OPINION

Fusion of tumour cells with bone marrow-derived cells: a unifying explanation for metastasis

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Abstract | The causes of metastasis remain elusive despite vast information on cancer cells. We posit that cancer cell fusion with macrophages or other migratory bone marrow-derived cells (BMDCs) provides an explanation. BMDC–tumour hybrids have been detected in numerous animal models and recently in human cancer. Molecular studies indicate that gene expression in such hybrids reflects a metastatic phenotype. Should BMDC–tumour fusion be found to underlie invasion and metastasis in human cancer, new approaches for therapy would surely follow.

In his 1911 article "About cell fusion with qualitatively abnormal chromosome distribution as cause for tumour formation" Aichel first proposed the fusion theory of tumour progression and exhorted future scientists to "study chromosomes from all angles" to investigate it further[1]. He proposed that the source of aneuploidy could be fusion of tumour-invading leukocytes with cancer cells, suggesting that a combination of extra chromosomes and the "qualitative differences" in chromosomes from the two cell types could lead to the metastatic phenotype (reviewed in REFS 2–4). Decades later, the same hypothesis — that metastasis is caused by leukocyte–tumour cell fusion — was proposed independently by Meckler[5,6] and by Goldenberg[7,8]. Several laboratories have now reported that hybrids produced by fusion *in vitro* or *in vivo* were aneuploid and of higher metastatic potential (reviewed in REF. 2–4). In 1984, LaGarde and Kerbel summarized the emerging concepts[9]: "[Tumour cell hybridization] can lead to major changes in gene expression. These processes can lead to the evolution of subpopulations of tumour cells having major losses or gains in their malignant aggressiveness and therefore represents a large-scale genetic mechanism capable of generating genotypic and phenotypic diversification. If the normal host cell happens to be a lymphoreticular-haematopoietic cell, it could donate this phenotype to cell types which otherwise do not normally express metastatic traits." There is now considerable evidence to support these concepts.

The pathways of invasion and metastasis have been under intense scientific scrutiny and much is now known about the steps involved[10,11]. However, the actual genesis of metastatic cells from within populations of non-metastatic cells of the primary tumour is not understood. What are the initiating mechanisms that cause a carcinoma or melanoma cell in the epithelium to free its adhesions to neighboring cells, adapt a migratory phenotype, cross the basal lamina into the dermis, intravasate into the blood circulatory system or lymphatics, extravasate, and form new tumours in lymph nodes and distant tissues or organs? The long-standing view is essentially Darwinian: the unstable cancer genome combined with host selective pressures generates metastatic cells in the otherwise non-metastatic primary tumour[12,13]. This view continues to provide the best framework for envisioning tumour progression. Yet it is difficult to imagine how this might occur through successive, stepwise mutations as generation of a metastatic phenotype would require activation and silencing of large numbers of genes in the primary tumour cell[10]. One solution to this problem lies in the activation of master regulatory genes that control multiple pathways and initiate pro-metastatic cascades[14]. This has been highlighted in reports that master regulators of epithelial–mesenchymal transition (EMT) in development, such as SNAIL (also known as SNAI1), SLUG (also known as SNAI2), secreted protein, acidic, cysteine-rich (SPARC) and TWIST (also known as TWIST1), have analogous roles in invasion and metastasis, in which they activate mesoderm-associated pathways of cellular adhesion and migration[10,14]. For example, in breast cancer TWIST activates microRNA-10b, which in turn causes increased expression of the pro-metastatic gene *RHOC*, with increased metastatic potential of the affected cells[14]. However, the mechanisms through which master regulators such as TWIST are themselves upregulated in cancer are not understood. We propose that at least in some cases this could be initiated by fusion of cancer cells with bone marrow-derived cells (BMDCs). Although a transition from epithelial to mesodermal gene expression is indeed a characteristic of invasion and metastasis, the expressed genes are often remarkably similar to those associated with migratory BMDCs, such as macrophages and other myeloid-lineage cells[3,4,15]. Fusion of migratory BMDCs and cancer cells with co-expression of both fusion partner genomes provides a potential explanation for this phenomenon[2–4,16].

In our opinion the fusion theory comes closer to a unifying explanation of tumour progression than any yet proposed. Fusion represents a non-mutational mechanism that could explain the aberrant gene expression patterns associated with malignant cells. Studies of macrophage–tumour cell fusions have demonstrated that genes from both parental partners are expressed in hybrid cells[17]. Gene expression in such cells reflects combinations of myeloid lineage genes along with those of the cancer cell lineage, all in a background of deregulated cell division. In fact, many molecules and traits associated with tumour progression are expressed by

healthy myeloid lineage cells, for example, angiogenesis, motility, chemotaxis and tropism, immune signalling, matrix degradation and remodelling, responses to hypoxia, and multi-drug resistance to chemotherapy[3,4]. Tumour fusion could also account for aneuploidy and genetic rearrangements in metastatic cells[2,18]. It is further possible that tumour–BMDC fusions are a source of cancer stem cells[19]. From studies in animal and human cancers there is little doubt that tumour hybrids are generated *in vivo* and that at least in animals they can be a source of metastases[2–4]. This Perspective reviews the molecular and cellular pathways that are activated following fusion of tumour cells with BMDCs, their expression in macrophages and other BMDCs, and their similarities to those governing tumour progression in animal and human cancer.

Cell fusion mechanisms

Cell fusion is a widespread phenomenon in biology[20]. The pathways vary between different cell types, suggesting that they have evolved separately in different systems[21–23]. However, there are many mechanistic similarities[24] and it was recently shown that myoblasts and macrophages use some of the same molecular components in fusion[25]. Fusion might occur following phagocytosis of cancer cells or apoptotic bodies by tumour-associated macrophages or other phagocytes[2]. Horizontal transfer of oncogenes during phagocytosis of cancer cells *in vitro* was demonstrated[26]. Cancer cell fusion can be induced by viruses[18,27]. Endometrial and breast cancers fuse by means of the protein syncytin (encoded by *ERVWE1*)[28]. Chronic activation of protein kinase AKT2 leads to multinucleation and cell fusion in human epithelial kidney cells[29]. Cell–cell invasion mechanisms of 'cellocytosis'[23] or 'entosis'[30] may also initiate fusion. A general requirement is that the two fusing membranes be in close contact. This is accomplished by receptor–ligand interactions, as seen in virus–cell fusions[18,27] and in macrophage–macrophage fusions in the formation of osteoclasts and giant cells[22,31]. Regarding macrophages, several genes are involved in fusion[32]. For osteoclast formation, three receptor systems involved in fusion are macrophage fusion receptor (MFR, also known as signal-regulatory protein α (SIRPA)), CD44 and dendritic cell-specific transmembrane protein (DC-STAMP, also known as TM7SF4)[22]. MFR and its ligand CD47 (thrombospondin 2 receptor) belong to the immunoglobulin superfamily[33]. MFR is expressed by myeloid cells and neurons whereas CD47 is expressed in many cell types. CD44, for which the fusion ligand is unknown, is also transiently expressed in an early stage in fusion. The extracellular domain of CD44 is cleaved by membrane type I matrix metalloproteinases, possibly bringing plasma membranes closer as a prelude to fusion[23,34]. DC-STAMP is a chemokine-like receptor that is essential for macrophage fusion to form osteoclasts and giant cells[31]. Although the DC-STAMP ligand is as yet undetermined, a candidate is the cytokine CCL2 (also known as monocyte chemoattractant protein 1 (MCP1)), which is an important component of osteoclast and giant cell formation[35,36].

Macrophages may thus fuse with cancer cells through their inherent fusion capabilities. Likewise, cancer cells may be prone to fusion because of aberrant expression of fusion-associated receptors or ligands. For example, CD44 is widely expressed in cancer, in which it is a cell surface receptor for hyaluronan and associated with poor outcome[37,38]. It is also a marker for putative solid tissue cancer stem cells in several different neoplasms (for an example see REF. 39). CD47 and CCL2 (and CCL2 receptors) are each expressed by many different cancers[40–42]. Close apposition of plasma cell membranes between macrophages and melanoma cells is readily observed in tumour biopsies, fulfilling one of the requirements for fusion[43].

Cancer cell fusion *in vivo*

Cancer cells fuse with many cell types *in vivo*, including stromal cells[44], epithelial cells[45] and endothelial cells[46–48]. There are more than 30 reports of tumour cell fusion with host cells and many of these implicate macrophages or other BMDCs as host fusion partners[2–4,45,49–53]. For example, when the MDAY or A9 mouse sarcomas were implanted in mice with allogeneic bone marrow transplants, hybrids between BMDCs and tumour cells were generated[51,54]. Another example was seen in the development of a spontaneous melanoma metastasis to the lungs in a Balb/c nude mouse[52] (FIG. 1). Balb/c mice are albino owing to a homozygous mutation in tyrosinase (*c*/*c*), the rate-limiting enzyme in melanogenesis. Although the melanoma clone implanted into these mice was genetically wild-type for tyrosinase (*C*/*C*), the cells produced little or no melanin in culture and formed amelanotic tumours in mice. Metastases, though infrequent, were generally small, amelanotic tumours in the lung, and were well tolerated by the mice[53]. However, in one experiment a mouse developed a melanin-producing *in transit* metastasis near the site of implantation in the tail dermis (FIG. 1a). Because of this the tail was amputated and the mouse was followed to see if distant metastases developed. After 5 weeks the mouse became moribund with a massive, highly pigmented pulmonary metastasis (FIG. 1c). DNA analyses showed that cells from the metastasis had a genotype of *C*/*c*, indicating they were hybrids formed from fusion of the implanted tumour cells (*C*/*C*) with host cells (*c*/*c*). Cells from the metastasis showed an average 30–40% increase in DNA content, increased chemotaxis *in vitro*, activation of *N*-acetylglucosaminyltransferase V (GnT-V, MGAT5, E.C.2.4.1.155), and production of β1,6-branched oligosaccharides (see below). They also produced 'coarse melanin' — autophagosomes containing melanosomes and other organelles (below). Small numbers of highly melanized, coarse melanin-producing cells were found within the original implanted tumour (FIG. 1b). These were not present in the cultured parental melanoma cells and were thus generated *in vivo*[52]. Morphologically identical cells were cultured from the metastasis and determined to be *C*/*c* hybrids with host cells, indicating that fusion and hybridization had occurred in the original implant. Histopathology studies of the original implant revealed that it was infiltrated with macrophages, supporting the possibility that macrophage–tumour fusion had occurred there.

