contain only bovine chromosomes and non-fused MDA-MB-231 cells (bottom left), which contain only human chromosomes.

Differential nuclear subcompartmentation of parental genomes in fused nuclei

In fused nuclei, the bC7 centromeres occurred clustered together in a nuclear subdomain and were not intermin-

gled with the hC17 centromeres (fig. 1c). We therefore examined whether the bovine and human genomes occupied different subcompartments within the same nucleus and took advantage of the observation that hybridizations with the bC7 probe at lower stringency resulted in detection of all bovine chromosomes, but not of human chromosomes. Thus, double FISH using Dig-labeled bC7 probe at low stringency in combination with a biotin-labeled human Alu sequence (BLUR8) probe demonstrated all bovine and all human chromosomes in contrasting colors. In most fused cells, two separate nuclei, one possessing human and the other possessing bovine chromosomes were detected (fig. 2e). In addition, fused nuclei were regularly observed. In such nuclei, human and bovine chromosomes did not intermingle but occupied distinct subcompartments (fig. 2a–c). A similar nuclear subcompartmentation of parental cell genomes is known to occur in plant cell hybrids as well as in mouse preimplantation embryos and is preserved through several rounds of cell division [15, 16]. We also observed metaphase figures containing bovine and human chromosomes (fig. 2d) as well as occasional trinuclear cells, usually containing two human nuclei and one bovine nucleus. Fusion of cells is likely to initially result in binuclear (and, more rarely, trinuclear) cells and mononuclear cells probably appear after mitotic division of bi- and, possibly, trinuclear cells. This notion is supported by the observation that mononuclear fused cells frequently occurred as pairs (figs 1b, c, 2a).

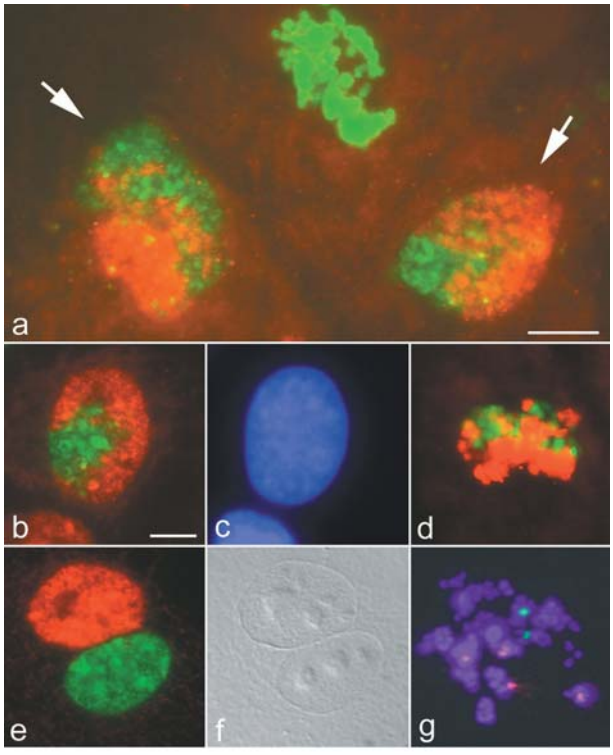

Figure 2. Spontaneous heterotypic cell fusion in vitro. (*a*) Co-culture of MDA-MB-231 and CPAE cells submitted to double FISH using probes and conditions permitting detection of all human chromosomes (red) and all bovine chromosomes (green). Note two nuclei containing human and bovine chromosomes in different subdomains (arrows). Also note the presence of a mitosis in a bovine endothelial cell (top). (*b*, *c*) Fused cell from co-culture of MDA-MB-231 and CPAE cells, submitted to double FISH as in *a* and counterstained for DNA (blue; *c*). Note the compartmentation of human and bovine chromosomes in different subdomains of the same nucleus. (*d*) Mitosis of a fused cell from a co-culture of MDA-MB-231 and CPAE cells stained as in a. Note the admixture of human and bovine chromosomes. (*e*, *f*) Binuclear, fused cell from a co-culture of MDA-MB-231 and CPAE cells stained as in a and observed in fluorescence (*e*) or in differential interference contrast (*f*). Note the presence of one nucleus containing bovine chromosomes (green) and one nucleus containing human chromosomes (red) in the same cell. (*g*) Mitotic figure detected in a colcemide-arrested co-culture of MDA-MB-231 and CPAE cells submitted to double FISH detecting human chromosome 17 (red) and bovine chromosome 7 (green). Note the admixture of both types of chromosomes in this metaphase. (*a*, *b*) Scale bar, 10 µm.

