Chapter 2

Fusion in Cancer: An Explanatory Model for Aneuploidy, Metastasis Formation, and Drug Resistance

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Abstract

Aneuploidy, metastasis formation, and drug resistance are major issues to overcome in most cancers. If there exists common underlying proceedings for the formation of these phenomena is still unknown. The searching and thereby better understanding of causal mechanisms could promote the generation of drugs targeting the ultimate cause of these cancer promoting events. The merging of a cancer cell with another cancer cell or normal cell could be one explanation how cancer cells could gain advantageous properties and escape eliminating cell fates thereby foster cancer progression. This chapter summarizes how cell–cell fusion could directly be involved in the pathogenesis of cancer and which often cancer associated mechanisms, like viral infections or chronic inflammation, are hitherto proposed to trigger cell fusion in cancer context.

Key words Cell fusion, Cancer, Inflammation, Aneuploidy, Metastasis, Drug resistance, Hybrid cells

1 Introduction

Cell fusion is a common biological phenomenon, involved in various processes like fertilization, placentation, osteoclast formation, tissue regeneration, and wound healing [1–5]. The incorporation of additional nuclei while merging of two or more cells serves mainly to provide beneficial cell functions and to expand tissue size [1, 2, 6]. But the fusion of cells can also be utilized in the evolution of malignancies, like infection of enveloped viruses to host cells [7]. Furthermore, a presumption that cell fusion plays a fundamental role in cancer already exists since 1911 when Otto Aichel, a German pathologist, first postulated the hypothesis. He proposed the joining of cancer cells with white blood cells as an explanation for chromosomal abnormalities and gain of leukocyte characteristics, like the ability to migrate through the blood stream [8]. Long time disregarded in cancer context, the first supportive data appeared in the early 1960s, when Barski and Cornefert noticed the occurrence of a new type of polyploid "M"

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cells in cocultures of mouse low and high tumor producing cell lines. These hybrid cell clones showed stable characteristics and seemed to have a selective advantage in mixed cultures in vitro. Moreover, after inoculating a mixed cell population in C3H mice, the hybrids even seemed to suppress low-cancer cell clones, and thus, nearly pure "M" cell tumors were obtained [9–11]. Likewise, Goldenberg et al. reported of both human and hamster genes, obtained in highly malignant tumor cells which were originated after injecting certain human cancer cells into cheeks pouches of hamsters [12, 13]. Despite skepticism against the impact of such fusion events in pathogenesis, several work groups further investigated cell fusion processes in cancer context in vitro and in vivo, providing more and more supporting evidence for the possible menace of this phenomenon in cancer progression [14–17].

2 Cell Fusion and Cancer

Whether there exists common underlying mechanism for the formation of multinucleated cells by fusion in different cell types is still unknown. Viral proteins catalyzing cell fusion play an important role in human evolution, since syncytin the envelope gene of a human endogenous defective retrovirus is known to be the major mediator of placental cytotrophoblast fusion in vivo. The originated syncytiotrophoblasts are multinucleated cells that build the outer layer of the trophoblast of an early primate embryo [6, 18]. The Syncitin-1 protein seems to be also involved in further important cell fusion processes in humans, like fusion of myoblasts into muscle fibers [19] and osteoclast formation [20]. Bone resorbing osteoclasts are equally large multinucleated cells, in this case formed through cell fusion of mononucleated precursors with monocytic origin [21]. But it is well known that there are other fusion factors like CD47 [22] and DC-STAMP [23] needed for monocyte fusion leading to osteoclast formation and beyond that, fusion factors seem to be characterized by enormous functional diversity [20, 22–24]. Knock out experiments in breast cancer cells and endothelial cells both expressing the syncytin-1 receptor ASCT-2 using antisense oligonucleotides or a specific blocking peptide (CHR) showed a partly inhibition of spontaneous cell fusion in cocultures in vitro. But neither the siRNA nor the CHR peptide was able to effect total inhibition of cancer cell–endothelial cell fusion providing strong evidence that syncytin-1 is admittedly involved in cell fusion, but not the unique fusogenic protein expressed by breast tumor cells [25]. Moreover, data of Hobolt-Pedersen and colleagues reflected selectivity in the choice for fusion partners based on the heterogeneity among the cells with respect to their maturation state as well as the expression pattern and cellular organization of fusion factors [26]. Recently, Zhou and Platt

published a detailed overview regarding currently known molecules evolved in certain steps essential for the process of bringing two mammalian cells in close proximity and priming them for cell fusion [27]. However, while virus- or otherwise catalyzed cell fusion in embryogenesis as well as during additional common physiological processes of development leads mostly to advantageous tissue remodeling, cell fusion occurring in oncogenesis points up the dark side. In light of these facts, some cell types appear to share molecular components required for cell fusion [28], but which molecules in particular are involved in (cancer) cell fusion leading to pathogenesis remains poorly understood.