BMDCs in human cancer and stem cell-like distribution patterns. The first and, as yet, sole confirmation of BMDC–tumour cell fusion in humans has been reported. Transcriptionally active malignant nuclei and normal nuclei were observed in tumour-associated osteoclasts from myeloma patients. In the osteoclast population, 30% of the nuclei were of malignant-cell origin, indicating a remarkably high incidence of osteoclast–tumour cell fusion[55]. The potential relevance of this finding to myeloma pathobiology is not yet known. Other studies have demonstrated the presence of donor genes in carcinoma cells of secondary malignancies arising after allogeneic haematopoietic stem cell (HSC) transplant; however, for largely technical reasons, definitive proof for or against donor–host fusion was lacking in each. In the first reported case, a renal cell carcinoma (RCC) developed in a child following an HSC transplant from his cancer-free brother[56]. A lymph node metastasis of this tumour (the only tissue available) was analysed by laser capture microscopy of tumour cells and PCR-based analyses for donor genes (FIG. 2). Carcinoma cells throughout the tumour contained the donor-specific A allele of the ABO

blood group, indicating that HSCs had in some manner become incorporated into the tumour. The patient history of radiation and immunosuppression before HSC transplant increased the likelihood that the tumour arose *de novo* in the patient and that donor BMDCs became incorporated through fusion with pre-existing tumour cells. However, because a suitable patient-specific DNA sequence was unavailable, evidence for donor and patient genes in the same cells was lacking[56]. Nonetheless, carcinoma cells throughout the tumour produced β1,6-branched oligosaccharides (FIG. 2), a risk factor in several cancers and a characteristic of other BMDC–tumour cell hybrids, as discussed below. In the second case[57], tumour cells from a primary papillary RCC (PRCC) arising after a male-to-female HSC transplant were found to exhibit a trisomy 17, a common abnormality in PRCC and other cancers[58] (FIG. 3). About 1% of the trisomy 17-containing tumour cells also contained the donor Y chromosome in the same nucleus[57]. As above, this combined with the patient history suggested that fusion had occurred between tumour cells and donor HSC cells after development of the tumour[57]. However, the possibility that the tumour was derived solely from a donor HSC, without fusion, followed by growth and widespread loss of the Y was not ruled out[59]. Nonetheless, it is of note that the Y- and trisomy 17-containing carcinoma cells were distributed within the tumour in pairs and clusters resembling post-mitotic daughter cells — a pattern that would be predicted for cancer stem cells[19,60]. Also, Y-containing carcinoma cells were localized to a region covering only about 10% of the tumour, suggesting a clonal emergence of these cells. Supporting this, Y-containing carcinoma cells differed from the majority of carcinoma cells in this tumour through their high expression of β1,6-branched oligosaccharides (FIG. 3). In other reports, Y-containing cancer cells were found in two cases of intestinal adenoma and one case of lung cancer in females who had previously received male HSC transplants[61]. XY fluorescence *in situ* hybridization of a limited number of these cells revealed no evidence of the XXY or XXXY cells that could have supported (but not proven) the presence of BMDC–tumour hybrids. The authors proposed that some BMDCs come to resemble cancer cells through "development mimicry" rather than being "direct seeds of the cancer"[61]. However, in the case above[57], as the donor Y chromosome was present in the same cells with a trisomy 17 it seems unlikely that HSC donor cells could have acquired this aneuploid karyotype

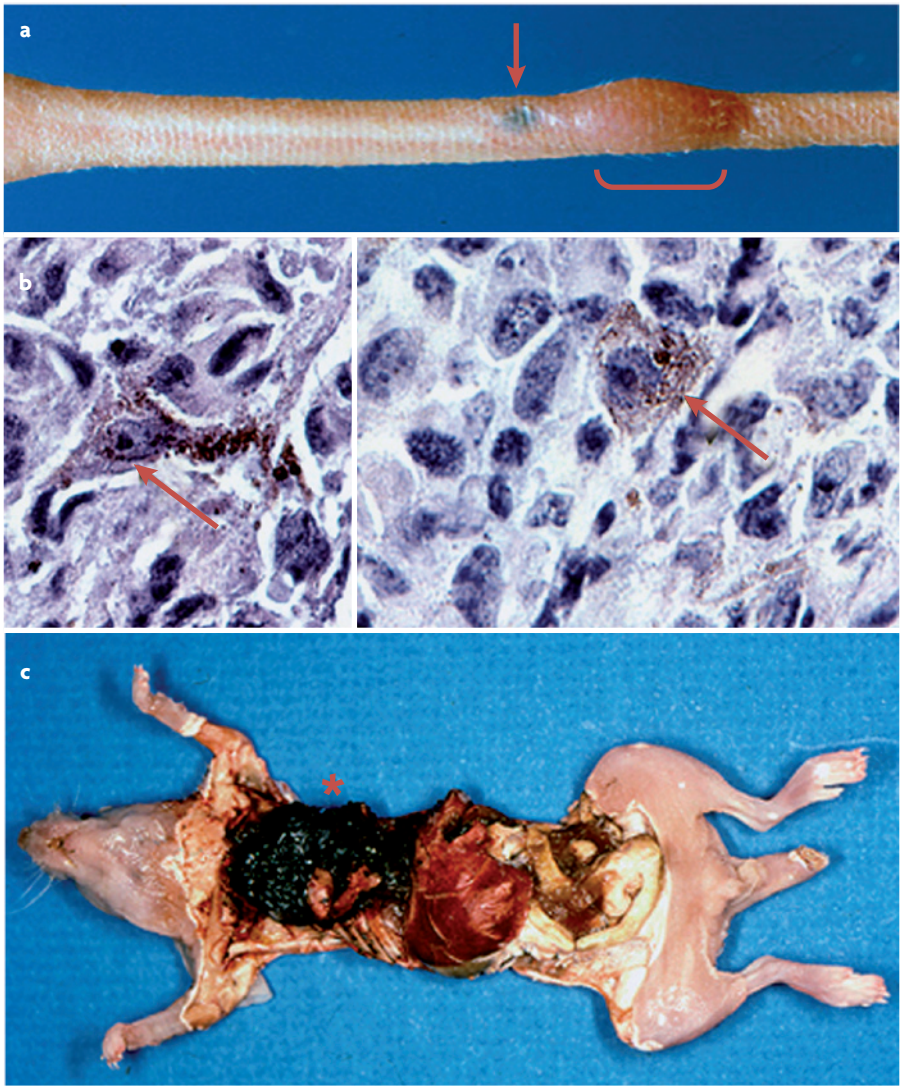

Figure 1 | **Spontaneous *in vivo* fusion in melanoma**[52]**.** Cells from a clone of the Cloudman S91 mouse melanoma were implanted subcutaneously in the tail of a Balb/c *nu/nu* mouse. The mice were albino due to a homozygous mutation in tyrosinase (*c/c*), the rate-limiting enzyme in melanogenesis. Although the melanoma clone was genetically wild-type for tyrosinase (*C/C*), the cells produced little or no melanin in culture and formed amelanotic tumours in mice. Metastases, though infrequent, were generally small, amelanotic tumours in the lung, and were well tolerated by the mice[53]. In one experiment (designed for other purposes), what appeared to be a melanin-producing *in transit* metastasis developed (**a**, arrow) near the site of implant (bracket). The tail was amputated and the implanted tumour was formalin-fixed, embedded in paraffin and sectioned serially. Small numbers of highly melanized, coarse melanin-producing cells were found within the implanted tumour that were not seen in cultures of the parental melanoma cells and had thus been generated *in vivo* (**b**, arrows). Five weeks after removal of the tail the mouse became moribund with a massive, highly pigmented pulmonary metastasis (**c**, asterisk). Cells from the metastasis were cloned in soft agar. DNA analyses revealed that 12 of 12 randomly picked clones had a genotype of *C/c*, indicating they were hybrids formed from fusion of the implanted tumour cells (*C/C*) with host cells (*c/c*). Cells from the metastasis showed an average 30–40% increase in DNA content, increased chemotaxis *in vitro*, activation of the glycosyltransferase GnT-V, and production of its enzymatic product, β1,6-branched oligosaccharides[52]. Like the pigmented cells found in the primary implant (**b**), they also produced 'coarse melanin' — autophagosomes containing melanosomes and other organelles[52]. Similar cells were cultured from the metastasis and were also seen in histolopathology sections of the pulmonary tumour. This indicated that the coarse melanin-containing cells originated in the primary implant through host–tumour cell fusion(s). Coarse melanin was also observed in another *in vivo* melanoma hybrid 'PADA'[84] and in experimentally fused macrophage–melanoma hybrids and is a common characteristic of human melanomas[159]. Reproduced, with permission, from REF. 52 © American Association for Cancer Research (2000).

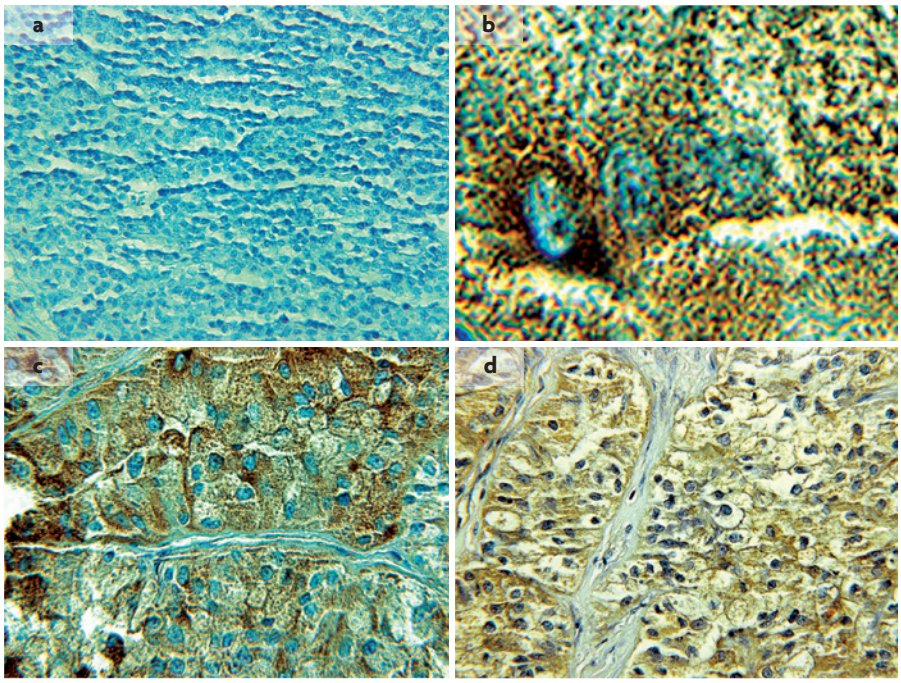

Figure 2 | **A renal cell carcinoma arising after allogeneic stem cell transplant.** These samples were taken from a lymph node metastasis arising in a boy after receiving a haematopoietic stem cell (HSC) transplant from his cancer-free brother[56]. Tumour cells throughout the metastasis contained the ABO blood group A allele of the HSC donor, suggesting that the cells were hybrids between donor HSC(s) and patient tumour cell(s), although direct transformation of bone marrow-derived cells into tumour cells was not ruled out. The sections were stained by lectin histochemistry for β1,6-branched oligosaccharides with the plant lectin leukocytic phytohaemagglutinin (LPHA), which exhibits high specificity for β1,6-branching on *N*-glycoproteins. β1,6-branched oligosaccharides were present in cells throughout the tumour, consistent with the wide distribution of the donor A allele[56]. **a** | Adjacent lymphocytes in the same sections were negative for LPHA staining. **b** | Higher power revealed that LPHA stained in a coarse vesicular, autophagosome-like pattern similar to that seen with coarse melanin in macrophage-melanoma hybrids (FIG. 1). **c**,**d** | Low-power fields demonstrating homogeneous staining of tumour cells for β1,6-branched oligosaccharides. A similar coarse vesicular, autophagosome-like staining pattern for β1,6-branched oligosaccharides is widespread in human cancer[159]. Reproduced, with permission, from REF. 56 © Nature Publishing Group (2004).

simply through mimicry of carcinoma cells, as mimicry would presumably not include genetic aberrations[61]. In another study of secondary solid tumours following female-to-male HSC transplants, tumour cells were found with two X chromosomes but no Y, suggesting they originated at least in part from the female donor BMDCs[62]. However, this study did not report on the potential presence of XXXY (tetraploid) or XXY (aneuploid) cells that might have been indicators of BMDC–tumour cell fusion[50,61,62], nor did it rule out the widespread loss of the Y chromosome that occurs in many cancers as an explanation for the XX karyotype of some carcinoma cells[50,59]. Nonetheless, as above[57] the XX tumour cells tended to be in clusters, suggesting a stem cell-like pattern within the tumours.