Fusions between cancer cells and endothelial cells in vivo

We next examined whether fusions between cancer cells and host cells occurred in vivo, using a model for lung metastasis of MDA-MB-231 cells in nude mice [17]. The mice were injected with MDA-MB-231 cells in the tail vein and sacrificed after 24 or 48 h. Lung sections were submitted to double FISH using probes that demonstrated mouse and human chromosomes in contrasting colors. Control hybridizations to human and mouse cells documented the species specificity of the probes, and hybridizations to lung sections of injected mice revealed the occurrence of cells with fused nuclei that contained both human and mouse DNA (fig. 3a–f). The presence of fused nuclei was further verified by optical sectioning in the confocal microscope (fig. 3a, d–f). As in the bovine/human hybrid cells described above, the two parental genomes occupied distinct subcompartments within the same nucleus in the mouse/human hybrid cells (fig. 3). Fused nuclei constituted 0.5–2% of all tumor cell nuclei detected in the lungs with no marked difference between animals sacrificed 24 or 48 h after injection. Hybridized sections were next stained for the endothelial cell marker vWf using indirect immunofluorescence. A few cells containing fused human/ mouse nuclei stained for this endothelial marker (fig. 3b, c), which, in addition, detected cells that contained one nucleus positive for human and one nucleus positive for mouse chromosomes. These data show that a population of intravascularly injected human breast cancer cells fuse with host cells to form hybrid cells containing both human and mouse chromosomes and expressing the endothelial marker vWf.

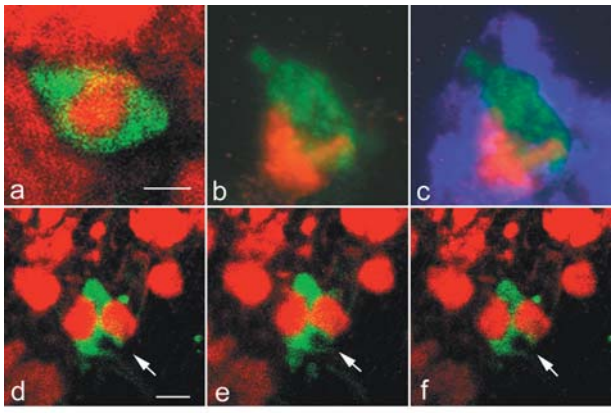

Figure 3. Spontaneous heterotypic cell fusion in vivo. (*a*) Confocal image of lung section from a nude mouse injected with MDA-MB-231 cells submitted to double FISH detecting all mouse chromosomes (red) and all human chromosomes (green). Note a fused cell with mouse and human chromosomes occupying different subdomains of the same nucleus. (*b*, *c*) Lung section from a nude mouse injected with MDA-MB-231 cells submitted to double FISH detecting mouse (red) and human (green) chromosomes and subsequently restained for vWf (blue; *b*: red-green double exposure; *c*: triple exposure). Note a cell containing a fused nucleus with a vWf-positive cytoplasm. (*d–f*) Three confocal sections at different levels through a fused nucleus (arrow), containing human (green) and mouse (red) chromosomes in distinct subdomains. (*a*, *d*) Scale bar, 10 µm.

Discussion

Life begins with the fusion between a sperm cell and an egg and, during ensuing development, more cell fusions occur, including fusions of cytotrophoblasts to form syncytiotrophoblasts, of myoblasts to form muscle fibers and of macrophages to form osteoclasts [reviewed in ref. 18]. In mammals, fusions after fertilization involve the merger of the same type of cells (homotypic fusions).