In summary, although host cell–cancer cell fusion has been demonstrated in animals, there is as yet far less information in human cancer. HSCs have been shown to incorporate into human cancers; however, the mechanisms of incorporation — fusion versus direct transformation — remain to be elucidated. In the limited number of cases so far, some of the HSCs incorporated into human solid tumours showed a clonal distribution pattern that might be expected for cancer stem cells, consistent with a recent proposal that BMDC–tumour cell fusion is a potential source of cancer stem cells[19].

Cancer cell fusion and the hybrid phenotype. Fusion-induced enhancement of metastasis and a differentiated trait such as melanin production is in contrast to previous studies in which hybrids that were formed *in vitro* between normal epithelial cells or fibroblasts and tumorigenic cancer cells were generally suppressed in tumorigenicity compared with the parental cancer cells[63–69], with some exceptions[70,71]. These important observations led to the concept of, and subsequent identification of, a number of different tumour suppressor genes that have been largely involved in control of progression through the cell cycle[69]. Differentiated traits were also suppressed in such hybrids. For example, polyethylene glycol- and Sendai virus-induced hybrids between fibroblasts and pigmented, tumorigenic melanoma cells were non-pigmented and non-tumorigenic[72–76]. The tendency of hybrids to lose chromosomes with successive cell divisions was exploited for chromosomal mapping of suppressor genes. However, when healthy leukocytes were used as fusion partners with cancer cells, co-activation of differentiated functions between parental genomes was seen, for example, in leukocyte–hepatoma hybrids[77,78], leukocyte–myeloma hybrids[79], immuglobulin-secreting hybridomas[80] and macrophage–melanoma hybrids discussed herein. Thus, unlike tumour-suppressive fibroblasts and epithelial cells, haematopoietic cells enhanced malignancy and differentiation when hybridized with transformed cells. Expression of genes from both parental lineages in cancer cell hybrids could explain many properties of metastatic cells[3,4]. For example, tropism to lymph nodes and organs and tissues such as bone marrow, brain, lung and liver is a common trait of macrophages and metastatic cells alike. Likewise, the notorious multidrug resistance of malignant cells to chemotherapy owing to high levels of p-glycoprotein[81] could reflect the fact that macrophages also express this phenotype[82].

Tumour–BMDC fusions might explain how common gene expression patterns emerge for different tumour types. We, and others, have found that when BMDC–tumour cell hybrids were isolated *in vitro* with no selective pressure other than for growth in drug-containing media, remarkably high numbers of them exhibited a metastatic phenotype in mice. Of 75 clones of polyethylene glycol-fused macrophage–melanoma hybrids isolated *in vitro*, about half showed increased chemotaxis *in vitro* and metastasis in mice[53,83,84]. Similar results were obtained in T-cell hybridomas from the fusion of healthy T lymphocytes with T lymphoma cells[85], and in hybrids between mouse T-cell lymphoma cells and bone marrow-derived macrophages or spleen lymphocytes[86,87]. High-frequency emergence of a common metastatic phenotype *in vitro* without host-selective pressure was surprising, particularly in view of the apparently chaotic nature of aneuploidy. In fact, little is

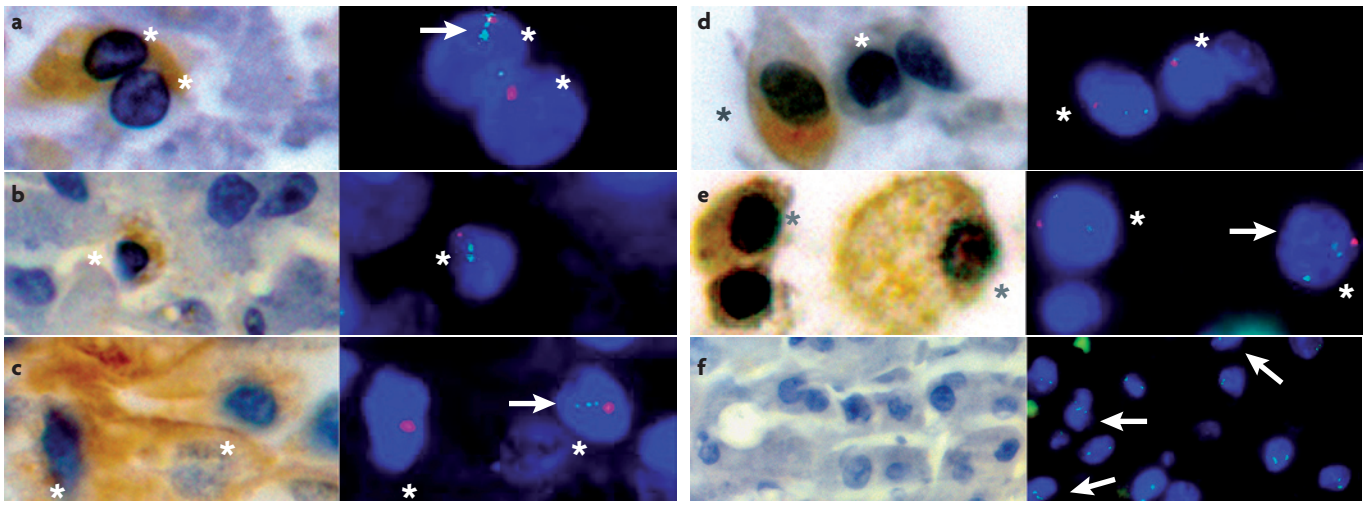

Figure 3 | **Tumour β1,6-branched oligosaccharides after allogeneic stem cell transplant.** The papillary renal cell carcinoma (PRCC) arose in the kidney of a female 2 years after she had received a male haematopoietic stem cell transplant from her cancer-free 15 year-old son[57]. Karyotypes revealed that some of the carcinoma cells contained a trisomy 17, a common abnormality for PRCC. Cells containing both the Y and ≥3 copies of chromosome 17 were localized to a small region covering about 10% of the section area where they comprised about 1% of the tumour cells. These cells were putative fusion hybrids between bone marrow-derived cells (BMDCs) and carcinoma cells, although direct transformation of BMDCs in carcinoma cells without fusion was not definitively ruled out[57]. Sections were stained with leukocytic phytohaemagglutinin (LPHA), a selective marker for β1,6-branched oligosaccharides. LPHA-positive cells were photographed, and the sections were processed by fluorescence *in situ* hybridization (FISH) for the Y (red) and 17 (green) chromosomes. **a**–**e** | Left: LPHA-positive carcinoma cells. Right: FISH analyses of the same section for the Y and 17. Arrows show cells containing both the Y and trisomy 17, demonstrating the presence of donor genes in the carcinoma nuclei. Asterisks denote Y-containing carcinoma cells in both left and right panels. **f** | A region that was devoid of LPHA-positive cells. Left: LPHA-negative carcinoma cells. Right: a FISH-labelled sequential section of the same region displaying only chromosome 17 and not the Y. Of the 70 LPHA-positive cells studied in this manner, 46 nuclei gave positive FISH signals, and of these 37 (80%) contained a Y chromosome. The majority of tumour cells were LPHA-negative and displayed 17 but not the Y [3,4,57]. Thus, tumour-incorporated BMDCs were the main source of tumour cell-associated β1,6-branched oligosaccharides for this tumour. As with other such cases discussed herein and for a wide number of human cancers[159], staining for β1,6-branched oligosaccharides revealed a coarse vesicular phenotype (for example, **e**, left). Reproduced, with permission, from REF. 57 © Nature Publishing Group (2005).

known of the regulation of gene expression in hybrids at the molecular level. Evidence that BMDC–tumour hybrids express many of the same genes associated with invasive and metastatic cancers and that these genes are also expressed by macrophages and other migratory BMDCs is summarized below.

SPARC. SPARC (also known as osteonectin and BM40) is a modulator of cell–matrix interactions during development and is a key component of wound healing, tissue repair and hard-tissue formation[88,89]. SPARC modulates cellular shape and as such is a counter-adhesive factor[89]. SPARC binds to several proteins of the extracellular matrix and is also a chaperone aiding proper folding of collagen in the endoplasmic reticulum[90]. In development, SPARC is expressed in late gastrulation during differentiation of invaginated epithelial cells into mesoderm[91]. Interestingly, SPARC is important in osteoclast formation[92,93]. In tissue macrophages SPARC is expressed in regions of neovascularization, for example, in wound repair[94] and degenerative aortic stenosis[95]. High SPARC expression is associated with tumour progression and poor outcome in melanoma and a number of carcinomas including breast, colorectal, ovarian and lung[96]. SPARC acts as a regulator of melanoma EMT by downregulating melanoma E-cadherin (also known as CDH1) with loss of homotypical adhesion, and stimulates motility and increases expression of mesenchymal markers such as matrix metalloproteinase MMP9 (REF. 97). The actions of SPARC are mediated through SNAIL, a transcription factor in the initiation of EMT during normal development and cancer[98].

The *SPARC* gene provides an example of gene regulation in BMDC–tumour fusion. In fusions between mouse macrophages or human blood monocytes and weakly metastatic mouse Cloudman S91 melanoma cells, unfused melanoma cells, macrophages and monocytes all expressed *SPARC* mRNA; however, the levels were 3–4-fold higher per μg total RNA in hybrids[17,99]. *SPARC* mRNA levels were highest in hybrids of high metastatic potential and lowest in weakly metastatic hybrids and parental melanoma cells. Moreover, hybrids between human monocytes and mouse melanoma cells expressed both human and mouse *SPARC* mRNA[17]. This indicated that genomes from cells of the two different developmental lineages were both activated. Thus, for *SPARC*, gene expression was enhanced by hybridization of tumour cells with macrophages, high expression was correlated with high metastatic potential, and *SPARC* mRNA was produced in hybrids from the genomes of both parental fusion partners. That increased SPARC expression was a characteristic of macrophage–melanoma hybrids provides a possible explanation for increased SPARC and SPARC-mediated pathways in human melanoma and other cancers. It is not known whether other regulators of EMT and development in addition to SPARC were expressed in macrophage–tumour cell fusion hybrids (transcription factors TWIST, SNAIL and others)[10,14]. However at least one, TWIST, is activated in macrophages and regulates inflammatory cytokine production[100,101]. By analogy to SPARC, this suggests that TWIST expression in some invasive carcinomas reflects expression of macrophage-lineage genes following macrophage–tumour cell fusion.

MCR1 and MET. The melanocortin 1 (MC1, melanocyte-stimulating hormone) receptor (MC1R) is activated by MC1 in healthy melanocytes and melanoma cells in which, through cyclic AMP-dependent mechanisms, it activates melanogenesis and regulates proliferation along with several other actions[102,103]. MC1R appears to have a role in melanoma progression, at least in part through its activation of the proto-oncogene *MET*, whose signalling pathway is a key regulator of metastasis in melanoma and many other cancers[104–106].

As with SPARC, gene expression for both *MC1R* and *MET* was increased in highly metastatic macrophage–melanoma hybrids[107,108]. Moreover, each was involved in the induction of chemotactic motility in hybrids[83,107]. Upregulated *MC1R* mRNA expression in hybrids was associated with increased cellular binding of its ligand MC1, and amplified responsiveness to MC1, as shown by increased chemotactic motility, dendricity and melanization[83,84]. Exposure of hybrids to MC1 also increased both the production of *MET* mRNA and responsiveness to hepatocyte growth factor (HGF) as a chemoattractant[108]. Thus the MC1–MC1R and HGF–MET pathways appeared to act together in a positive autocrine loop to control chemotaxis and other functions in hybrid cells. This same relationship appears to be operative in malignant melanoma[105]. In melanoma, MET and MC1R are each regulated through the master transcription factor microphthalmia-associated transcription factor (MITF)[104], which itself is associated with tumour progression[109]. Although it was not determined whether MITF was upregulated in experimental macrophage–melanoma hybrids, this appears to have been the case, as levels of the mRNAs for both *MET* and *MC1R* were increased, an expected consequence of increased MITF[104,109,110]. High expression of MITF[111], MET[112,113] and MC1R[104,114–117] are all characteristics of monocytes, macrophages and other BMDCs.

GnT-V and β1,6-branched oligosaccharides. GnT-V is a Golgi complex enzyme that is highly expressed in myeloid cells and metastatic cancer cells. GnT-V and its enzymatic products, β1,6-branched oligosaccharides conjugated to *N*-glycoproteins, are associated with poor outcome in melanoma[43,118] and carcinomas of the breast[119,120], colon[121,122] lung[123] and endometrium[124]. β1,6-branched oligosaccharides were first purified from granulocytes[125]. From structural analyses they are composed of poly-*N*-acetyllactose amines that are carriers of sialyl lewis^x antigen (sialyl le^x) and thereby used by both leukocytes and metastatic cancer cells for binding to E-selectin (SELE) and/or galectin 3 (also known as lectin, galactoside-binding, soluble 3 (LGALS3)) on endothelial cells during systemic migration[125,126].

GnT-V mRNA, protein and/or enzymatic activity were increased in highly metastatic macrophage–melanoma hybrids *in vitro*[127], and in host–tumour fusions in both lymphomas and melanomas growing in mice[51,128,129]. In human cancer, β1,6-branched oligosaccharide production was a characteristic of putative BMDC–tumour hybrids in the two RCCs discussed above that developed after allogeneic HSC transplant[56,57] (FIGS 2,3). Moreover, multiple pathways in invasion and metastasis that are regulated by GnT-V were increased in macrophage–melanoma hybrids, such as motility-associated integrin subunits, cell surface expression of lysosomal-associated membrane protein 1 (LAMP1) and autophagy.

Motility-associated integrins. The integrin subunits α2, α3, α5, α6, αv, β1 and β3 are all involved with migration of leukocytes and cancer cells. These same integrin subunits were significantly upregulated at the protein level in metastatic macrophage–melanoma hybrids compared with weakly metastatic hybrids and parental melanoma cells[127] (J.M.P. and A.K.C., unpublished data). Following stimulation with MC1, protein levels were further increased in highly metastatic hybrids. These results correlated with findings that metastatic hybrids had acquired an MC1-inducible chemotactic phenotype that was directed toward fibronectin (FN1) through the action of integrin α5β1 (REF. 83). Of great interest, all the above subunits have been identified as substrates for GnT-V and their actions are strongly affected by their glycosylation status with β1,6-branched oligosaccharides[129–139]. For example, in human fibrosarcoma cells addition of β1,6 branched oligosaccharides onto the β1 integrin subunit by GnT-V reduced α5β1 integrin clustering and stimulated cell migration[139]. Further, the above integrin subunits are each involved in metastasis. Levels of the α3β1 integrin are increased and associated with increased migration and invasion in several types of metastatic cancers[140]. α5β1 is a well-characterized receptor for fibronectin that is overexpressed in metastasis[141–143]. Upregulation of αvβ3, a vitronectin (VTN) receptor, was described in various cancers including malignant melanoma and glioblastoma[141,144–146].

Expression of the β1-integrin subunit is a key component of melanoma metastasis[147]. The above integrins and integrin subunits are also highly expressed in macrophages, in which they are involved with many functions, including cell adhesion and migration, signal transduction, cell–cell recognition and phagocytosis[148–152].

Cell surface expression of LAMP1. LAMP1 is a preferred substrate for GnT-V and therefore a major carrier of sialyl le^x and poly-*N*-acetyllactose amines that bind to E-selectins and galectins[130]. Cell surface LAMP1 thus mediates binding to endothelial cells by both leukocytes and cancer cells[153–155]. Macrophage–melanoma hybrids showed increased expression of cell surface LAMP1 (REF. 127). This was seen in highly metastatic macrophage–melanoma hybrids as well as peritoneal macrophages compared with that in parental melanoma cells and less metastatic hybrids.

Autophagy and course melanin. As mentioned, the spontaneous mouse melanoma–host hybrid described above showed a high level of autophagy and coarse melanin[52] (FIG. 1). This was also a characteristic of another spontaneous melanoma–host hybrid described previously ('PADA')[84] and of macrophage–melanoma hybrids fused *in vitro*[52,53,84]. Electron microscope studies revealed that melanin was localized largely to heavily melanized melanosomes packaged in autophagosomes. Autophagosomes were verified by the presence of double limiting membranes and heterogeneous morphologies. They were also strongly positive for β1,6-branched oligosaccharides, implicating a role for GnT-V in their formation[156–158]. These were surprising findings because healthy melanocytes do not appear to use GnT-V in melanogenesis and the melanosomes are not packaged in autophagosomes but exist singly in the cytoplasm. That several independently isolated melanoma hybrids all showed high levels of autophagy and coarse melanin raised the question as to whether this trait might be a signature of BMDC–melanoma fusion in human melanoma. Although coarse melanin in melanoma had been known to pathologists for more than a century and was shown to be due to autophagy (reviewed in REF. 159), its frequency in human cancers had not been evaluated[160]. Analyses of several hundred cases have revealed that it is a common trait, expressed by 85% or more of melanomas[43,159]. It was further determined that coarse melanin-producing melanoma cells and melanophages (macrophages with

autophagolysosomal vesicles containing undigested melanin) account for the well-known hypermelanotic regions of cutaneous malignant melanoma used in clinical diagnosis[43,159]. As in macrophage–melanoma hybrids, coarse melanin vesicles in human melanomas contained β1,6-branched oligosaccharides[43,159]. In cutaneous malignant melanoma, β1,6-branched oligosaccharide-positive, coarse melanin-producing melanoma cells emerge clonally as 'nests' within the *in situ* tumour and have the capacity for invasion into the dermis[43, 159]. This is consistent with BMDC–tumour cell fusion as an explanation for the appearance of these cells (FIG. 1b). Moreover, β1,6-branched oligosaccharide-positive coarse vesicles without melanin were common in all 22 types of human cancers studied and predicted worse outcome in primary breast carcinomas[119,159]. Although it is not certain that the coarse vesicular structures seen in other neoplasms were always due to autophagy, it nonetheless suggested that high levels of autophagy might be widespread, if not universal, in cancer. This was supported by separate molecular genetic studies also indicating that high levels of autophagy are common in cancer, in which they are associated with tumour survival and progression[161–166]. This could seem counterintuitive as autophagy has long been thought to be a catabolic event associated with cell death. However, more recent evidence indicates that autophagy can act as a pro-survival factor by producing a useable energy source for cancer cells deprived of an adequate blood supply. Thus autophagy might help drive metastatic progression where cells can produce nutrients distant from the primary tumour and its nutrient support system[164,166].

Whether through BMDC–melanoma cell fusion or some other mechanism, the generation of β1,6-branched oligosaccharide-positive coarse melanin appears to account in part for the well-known immunogenicity of malignant melanoma. These highly melanized melanoma cells are immunogenic and attractive to macrophages[43]. One immune escape mechanism appears to involve the generation of variant tumour cells that no longer attract macrophages, for example through loss of melanin production and generation of amelanotic variants[176]. In cutaneous malignant melanoma, dermal nests of melanophage-free melanoma cells with reduced or absent melanin were nonetheless positive for β1,6-branched oligosaccharides and associated with worse patient outcome[43].

Could autophagy in human cancer result from fusions between cancer cells and macrophages or other phagocytes? In fact, macrophages express active autophagy as a part of the pathway for digestion of phagocytosed microorganisms and cells[167,168]. Autophagy in macrophages is linked to phagocytosis, interestingly, another characteristic of metastatic cancers[169–173]. Moreover, macrophage vesicles, like those in experimental macrophage–melanoma hybrids and cancer cells, are positive for β1,6-branched oligosaccharides[118,119,159]. Therefore, activation of phagocytic and autophagic pathways in human cancers could reflect expression of imprinted genes of myeloid lineage in macrophage–tumour cell fusion hybrids. We suggest that, should cancer cell autophagy be linked to phagocytosis as it is in macrophages, nutrients could be continuously phagocytosed from external sources and digested through autophagy, rendering metastatic cells constitutively independent of a direct blood supply.