Cell fusions must be tightly regulated, but the regulatory mechanisms are, as yet, poorly understood. Proteins (fusogens) that reduce the repelling forces between membrane lipid bilayers are needed for cell fusions to occur. Fusogens enhance fusions between viruses and cells [19], between intracellular membranes [20] and between cells [21]. The barriers preventing cell fusion can be broken by addition of extraneous agents like Sendai virus or polyethylene glycol. By this approach, heterotypic cell fusions can be produced, resulting, e. g. in monoclonal-antibody-secreting hybridomas [22]. In this case, fusions between normal antibody-producing spleen cells and malignant myeloma cells result in cells that retain the unrestrained proliferation of the myeloma partner and the antibody production of the spleen cell partner. Additionally, fusions induced to occur between macrophages and cancer cells have been found to increase the metastatic capability of the cells [23–25]. In contrast, fusions induced to occur between cancer cells and fibroblasts ablate the malignant phenotype due to the production of tumor suppressors by the fibroblast genome [26, 27]. Thus, depending upon the genetic make-up of the fusion partners, different outcomes will ensue from fusions between malignant and normal cells.

Since 1911, the possibility has been considered that cancer cells may spontaneously fuse with host cells to produce hybrids with supernumerary chromosomes that could evolve into cells more malignant than the original cancer cells [28–30]. Indeed, studies have documented that cells expressing a mixed phenotype appear in co-cultures of certain types of malignant and normal cells [31, 32]. In addition, transplanted tumor cells of animal [33–36] or human [37] origin may acquire phenotypic characteristics of normal host cells. In some of these studies, data suggested that the putative fusion partner was of macrophage origin while, in most cases, definite identification was not achieved. Importantly, however, recent data have suggested that the interpretation of some of these data may be more complicated than originally believed. Thus, uptake of foreign DNA through phagocytosis of apoptotic bodies may lead to acquisition of mixed cell traits [38–40].

Our present data provide definite evidence that human breast cancer cells spontaneously fuse with endothelial cells resulting in viable and actively dividing hybrid cells that contain chromosomes and protein markers characterizing both fusion partners. Most fusions produced bi- or trinuclear cells with the parental genomes segregated into normal, non-apoptotic nuclei. No evidence of apoptotic bodies was detected. Mononuclear cells are likely to form subsequent to mitosis of the bi- or trinuclear cells. Even within mononuclear hybrid cells, the parental genomes were segregated into different subcompartments in a pattern identical to that observed in plant cell hybrids and in mouse embryos [15, 16]. In the in vivo studies, a population of bi- and mononuclear hybrid cells was shown to express the endothelial cell marker vWf. Whether vWf-negative hybrid cells represent hybrids formed between cancer cells and other host cells or if some hybrid cells cease producing vWf following fusion remains unknown. However, since expression of vWf is attenuated and eventually lost during serial passaging of endothelial cells, the latter explanation seems plausible. Importantly, through fusions with endothelial cells, cancer cells may temporarily or permanently acquire endothelial cell characteristics that enhance their capability to traverse the endothelial barrier and metastasize. Such characteristics include the production of endothelial cell markers as well as of proteases that are capable of degrading the vascular basement membrane [cf. ref. 41]. The fusions observed seem likely to reflect ectopic expression of fusogens by cancer cells. However, the observation that certain endothelial cell types (HUVECs, CPAE) are more prone to fusion than others (EJG) suggests that some complementarity between cancer cell fusogens and endothelial cell surface molecules must underlie this specificity.

Acknowledgements. Grant support was from the Danish MRC and Cancer Foundation. We thank H. Ruby, G. Mikkelsen, A. F. Petersen, Z. Rasmussen and Ö. B. Özdas for expert technical support.