In summary, metastatic macrophage–melanoma hybrids show high expression of SPARC, MET, MC1R, integrin subunits α3, α5, α6, αv, β1, β3, cell-surface LAMP1 and GnT-V and high levels of autophagy. This is paralleled in melanoma, and in a number of other cancers in which these molecules are associated with a migratory phenotype, enhanced survival, metastasis and poor outcome. Central to the metastatic phenotype is GnT-V which, through addition of β1,6-branched oligosaccharides to several of the above proteins, causes many phenotypic changes, including increased chemotaxis, melanogenesis and possibly autophagy. Expression of MC1R, MITF, MET, motility-related integrins, cell-surface LAMP1 and GnT-V, and high levels of autophagy are also characteristic of monocytes and macrophages and other BMDCs. Thus, expression of these molecules in cancer could be a result of fusion of cancer cells with migratory BMDCs and co-expression of imprinted genes from both parental fusion partners. Although these molecules and traits are of course not the only factors involved in tumour progression, their high expression in BMDC–tumour hybrids provides a framework for understanding how fusion can explain metastasis (FIG. 4).

Problems and pitfalls

To prove fusion and genomic hybridization requires identification of genes or chromosomes from both of the putative fusion partners in the same cell or cells. Hence, fusion has been well-documented in tumour xenografts in animals where hybrids were identified by the presence of both tumour and host genes. Little is yet known of the extent of cancer cell fusion in humans. Although a few human cases have recently been reported[55–57,61,62], only one of these, involving macrophage–myeloma

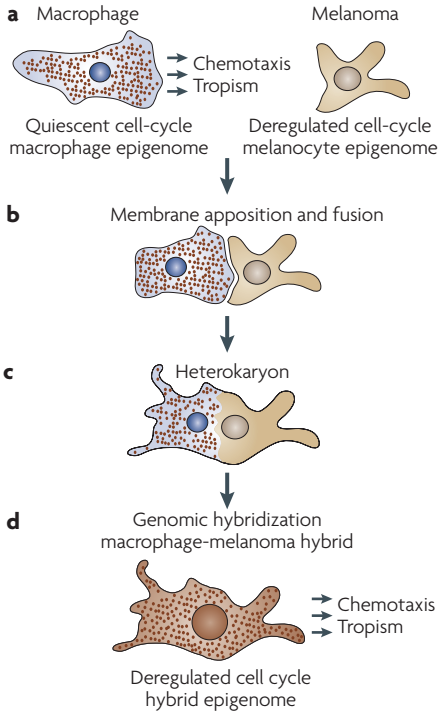

Figure 4 | **A model for generation of a metastatic phenotype following fusion of a melanoma cell with a macrophage. a** | A macrophage is attracted to a non-migratory melanoma cell *in situ*. The epigenomes of the two cells reflect their myeloid and melanocytic lineages respectively. The melanoma cell produces 'fine' or 'dusty' melanin — individual melanosomes in the cytoplasm, generally with a golden-brown colour. Melanoma-associated macrophages are known as melanophages because they are laden with autophagolysosomal vesicles containing residual melanin from engulfed and digested melanoma cells, and thus at times are difficult to distinguish from melanoma cells at the light microscope level[43,159,160]. **b** | The macrophage and melanoma plasma membranes form close appositional contacts, normally as a prelude to ingestion and destruction of the melanoma cell[43]. However in some cases the two cells fuse. **c** | Following fusion a heterokaryon is formed with the two nuclei separate in the cytoplasm. **d** | Genomic hybridization occurs and a mononuclear macrophage–melanoma hybrid emerges. From studies of macrophage–melanoma hybrids generated experimentally *in vitro* and of melanoma–host hybrids generated spontaneously in mice, such hybrids have a deregulated cell cycle, are aneuploid and exhibit epigenomes of both parental lineages. Some exhibit the myeloid capability for chemotaxis *in vitro* and tropism *in vivo*, common characteristics of metastatic cells.

fusion in osteoclast formation, definitively proved fusion[55]. The use of myeloma clone-specific immunoglobulin rearrangements as parental markers of myeloma cells can thus be used to further investigate questions of fusion in myeloma[55]. Other studies have suggested that incorporation of BMDCs into tumour cells can occur through differentiation or neoplastic transformation without fusion[61,62,174]. It is possible that both mechanisms are operative in cancer as well as in healthy tissue regeneration and repair, and this remains to be resolved. The use of allogeneic HSC transplants in medicine followed by the unfortunate development of secondary malignancies provides a potential source of pathology material for study[56,57]. However, such cases are in limited supply and it will take some time to determine the extent of fusion in human cancer by this technique alone. Another problem is that the frequency of cancer cell fusion may be low, as it is in culture (~1 in 10^5–10^7 non-fused cells), making fusion events difficult if not impossible to follow *in vivo*[53]. Also, depending on the time when a particular tumour is analysed, the number of hybrid cells could range from none, should hybridization not have occurred, to 100% if hybrids had overgrown a pre-existing tumour or initiated a new tumour, for example, a metastasis. Further, hybrid cells in a tumour could result from a single progenitor hybrid or from multiple hybrids formed from separate fusions. It is thus difficult to study the molecular mechanisms of cancer cell fusion *in vivo*, or to estimate its frequency. Until more progress is made in these and other areas, the effect of BMDC incorporation into human tumours, whether by fusion or other mechanisms, remains to be determined.

Concluding remarks

Tumour cell–BMDC fusion as a source of metastatic cells would imply that prevention of fusion or of early, rate-limiting post-fusion events might prevent metastasis (for example see REF. 175). With better understanding should come better strategies for targeting vulnerable steps in fusion and the generation of hybrids. Post-fusion events and hybrid formation could present other fruitful areas of focus, for example, molecular steps governing the integration of parental fusion partner genes into hybrid genomes, or those involved with activation of master regulatory genes that are rate-limiting in the development of a migratory phenotype. Early post-fusion cells are also likely to express unique antigenic profiles, making them susceptible to immunotherapy.

The cancer cell–BMDC fusion theory presents a unifying explanation for tumour progression. It seems that this theory is not only possible but likely to be correct to at least some degree, with the remaining question being how extensively does it contribute to progression of human cancers? In our opinion the theory deserves far more attention from the cancer research community than it currently receives. Should cancer cell–BMDC fusion be determined to drive tumour progression in humans, surely new therapeutic strategies would follow.

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1. Aichel, O. in *Vorträge und Aufsätze über Entvickelungsmechanik Der Organismen* Chapter XIII (ed Roux, W.) 92–111 (Wilhelm Engelmann, Leipzig, 1911).
2. Pawelek, J. M. Tumour cell hybridization and metastasis revisited. *Melanoma Res.* **10**, 507–514 (2000).
3. Pawelek, J. M. Tumour-cell fusion as a source of myeloid traits in cancer. *Lancet Oncol.* **6**, 988–993 (2005).
4. Pawelek, J. *et al.* Co-opting macrophage traits in cancer progression: a consequence of tumor cell fusion? *Contrib. Microbiol.***13**, 138–155 (2006).
5. Mekler, L. B. [A general theory of oncogenesis.] Materials of Symposia on General Immunology. The Club of Immunologists of NF Gamaleya Inst of Epidemiology and Microbiology **3**, 91–100 (1968) (in Russian).
6. Mekler, L. B. [Hybridization of transformed cells with lymphocytes as 1 of the probable causes of the progression leading to the development of metastatic malignant cells.] *Vestn Acad. Med. Nauk. SSR (Bulletin of the USSR Acad Med Sci)* **26**, 80–89 (1971) (in Russian).
7. Goldenberg,.DM. [On the progression of malignity: a hypothesis.] *Klin. Wschr.* **46**, 898 (1968) (in German).
8. Goldenberg, D. M. & Gotz, H. On the 'human' nature of highly malignant heterotransplantable tumors of human origin. *Europ. J. Cancer* **4**, 547–548 (1968).
9. Lagarde, A. E. & Kerbel, R. S. Somatic cell hybridization *in vivo* and *in vitro* in relation to the metastatic phenotype. *Biochim. Biophys. Acta* **823**, 81–110 (1984).
10. Gupta, P. B., Mani, S., Yang, J., Hartwell, K. & Weinberg, R. A. The evolving portrait of cancer metastasis *Cold Spring Harb. Symp. Quant. Biol.* **6**, 291–297 (2005).
11. Chambers, A. F., Groom, A. C. & MacDonald, I, C. Dissemination and growth of cancer cells in metastatic sites. *Nature Rev. Cancer* **2**, 563–572 (2002).
12. Nowell, P. C. The clonal evolution of tumor cell populations. *Science* **194**, 23–28 (1976).
13. Fidler, I. J. & Kripke, M. L. Metastasis results from preexisting variant cells within a malignant tumor. *Science* **197**, 893–895 (1977).
14. Ma, L., Teruya-Feldstein, J. & Weinberg, R. A. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* **449**, 682–688 (2007).
15. Chakraborty, A. K. & Pawelek, J. M. GnT-V, macrophages, and cancer metastasis: A common link. *Clin. Exp. Metastasis* **20**, 365–373 (2003).
16. Munzarova, M., Lauerova, L. & Capkova, J. Are advanced malignant melanoma cells hybrids between melanocytes and macrophages? *Melanoma Res.* **2**, 127–129 (1992).
17. Chakraborty. A. K., de Freitas Sousa, J., Espreafico, E. M. & Pawelek, J. M. Human monocyte × mouse melanoma fusion hybrids express human gene. *Gene* **275**, 103–106 (2001).
18. Duelli, D. & Lazebnik, Y. Cell-to-cell fusion as a link between viruses and cancer. *Nature Rev. Cancer* **7**, 968–976
19. Bjerkvig, R., Tysnes, B. B., Aboody, K. S., Najbauer, J. & Terzis, A. J. Opinion: the origin of the cancer stem cell: current controversies and new insights. *Nature Rev. Cancer* **5**, 899–904. Erratum in *Nature Rev. Cancer* **5**, 995 (2005).
20. Sapir, A., Avinoam, O., Podbilewicz, B. & Chernomordik, L. V. Viral and developmental cell fusion mechanisms: conservation and divergence. *Dev Cell.* **14**, 11–21 (2008).
21. Chen, E. H. & Olson, E. N. Unveiling the mechanisms of cell–cell fusion. *Science* **308**, 369–373 (2005).
22. Vignery, A. Macrophage fusion: the making of osteoclasts and giant cells. *J. Exp. Med.* **202**, 337–340 (2005).
23. Vignery, A. Macrophage fusion: are somatic and cancer cells possible partners? *Trends Cell Biol.* **4**, 188–193 (2005).
24. Chen, E. H., Grote, E., Mohler. W. & Vignery, A. Cell–cell fusion. *FEBS Lett.* **581**, 2181–2193 (2007).
25. Pajcini, K. V., Pomerantz, J. H., Alkan, O., Doyonnas, R. & Blau, H. M. Myoblasts and macrophages share molecular components that contribute to cell–cell fusion. *J. Cell Biol.* **180**, 1005–1019 (2008).
26. Holmgren, L., Bergsmedh, A. & Spetz, A. L. Horizontal transfer of DNA by the uptake of apoptotic bodies. *Vox Sang.* **83** (Suppl. 1), 305–306 (2002).
27. Duelli, D. M. *et al.* A virus causes cancer by inducing massive chromosomal instability through cell fusion. *Curr. Biol.* **17**, 431–437 (2007).
28. Larsson, L. I., Bjerregaard, B., Wulf-Andersen, L. & Talts, J. F. Syncytin and cancer cell1 fusions. *Sci. World J.* **7**, 1193–1197 (2007).
29. Jin, J. & Woodgett, J. R. Chronic activation of protein kinase Bβ/Akt2 leads to multinucleation and cell fusion in human epithelial kidney cells: events associated with tumorigenesis. *Oncogene* **24**, 5459–5470 (2005).
30. Overholtzer, M. *et al.* A nonapoptotic cell death process, entosis, that occurs by cell-in-cell invasion. *Cell* **131**, 966–979 (2007).
31. Yagi, M., Miyamoto, T., Toyama, Y. & Suda, T. Role of DC-STAMP in cellular fusion of osteoclasts and macrophage giant cells. *J. Bone Miner. Metab.* **24**, 355–358 (2006).
32. Teitelbaum, S. L. & Ross, F. P. Genetic regulation of osteoclast development and function. *Nature Rev. Genet.* **4**, 638–649 (2003).
33. Han, X. *et al.* CD47, a ligand for the macrophage fusion receptor, participates in macrophage multinucleation. *J. Biol. Chem.* **275**, 37984–37992 (2000).
34. Kajita, M. *et al.* Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. *J. Cell Biol.* **153**, 893–904 (2001).
35. Kyriakides, T. R., *et al.* The CC chemokine ligand, CCL2/MCP1, participates in macrophage fusion and foreign body giant cell formation. *Am. J. Pathol.* **165**, 2157–2166 (2004).
36. Kim, M. S., Magno, C. L., Day, C. J. & Morrison, N. A. Induction of chemokines and chemokine receptors CCR2b and CCR4 in authentic human osteoclasts differentiated with RANKL and osteoclast like cells differentiated by MCP-1 and RANTES. *J. Cell. Biochem.* **97**, 512–518 (2006).
37. Marhaba, R. & Zöller, M. CD44 in cancer progression: adhesion, migration and growth regulation. *J. Mol. Histol* **35**, 211–231 (2004).
38. Götte, M. & Yip, G. W. Heparanase, hyaluronan, and CD44 in cancers: a breast carcinoma perspective. *Cancer Res* **66**, 10233–10237(2006).
39. Dalerba, P. *et al.* Phenotypic characterization of human colorectal cancer stem cells. *Proc. Natl Acad. Sci. USA* **104**, 10158–10163 (2007).
40. Zijlmans, H. J. *et al.* The absence of CCL2 expression in cervical carcinoma is associated with increased survival and loss of heterozygosity at 17q11.2. *J. Pathol.* **208**, 507–517 (2006.
41. Baier, P. K., Eggstein, S., Wolff-Vorbeck, G., Baumgartner, U. & Hopt, U. T. Chemokines in human colorectal carcinoma. *Anticancer Res.* **25**, 3581–3584 (2005).
42. Rendlew-Danielsen, J. M., *et al.* Dysregulation of CD47 and the ligands thrombospondin 1 and 2 in multiple myeloma. *Br. J. Haematol.* **138**, 756–760 (2007).

43. Handerson, T. *et al.* Melanophages reside in hypermelanotic, aberrantly glycosylated tumor areas and predict improved outcome in primary cutaneous malignant melanoma. *J. Cutaneous Pathol.* **34**, 667–738 (2007).
44. Jacobsen, B. M., *et al.* Spontaneous fusion with, and transformation of mouse stroma by, malignant human breast cancer epithelium. *Cancer Res.* **66**, 8274–8279 (2006).
45. Rizvi, A. Z., *et al.* Bone marrow-derived cells fuse with normal and transformed intestinal stem cells. *Proc. Natl Acad. Sci. USA* **103**, 6321–6325 (2006).
46. Mortensen, K., Lichtenberg, J., Thomsen, P. D. & Larsson, L. I. Spontaneous fusion between cancer cells and endothelial cells. *Cell. Mol. Life Sci.* **61**, 2125–2131 (2004).
47. Bjerregaard, B., Holck, S., Christensen, I. J. & Larsson, L. I. Syncytin is involved in breast cancer-endothelial cell fusions. *Cell. Mol. Life Sci.* **63**, 1906–1911 (2006).
48. Streubel, B. *et al.* Lymphoma-specific genetic aberrations in microvascular endothelial cells in B-cell lymphomas. *N. Engl. J. Med.* **351**, 250–259 (2004).
49. Alison, M. R., Lovell, M. J., Direkze, N. C., Wright, N. A. & Poulsom, R. Stem cell plasticity and tumour formation. *Eur. J. Cancer* **42**, 1247–1256 (2006).
50. Herzog, E. L., *et al.* Lung-specific nuclear reprogramming is accompanied by heterokaryon formation and Y chromosome loss following bone marrow transplantation and secondary inflammation. *FASEB J.* **21**, 2592–12601 (2007).
51. Kerbel, R. S., Lagarde, A. E., Dennis, J. W. & Donaghue, T. P. Spontaneous fusion *in vivo* 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 (1983).
52. Chakraborty, A. K. *et al.* A spontaneous murine melanoma lung metastasis comprised of host × tumor hybrids. *Cancer Res.* **60**, 2512–2519 (2000).
53. Rachkovsky, M. S. *et al.* Melanoma × macrophage hybrids with enhanced metastatic potential. *Clin. Exp. Metastasis* **16**, 299–312 (1998).
54. Wiener, F., Fenyö, E. M. & Klein, G. Tumor-host cell hybrids in radiochimeras. *Proc. Natl Acad. Sci. USA* **7**, 148–152 (1974).
55. Andersen, T. L. *et al.* Osteoclast nuclei of myeloma patients show chromosome translocations specific for the myeloma cell clone: a new type of cancer-host partnership? *J. Pathol.* **211**, 10–17 (2007).
56. Chakraborty, A. *et al.* Donor DNA in a renal cell carcinoma metastasis from a bone marrow transplant recipient. *Bone Marrow Transplant.* **34**, 183–186 (2004).
57. Yilmaz, Y., Lazova, R., Qumsiyeh, M., Cooper, D. & Pawelek, J. Donor Y chromosome in renal carcinoma cells of a female BMT recipient: visualization of putative BMT-tumor hybrids by FISH. *Bone Marrow Transplant.* **35**, 1021–1024 (2005).
58. Salama, M. E., Worsham, M. J. & DePeralta-Venturina, M. Malignant papillary renal tumors with extensive clear cell change: a molecular analysis by microsatellite analysis and fluorescence *in situ* hybridization. *Arch. Pathol. Lab. Med.***127**, 1176–1181 (2003).
59. Lau, L. C., Tan, P. H., Chong, T. W., Foo, K. T. & Yip, S. Cytogenetic alterations in renal tumors: a study of 38 Southeast Asian patients. *Cancer Genet. Cytogenet.***175**, 1–7 (2007).
60. Guo, W., Lasky, J. L. 3rd & Wu, H. Cancer stem cells. *Pediatr. Res.* **59**, 59R–64R (2006).
61. Cogle, C. R. *et al.* Bone marrow contributes to epithelial cancers in mice and humans as developmental mimicry. *Stem Cells* **25**, 1881–1887 (2007).
62. Avital, I. *et al.* Donor-derived human bone marrow cells contribute to solid organ cancers developing after bone marrow transplantation. *Stem Cells* **25**, 2903–2909 (2007).
63. Wiener, F., Klein, G. & Harris, H. The analysis of malignancy by cell fusion. *J. Cell Sci.* **15**, 177–183 (1974).
64. Stanbridge, E. J. Suppression of malignancy in human cells. *Nature* **260**, 17–20 (1976).
65. Sidebottom, E., The analysis of malignancy by cell fusion. *In Vitro* **16**, 77–86 (1980).
66. Ramshaw, I. A., Carlsen, S., Wang, H. & Badenoch-Jones, P. The use of cell fusion to analyse factors involved in tumour cell metastasis. *Int. J. Cancer* **32**, 471–478 (1983).
67. Harris, H. The analysis of malignancy by cell fusion: the position in 1988. *Cancer Res.,* **48**, 3302–3306 (1988).
68. Weinberg, A. S. Tumor suppressor genes. *Science* **254**, 1138–1146 (1991).
69. Levine, A. J. In *The Molecular Basis of Cancer*. (eds Mendelsohn, J., Howley, P. M., Israel, M. A. & Liotta, L. A) 86–104 (WB Saunders, Philadelphia, 1995).
70. Scaletta, L. J. & Ephrussi, B. Hybridization of normal and neoplastic cells *in vitro*. *Nature* **205**, 1169 (1965).
71. Defendi, V., Ephrussi, B., Koprowski, H. & Yoshida, M. C. Properties of hybrids between polyoma-transformed and normal mouse cells. *Proc. Natl Acad. Sci. USA* **57**, 299–305 (1967).
72. Jonasson, J., Povey, S. & Harris, H. The analysis of malignancy by cell fusion. VII. Cytogenetic analysis of hybrids between malignant diploid cells and of tumours derived from them. *J. Cell Sci.* **24**, 217–254 (1977).
73. Davidson, R. L., Ephrussi, R. L. B. & Yamamoto, K. Regulation of pigment synthesis in mammalian cells, as studied by somatic hybridization. *Proc. Natl Acad. Sci. USA* **56**, 1437–1440 (1966).
74. Powers, T. P. & Davidson, R. L. Coordinate extinction of melanocyte-specific gene expression in hybrid cells. *Som. Cell Mol. Gen.* **22**, 41–56 (1996).
75. Gourdeau, H. & Fournier, R. E.K, Genetic analysis of mammalian cell differentiation. *Ann. Rev. Cell Biol.* **6**, 69–94 (1990).
76. Powers, T. P., Shows, T. B. & Davidson, R. L. Pigment-cell-specific genes from fibroblasts are transactivated after chromosomal transfer into melanoma cells. *Mol. Cell Biol.* **14**, 1179–1190 (1994).
77. Darlington, G. J., Bernhard, H. P. & Ruddle, F. H. Human serum albumin phenotype activation in mouse hepatoma — human leukocyte cell hybrids. *Science* **185**, 859–862 (1974).
78. Malawista, S. E. & Weiss, M. C. Expression of differentiated function in hepatoma cell hybrids: high frequency of induction of mouse albumin production in rat hepatoma-mouse lymphoblast hybrids. *Proc. Natl Acad. Sci. USA* **71**, 927–931 (1974).
79. Giacomoni, D. Tumorigenicity and intracisternal A-particle expression of hybrids between murine myeloma and lymphocytes. *Cancer Res.* **39**, 4481–4484 (1979).