1 Sporn M. B. (1996) The war on cancer. Lancet **347:** 1377–1381
2 Quigley J. P. and Armstrong P. B. (1998) Tumor cell intravasation alu-cidated: the chick embryo opens the window. Cell **94:** 281–284
3 Hanahan D. and Folkman J. (1996) Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell **86:** 353–364
4 Holmgren L., O'Reilly M. S. and Folkman J. (1995) Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. Nat. Med. **1:** 149–153
5 Chang Y. S., Tomaso E. di, McDonald D. M., Jones R., Jain R. K. and Munn L. L. (2000) Mosaic blood vessels in tumors: frequency of cancer cells in contact with flowing blood. Proc. Natl. Acad. Sci. **97:** 14608–14613
6 Maniotis A. J., Folberg R., Hess A., Seftor E. A., Gardner L. M., Pe'er J. et al. (1999) Vascular channel formation by human melanoma cells in vivo and in vitro: vasculogenic mimicry. Am. J. Pathol. **155:** 739–752
7 Mortensen K., Holck S., Christensen I. J., Skouv J., Hougaard D. M., Blom J. et al. (1999) Endothelial cell nitric oxide synthase expression in peritumoral microvessels is a favourable prognostic indicator in breast cancer. Clin. Cancer Res. **5:** 1093–1097
8 Mortensen K., Skouv J., Hougaard D. M. and Larsson L.-I. (1999) Endogenous endothelial cell nitric oxide synthase modulates apoptosis in cultured breast cancer cells and is transcriptionally regulated by p53. J. Biol. Chem. **274:** 37679–37684
9 Kong L., Dunn G. D., Keefer L. K. and Korthuis R. J. (1996) Nitric oxide reduces tumor cell adhesion to isolated rat postcapillary venules. Clin. Exp. Metastasis **14:** 335–343
10 Wang H. H., McIntosh A. R., Hasinoff B. B., Rector E. S., Ahmed N., Nance D. M. et al. (2000) B16 melanoma cell arrest in the mouse liver induces nitric oxide release and sinusoidal cytotoxicity: a natural hepatic defense against metastasis. Cancer Res. **60:** 5862–5869
11 Larsson L.-I. (1988) Immunocytochemistry, Theory and Practice, CRC, Boca Raton
12 Viuff D., Rickords L., Offenberg H., Hyttel P., Avery B., Greve T. et al. (1999) A high proportion of bovine blastocysts produced in vitro are mixoploid. Biol. Reprod. **60:** 1273–1278
13 Deininger P. L., Jolly D. J., Rubin C. M., Friedmann T. and Schmid C. W. (1981) Base sequence studies of 300 nucleotide renatured repeated human DNA clones. J. Mol. Biol. **151:** 17–33
14 Thomsen P. D., Høyheim B. and Christensen K. (1996) Recent fusion events during evolution of pig chromosomes 3 and 6 identified by comparison with the babirusa karyotype. Cytogenet. Cell. Genet. **73:** 203–208
15 Heslop-Harrison J. S. and Flavell R. B. (1993) The Chromosome, pp. 223–224, Bios, Oxford
16 Mayer W., Smith A., Fundele R. and Haaf T. (2000) Spatial separation of parental genomes in preimplantation mouse embryos. J. Cell Biol. **148:** 629–634
17 Price J. E., Polyzos A., Zhang R. D. and Daniels L. M. (1990) Tumorigenicity and metastasis of human breast carcinoma cell lines in nude mice. Cancer Res. **50:** 717–721
18 Witze E. and Rothman J. H. (2002) Cell fusion: an EFFicient sculptor. Curr. Biol. **12:** R467–R469
19 Stegman T. (2001) Membrane fusion mechanisms: the influenza hemagglutinin paradigm and its implications for intracellular fusion. Traffic **1:** 598–604
20 Rothman J. E. (2002) The machinery and principles of vesicle transport in the cell. Nat. Med. **8:** 1059–1062
21 Mohler W. A., Shemer G., Campo J. J. del, Valansi C., Opoku-Serebuoh E., Scranton V. et al. (2002) The type I membrane protein EFF-1 is essential for developmental cell fusion. Dev. Cell **2:** 355–362
22 Kohler G. and Milstein C. (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. Nature **256:** 495–497