80. Kohler, G. & Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* **256**, 495–497 (1975).
81. Gottesman, M. M. & Ling, V. The molecular basis of multidrug resistance in cancer: the early years of P-glycoprotein research. *FEBS Lett.* **580**, 998–1009 (2006).
82. Lemaire, S., Van Bambeke, F., Mingeot-Leclercq, M. P. & Tulkens, P. M. Modulation of the cellular accumulation and intracellular activity of daptomycin towards phagocytized *Staphylococcus aureus* by the P-glycoprotein (MDR1) efflux transporter in human THP-1 macrophages and madin-darby canine kidney cells. *Antimicrob. Agents Chemother.* **51**, 2748–2757 (2007).
83. Rachkovsky, M. & Pawelek, J. Acquired melanocyte stimulating hormone-inducible chemotaxis following macrophage fusion with Cloudman S91 melanoma cells. *Cell Growth Differ.* **10**, 515–524 (1999).
84. Pawelek, J. *et al.* Altered N-glycosylation in macrophage x melanoma fusion hybrids. *Cell. Mol. Biol.* **45**, 1011–1027 (2000).
85. Roos, E., La Riviere, G., Collard, J. G., Stukart, M. J. & De Baetselier, P. Invasiveness of T-cell hybridomas *in vitro* and their metastatic potential *in vivo*. *Cancer Res.* **45**, 6238–6243 (1985).
86. Larizza, L., Schirrmacher, V., Stöhr, M., Pflüger, E. & Dzarlieva, R. Inheritance of immunogenicity and metastatic potential in murine cell hybrids from the T-lymphoma ESb08 and normal spleen lymphocytes. *J. Natl Cancer Inst.* **72**, 1371–1381 (1984).
87. Larizza, L. *et al.* 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**, 699–707 (1984).
88. Lane, T. F. & Sage, E. H. The biology of SPARC, a protein that modulates cell-matrix interactions. *FASEB J.* **8**, 163–173 (1994).
89. Bradshaw, A. D. & Sage, E. H. SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. *J. Clin. Invest.* **107**, 1049–1054 (2001).
90. Martinek, N., Shahab, J., Sodek, J. & Ringuette, M. Is SPARC an evolutionarily conserved collagen chaperone? *J. Dent. Res.* **86**, 296–305 (2007).
91. Damjanovski, S., Huynh, M. H., Motamed, K., Sage, E.H & Ringuette, M. Regulation of SPARC expression during early Xenopus development: evolutionary divergence and conservation of DNA regulatory elements between amphibians and mammals. *Dev. Genes Evol.* **207**, 453–461 (1998).
92. Fujita, T. *et al.* SPARC stimulates the synthesis of OPG/OCIF, MMP-2 and DNA in human periodontal ligament cells. *J. Oral Pathol. Med.* **31**, 345–352 (2002). Erratum in *J. Oral Pathol. Med.* **31**, 504 (2002).
93. Mansergh, F. C. *et al.* Osteopenia in Sparc (osteonectin)-deficient mice: characterization of phenotypic determinants of femoral strength and changes in gene expression. *Physiol. Genomics*. **32**, 64–73 (2007).
94. Reed, M. J. *et al.* Differential expression of SPARC and thrombospondin 1 in wound repair: immunolocalization and *in situ* hybridization. *J. Histochem. Cytochem.* **41**, 1467–1477 (1993).
95. Charest, A. *et al.* Distribution of SPARC during neovascularisation of degenerative aortic stenosis. *Heart* **92**, 1844–1849 (2006).
96. Robert, G. *et al.* SPARC represses E-cadherin and induces mesenchymal transition during melanoma development. *Cancer Res.* **66**, 7516–7523 (2006).
97. Alonso, S. R. *et al.* A high-throughput study in melanoma identifies epithelial–mesenchymal transition as a major determinant of metastasis. *Cancer Res.* **67**, 3450–3460 (2007).
98. Barrallo-Gimeno, A. & Nieto, M. A. The Snail genes as inducers of cell movement and survival: implications in development and cancer. *Development* **132**, 3151–3161 (2005).
99. Chakraborty, A. K. & Yamaga, S. Differential gene expression in genetically matched mouse melanoma cells with different metastatic potential. *Gene* **315**, 165–175 (2003).
100. Sharif, M. N. *et al.* Twist mediates suppression of inflammation by type I IFNs and Axl. *J. Exp. Med.* **203**, 1891–1901 (2006).
101. Sosi, D., Richardson, J. A., Yu, K., Ornitz, D. M. & Olson, E. N. Twist regulates cytokine gene expression through a negative feedback loop that represses NF-κB activity. *Cell* **112**, 169–180 (2003).
102. Carlson, J. A., Linette, G. P., Aplin, A., Ng, B. & Slominski, A. Melanocyte receptors: clinical implications and therapeutic relevance. *Dermatol. Clin.* **25**, 541–557, viii–ix (2007).
103. Kanetsky, P. A. *et al.* Population-based study of natural variation in the melanocortin-1 receptor gene and melanoma. *Cancer Res.* **66**, 9330–9337 (2006).
104. McGill, G. G., Haq, R., Nishimura, E. K. & Fisher, D. E. c-Met expression is regulated by Mitf in the melanocyte lineage. *J. Biol. Chem.* **281**, 10365–10373 (2006).
105. Beuret, L. *et al.* Up-regulation of MET expression by α-melanocyte-stimulating hormone and MITF allows hepatocyte growth factor to protect melanocytes and melanoma cells from apoptosis. *J. Biol. Chem.* **282**, 14140–14147 (2007).
106. Boccaccio, C. & Comoglio, P. M. Invasive growth: a MET-driven genetic programme for cancer and stem cells. *Nature Rev. Cancer.* **6**, 637–645 (2006).
107. Chakraborty, A. K. *et al.* Upregulation of mRNA for the melanocortin-1 receptor but not for melanogenic proteins in macrophage x melanoma fusion hybrids exhibiting increased melanogenic and metastatic potential. *Pig. Cell Res.* **12**, 355–366 (1999).
108. Chakraborty, A. K. *et al.* Expression of c-Met proto-oncogene in metastatic macrophage × melanoma fusion hybrids: implication of its possible role in MSH-induced motility. *Oncol. Res.* **14**, 163–174 (2003).
109. Levy, C., Khaled, M. & Fisher, D. E. MITF: master regulator of melanocyte development and melanoma oncogene. *Trends Mol. Med.* **12**, 406–414 (2006).
110. Garraway, L. A. *et al.* Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. *Nature* **436**, 117–122 (2005).
111. Bronisz, A. *et al.* Microphthalmia-associated transcription factor interactions with 14–3–3 modulate differentiation of committed myeloid precursors. *Mol. Biol. Cell* **17**, 3897–3906 (2006).
112. Beilmann, M. *et al.* Neoexpression of the c-met/hepatocyte growth factor-scatter factor receptor gene in activated monocytes. *Blood* **90**, 4450–4458 (1997).
113. Gaasch, J. A., Bolwahnn, A. B. & Lindsey, J. S. Hepatocyte growth factor-regulated genes in differentiated RAW 264.7 osteoclast and undifferentiated cells. *Gene* **369**, 142–152 (2006).