23 Chakraborty A. K., Pawelek J., Ikeda Y., Miyoshi E., Kolesnikova N., Funasaka Y. et al. (2001) Fusion hybrids with macrophage and melanoma cells upregulate N-acetylglucosaminyltransferase V, beta1–6 branching and metastasis. Cell Growth Differ. **12:** 623–630
24 Larizza L., Schirrmacher V. and Pfluger E. (1984) Acquisition of high metastatic capacity after in vitro fusion of a non-metastatic tumor cell line with a bone marrow-derived macrophage. J. Exp. Med. **160:** 1579–1584
25 Rachkovsky M., Sodi S., Chakraborty A. K., Avissar Y., Bolognia J. L., Madison-McNiff J. et al. (1998) Melanoma × macrophage hybrids with enhanced metastatic potential. Clin. Exp. Metastasis **16:** 299–312
26 Weinberg R. A. (1991) Tumor suppressor genes. Science **254:** 1138–1146
27 Harris H. (1988) The analysis of malignancy by cell fusion: the position in 1988. Cancer Res. **48:** 3302–3306
28 Aichel O. (1911) Über Zellverschmelzung mit qualitative abnormer chromosomenverteilung. In: Vorträge und Aufsätze über Entwicklungsmechanik der Organismen, pp. 1–115, Roux W. (ed.), Engelmann, Leipzig
29 Munzarova M., Lauerova L. and Capkova J. (1992) Are advanced malignant melanoma cells hybrids between melanocytes and macrophages? Melanoma Res. **2:** 127–129
30 Duelli D. and Lazebnik Y. (2003) Cell fusion a hidden enemy? Cancer Cell **3:** 445–448
31 Wakeling W. F., Greetham J. and Bennett D. C. (1994) Efficient spontaneous fusion between some co-cultured cells, especially murine melanoma cells. Cell Biol. Int. **18:** 207–210
32 Busund L. T. R., Killie M. K., Bartnes K., Olsen R. and Seljelid R. (2002) Spontaneous hybridization of macrophages and Meth A sarcoma cells. Int. J. Cancer **98:** 573–581
33 Busund L. T. R., Killie M. K., Bartnes K. and Seljelid R. (2003) Spontaneously formed tumorigenic hybrids of Meth A sarcoma cells and macrophages in vivo. Int. J. Cancer **106:** 153–159
34 Chakraborty A. K., Sodi S., Rachkovsky M., Kolesnikova N., Platt J. T., Bolognia J. L. et al. (2000) A spontaneous murine melanoma lung metastasis comprised of host × tumor hybrids. Cancer Res. **60:** 2512–2519
35 Kerbel R. S., Lagarde A. E., Dennis J. W. and Donaghue T. P. (1983) Spontaneous fusion between normal host and tumor cells: possible contribution to tumor progression and metastasis studied with a lectin-resistant mutant tumor. Mol. Cell. Biol. **3:** 523–538
36 Larizza L., Schirrmacher V., Graf L., Pfluger E., Peres-Martinez M. and Stohr M. (1984) Suggestive evidence that the highly metastatic variant ESb of the T-cell lymphoma Eb is derived from spontaneous fusion with a host macrophage. Int. J. Cancer **34:** 699–702
37 Goldenberg D. M., Pavia R. A. and Tsao M. C. (1974) In vivo hybridisation of human tumour and normal hamster cells. Nature **250:** 649–651
38 Holmgren L., Szeles A., Rajnavolgyi E., Folkman J., Klein G., Ernberg I. et al. (1999) Horizontal transfer of DNA by the uptake of apoptotic bodies. Blood **93:** 3956–3963
39 Taille A. de la, Chen M.-W., Burchardt M., Chopin D. K. and Buttyan R. (1999) Apoptotic conversion: evidence for exchange of genetic information between prostate cancer cells mediated by apoptosis. Cancer Res. **59:** 5461–5463

40 Bergsmedh A., Szeles A., Henriksson M., Bratt A., Folkman M. J., Spetz A.-L. et al. (2001) Horizontal transfer of oncogenes by uptake of apoptotic bodies. Proc. Natl. Acad. Sci. USA **98:** 6407–6411

41 Yan L., Moses M. A., Huang S. and Ingber D. E. (2000) Adhesion-dependent control of matrix metalloproteinase-2 activation in human capillary endothelial cells. J. Cell Sci. **113:** 3979–3987