114. Lam, C. W., Getting, S. J. & Perretti, M. *In vitro* and *in vivo* induction of heme oxygenase 1 in mouse macrophages following melanocortin receptor activation. *J. Immunol.* **174**, 2297–2304 (2005).
115. Lam, C. W., Perretti, M. & Getting, S. J. Melanocortin receptor signaling in RAW264.7 macrophage cell line. *Peptides* **27**, 404–412 (2006).
116. Manna, S. K., Sarkar, A. & Sreenivasan, Y. α-Melanocyte-stimulating hormone down-regulates CXC receptors through activation of neutrophil elastase. *Eur. J. Immunol.* **36**, 754–769 (2006).
117. Taylor, A. W. The immunomodulating neuropeptide α-melanocyte-stimulating hormone (α-MSH) suppresses LPS-stimulated TLR4 with IRAK-M in macrophages. *J. Neuroimmunol.* **162**, 43–50 (2005).
118. Fernandes, B., Sagman, U., Auger, M., Demetrio, M. & Dennis, J. W. β1,6-branched oligosaccharides as a marker of tumor progression in human breast and colon neoplasia. *Cancer Res.* **51**, 718–723 (1991).
119. Handerson, T., Camp, R., Harigopal, M., Rimm, D. & Pawelek, J. β1,6-Branched oligosaccharides are associated with metastasis and predict poor outcome in breast carcinoma. *Clin. Cancer Res.* **11**, 2969–2973 (2005).
120. Seelentag, W. K. *et al.* Pronostic value of β1,6-branched oligosaccharides in human colorectal carcinoma. *Cancer Res.* **58**, 5559–5564 (1998).
121. Murata, K. *et al.* Expression of *N*-acetylglucosaminyltransferase V in colorectal cancer correlates with metastasis and poor prognosis. *Clin. Cancer Res.* **6**, 1772–1777 (2000).
122. Dosaka-Akita, H. *et al.* Expression of *N*-acetylglucosaminyltransferase V is associated with prognosis and histology in non-small cell lung cancers. *Clin. Cancer Res.* **10**, 1773–1779 (2004).
123. Fukuda, M., Spooncer, E., Oates, J. E., Dell, A. & Klock, J. C. Structure of sialylated fucosyl lactosaminoglycan isolated from human granulocytes. *J. Biol. Chem.* 25 10925–10935 (1984).
124. Yamamoto, E. *et al.* Expression of *N*-acetyl-glucosaminyltransferase V in endometrial cancer correlates with poor prognosis. *Br. J. Cancer* **97**, 1538–1544 (2007).
125. Fukuda, M., Spooncer, E., Oates, J. E., Dell, A. & Klock, J. C. Structure of sialylated fucosyl lactosaminoglycan isolated from human granulocytes. *J. Biol. Chem.* **259**, 10925–10935 (1984).
126. Mizoguchi, A., Takasaki, S., Maeda, S. & Kobata, A. Changes in asparagine-linked sugar chains of human promyelocytic leukemic cells (HL-60) during monocytoid differentiation and myeloid differentiation. Decrease of high-molecular-weight oligosaccharides in acidic fraction. *J. Biol. Chem.* **259**, 11949–11957 (1984).
127. Chakraborty, A. K. *et al.* Fusion hybrids with macrophage and melanoma cells up-regulate *N*-acetylglucosaminyltransferase V, β1–6 branching, and metastasis. *Cell Growth Differentiation* **12**, 623–630 (2001).
128. Dennis, J., Waller, C. A. & Schirrmacher, V. Identification of asparagine-linked oligosaccharides involved in tumor cell adhesion to laminin and type IV collagen. *J. Cell Biol.* **99**, 1034–1044 (1984).
129. Demetriou, M., Nabi, I. R., Coppolino, M., Dedhar, S. & Dennis, J. W. Reduced contact-inhibition and substratum adhesion in epithelial cells expressing GlcNAc-transferase, V. *J. Cell Biol.* **130**, 383–392 (1995).
130. Saitoh, O., Wang, W. C., Lotan, R. & Fukuda, M. Differential glycosylation and cell surface expression of lysosomal membrane glycoproteins in sublines of a human colon cancer exhibiting distinct metastatic potentials. *J. Biol. Chem.* **267**, 5700–5711 (1992).
131. Chammas, R., Veiga, S. S., Travassos, L. R. & Brentani, R. R. Functionally distinct roles for glycosylation of α and β integrin chains in cell matrix interactions. *Proc. Natl Acad. Sci. USA* **90**, 1795–1799 (1993).
132. Zheng, M., Fang, H. & Hakomori, S. Functional role of *N*-glycosylation in α5β1 integrin receptor. De-*N*-glycosylation induces dissociation or altered association of α5 and β1 subunits and concomitant loss of fibronectin binding activity. *J. Biol. Chem.* **269**, 12325–12331, (1994).
133. Leppa, S., Heino, J. & Jalkanen, M. Increased glycosylation of β1 integrin affects the interaction of transformed s115 mammary epithelial cells with laminin-1. *Cell Growth Differ.* **6**, 853–861, (1995).
134. Dennis, J. W., Granovsky, M. & Warren, C. E. Glycoprotein glycosylation and cancer progression. *Biochim. Biophys. Acta* **1473**, 21–34 (1999).
135. Yamamoto, H. *et al.* β1,6 *N*-acetyl-glucosamine bearing *N*-glycans in human gliomas; implications for role in regulating invasivity. *Cancer Res.* **60**, 134–142 (2000).
136. Ochwat, D., Hoja-Lukowicz, D. & Litynska, A. *N*-glycoproteins bearing β1,6-branched oligosaccharides from the A375 human melanoma cell line analysed by tandem mass spectrometry. *Melanoma Res.* **14**, 479–485 (2004).
137. Guo, H.-B., Lee, I., Kamar, M., Akiyama, S. K. & Pierce, M. Aberrant *N*-glycosylation of β1 integrin causes reduced α5β1 integrin clustering and stimulates cell migration. *Cancer Res.* **62**, 6837–6845 (2002).
138. Poche, E., Litysk, A., Amoresano, A. & Casbarra, A. Glycosylation profile of integrin α3β1 changes with melanoma progression. *Biochim. Biophys. Acta Mol. Cell Res.* **1643**, 113–123 (2003).
139. Jasiulionis, M. G., Chammas, R., Ventura, A. M., Travassos, L. R. & Brentani, R. R. α6β1-Integrin, a major cell surface carrier of β1-6-branched oligosaccharides, mediates migration of EJ-*ras*-transformed fibroblasts on laminin-1 independently of its glycosylation state. *Cancer Res.* **56**, 1682–1689 (1996).
140. Giannelli, G. *et al.* Role of the α3β1 and α6β4 integrins in tumor invasion. *Clin. Exp. Metastasis* **19**, 217–230 (2002).
141. Danen, E. H. J. *et al.* Emergence of α5β1 fibronectin- and αvβ3 vitronectin-receptor expression in melanocytic tumour progression. *Histopathology* **24**, 249–256 (1994).
142. Natali, P. G., Nicotra, M. R., Di Filippo, F. & Bigotti, A. Expression of fibronectin, fibronectin isoforms and integrin receptors in melanocytic lesions. *Br. J. Cancer* **71**, 1243–1247 (1995).
143. Galbraith, C. G., Yamada, K. M. & Galbraith, J. A. Polymerizing actin fibers position integrins primed to probe for adhesion sites. *Science* **315**, 992–995 (2007).
144. Gladson, C. L. & Cheresh, D. A. Glioblastoma expression of vitronectin and the αvβ3 integrin. Adhesion mechanism for transformed glial cells. *J. Clin. Invest.* **88**, 1924–1932 (1991).
145. Natali, P. G., *et al.* Clinical significance of αvβ3 integrin and intercellular adhesion molecule-1 expression in cutaneous malignant melanoma lesions. *Cancer Res.* **57**, 1554–1560 (1997).
146. Wong, N. C. *et al.* αv integrins mediate adhesion and migration of breast carcinoma cell lines. *Clin. Exp. Metastasis* **16**, 50–61 (1998).
147. Juliano, R. L. The role of β1 integrins in tumors. *Semin. Cancer Biol.* **4**, 277–283 (1993).
148. Ammon, C. *et al.* Comparative analysis of integrin expression on monocyte-derived macrophages and monocyte-derived dendritic cells. *Immunology* **100**, 364–369 (2000).
149. Aplin, A. E., Howe, A., Alahari, S. K. & Juliano, R. L. Signal transduction and signal modulation by cell adhesion receptors: the role of integrins, cadherins, immunoglobulin-cell adhesion molecules, and selectins. *Pharmacol. Rev.* **50**, 197–263 (1998).
150. Elsegood, C. L. *et al.* M-CSF induces the stable interaction of cFms with αVβ3 integrin in osteoclasts. *Int. J. Biochem. Cell Biol.* **38**, 1518–1529 (2006).
151. Shinji, H. *et al.* Expression and distribution of very late antigen-5 in mouse peritoneal macrophages upon ingestion of fibronectin-bound *Staphylococcus aureus*. *Microbiol. Immunol.* **51**, 63–171 (2007).
152. Kurita-Taniguchi, M. *et al.* Molecular assembly of CD46 with CD9, α3-β1 integrin and protein tyrosine phosphatase SHP-1 in human macrophages through differentiation by GM-CSF. *Mol. Immunol.* **38**, 689–700 (2002).
153. Chang, M. H. *et al.* Transthyretin interacts with the lysosome-associated membrane protein (LAMP-1) in circulation. *Biochem. J.* **382**, 481–489 (2004).
154. Sawada, R., Lowe, J. B. & Fukuda, M. E-selectin-dependent adhesion efficiency of colonic carcinoma cells is increased by genetic manipulation of their cell surface lysosomal membrane glycoprotein-1 expression levels. *J. Biol. Chem.* **268**, 12675–12681 (1993).
155. Sarafian, V. *et al.* Expression of Lamp-1 and Lamp-2 and their interactions with galectin-3 in human tumor cells. *Int. J. Cancer* **75**, 105–111 (1998).
156. Chakraborty, A. K. & Pawelek, J. M. β1,6-branched oligosaccharides regulate melanin content and motility in macrophage-melanoma fusion hybrids. *Melanoma Res.* **17**, 9–16 (2007).
157. Rupani, R., Handerson, T. & Pawelek, J. Co-localization of β1,6-branched oligosaccharides and coarse melanin in macrophage-melanoma fusion hybrids and human melanoma cells *in vitro*. *Pig. Cell Res.* **17**, 281–288 (2004).
158. Hariri, M. *et al.* Biogenesis of multilamellar bodies via autophagy. *Mol. Biol. Cell* **11**, 255–268 (2000).
159. Handerson, T. & Pawelek, J. β1,6-branched oligosaccharides and coarse vesicles: A common and pervasive phenotype in melanoma and other human cancers. *Cancer Res.* **63**, 5363–5369 (2003).
160. Clark, W. H. *et al.* Current concepts of the biology of human cutaneous malignant melanoma. *Adv. Cancer Res.* **24**, 267–338 (1977).
161. Hait, W. N., Jin, S. & Yang, J.-M. A matter of life or death (or both): understanding autophagy in cancer. *Clin. Cancer Res.* **12**, 1961–1965 (2006).
162. Hait, W. N., Wu, H., Jin, S. & Yang, J. M. Elongation factor-2 kinase: its role in protein synthesis and autophagy. *Autophagy* **2**, 294–296 (2006).
163. Hait, W. N., Jin, S. & Yang, J. M. Elongation factor-2 kinase regulates autophagy in human glioblastoma cells. *Clin. Cancer Res.* **12**, 1961–1965 (2006).
164. Mathew, R., Karantza-Wadsworth, V. & White, E. Role of autophagy in cancer. *Nature Rev. Cancer* **7**, 961–967 (2007).
165. Degenhardt, K. *et al.* Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell* **10**, 51–64 (2006).
166. Levine, B. Cell biology: autophagy and cancer. *Nature* **446**, 745–747 (2007).
167. Amer, A. O. & Swanson, M. S. Autophagy is an immediate macrophage response to *Legionella pneumophila*. *Cell Microbiol.* **7**, 765–778 (2005).
168. Amer, A. O., Byrne, B. G. & Swanson, M. S. Macrophages rapidly transfer pathogens from lipid raft vacuoles to autophagosomes. *Autophagy* **1**, 53–58 (2005).
169. Lugini, L. *et al.* Cannibalism of live lymphocytes by human metastatic but not primary melanoma cells. *Cancer Res.* **66**, 3629–3638 (2006).
170. Lugini, L. *et al.* Potent phagocytic activity discriminates metastatic and primary human malignant melanomas: a key role of ezrin. *Lab. Invest.* **83**, 1555–1567 (2003).
171. Damiani, M. T. & Colombo, M. I. Microfilaments and microtubules regulate recycling from phagosomes. *Exp. Cell Res.* **289**, 152–161 (2003).
172. Coopman, P. J., Do, M. T., Thompson, E. W. & Mueller, S. C. Phagocytosis of cross-linked gelatin matrix by human breast carcinoma cells correlates with their invasive capacity. *Clin. Cancer Res.* **4**, 507–515 (1998).
173. Montcourrier, P. *et al.* Characterization of very acidic phagosomes in breast cancer cells and their association with invasion. *J. Cell Sci.* **107**, 2381–2391 (1994).
174. Houghton, J. *et al.* Gastric cancer originating from bone marrow-derived cells. *Science* **306**, 1568–1571 (2004).
175. Parris, G. E. 2-Deoxy-D-glucose as a potential drug against fusogenic viruses including HIV. *Med. Hypotheses* **70**, 776–782 (2008).
176. Halaban, R. *et al.* Aberrant retention of tyrosinase in the endoplasmic reticulum mediates accelerated degradation of the enzyme and contributes to the dedifferentiated phenotype of amelanotic melanoma cells. *Proc. Natl Acad. Sci. USA* **94**, 6210–6215 (1997).

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene

AKT2 | CCL2 | CD44 | CD47 | CDH1 | *ERVWE1* | FN1 | HGF | LAMP1 | LGALS3 | MC1R | *MET* | MITF | MMP9 | *RHOC* | SELE | SIRPA | SNAI1 | SNAI2 | SPARC | TM7SF4 | TWIST1 | VTN

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