3 Inflammation and Cell Fusion

To finally understand cell fusion involved in cancer progression and its significance, one has to investigate the stimuli and the underlying mechanisms cells could use to gain new properties or to escape eliminating cell fates. For the moment, the most accepted hypothesis proposes inflammation as a trigger for cell fusion [29–31]. Chronic inflammation can lead to recruitment of HPSCs, MSCs and cells of the myelomonocytic lineage and subsequently tissue restoration by cell fusion in a late phase of physiological wound healing [32–37]. Transplantation experiments with bone marrow derived cells (BMDCs) gave evidence of fusion with cells of diverse tissues [32], but recently published data indicated an about 10 to 100-fold increased frequency of cell fusion events in liver, brain, and intestinal tissue in chronic inflammatory conditions [29–31, 38].

A correlation between inflammation, cell fusion, and cancer initiation is not far to seek, since the tumor environment resembles chronically inflamed tissue [32–35, 39]. The high fusogenic potential provided by chronically inflamed tissue for regeneration through cell fusion indicates that cell fusion between tumor cells and tumor cells or tumor infiltrating normal cells could be a common process in cancer progression [29, 38, 40]. Published data of Davies and coworkers provided in vivo evidence of inflammation enhanced fusion of bone marrow transplant derived BMDCs with intestinal progenitors in the intestine of a mouse inflammation model [29]. Since data of Rizvi and colleagues presume that also progenitor cells or even stem cells could be fusion partners for recruited cells of bone marrow origin [41], one may assume that CSCs as well as CSC-derived tumor progenitor cells could be target cells for cell fusion events, too. An impact of cell fusion in tumorigenesis catalyzed by infection of pathogens like viruses has been speculated by Duelli and Lazebnik [42]. Because virus mediated cell fusion is normally a tightly controlled process and restricted to a few cell types [43, 44], they supposed that most

arising hybrids are due to accidental cell fusion and hence likely to be abnormal [42]. Zur Hausen summarized that more than 20 % of the cancer burden worldwide can currently be linked to infectious agents, including viruses, bacteria or parasites [45]. Therefore, infectious agents could be a primary cause in several tumors, but if this abundance can be traced back to virus infections catalyzing cell fusion of tumor cells with normal cells or tumor cells with other tumor cells remains to be elusive.

To investigate whether common inflammatory gene signatures are shared among different tissues and inflammatory disorders Wang et al. analyzed the gene expression profiles of distinct rodent inflammatory disease models. Thereby they identified a common gene signature named "inflammatome" because of the significantly enrichment of genes involved in inflammation. Interestingly, the "inflammatome" also contained many inflammation-related genes proposed as cancer therapeutic targets, like TGFβ or VEGF [46, 47]. Furthermore, after comparing the gene signature with several known drug targets, candidate disease-associated genes from genome-wide association studies, and co-expression network modules developed from independent mouse and human cohorts, they suggested a causal nature of the "inflammatome" signature for several disorders including cancer and propagated 151 key regulatory driver genes [47]. With relation to the possible correlation between inflammation, cell fusion, and cancer initiation it would be interesting to investigate if some of these "inflammatome" genes also provide a key impact on cell fusion processes.

4 Aneuploidy

To understand the causality of cell fusion in tumorigenesis, one can question what was first—the already existing aneuploid (cancer) cell or the merging of two or more cells gave initially birth to the aneuploid cell. If two or more cells fuse together, they can either build heterokaryons—containing both nuclei separately—or the nuclei fuse generating synkaryons [48] (Fig. 1). In human tumors both can occur [49–51]. One prominent trigger for cell fusion might be high risk oncogenic human Papillomavirus (HPV) infection, the main cause of cervical cancer [52]. Binucleated cells are strongly observed in almost any HPV-positive low-grade squamous intraepithelial lesions before leading to accumulation of chromosomal aberrations in polyploid cells and co-overexpression of p53 and bcl-2 to high-grade squamous intraepithelial lesions [49, 53, 54]. Blau and colleagues reported the activation of previously silent genes typical of mature Purkinje neurons and repression of hematopoietic genes in nuclei of BMDCs in Purkinje heterokaryons generated in parabionts, indicating the use of cell fusion for nuclear reprogramming of BMDCs to a Purkinje

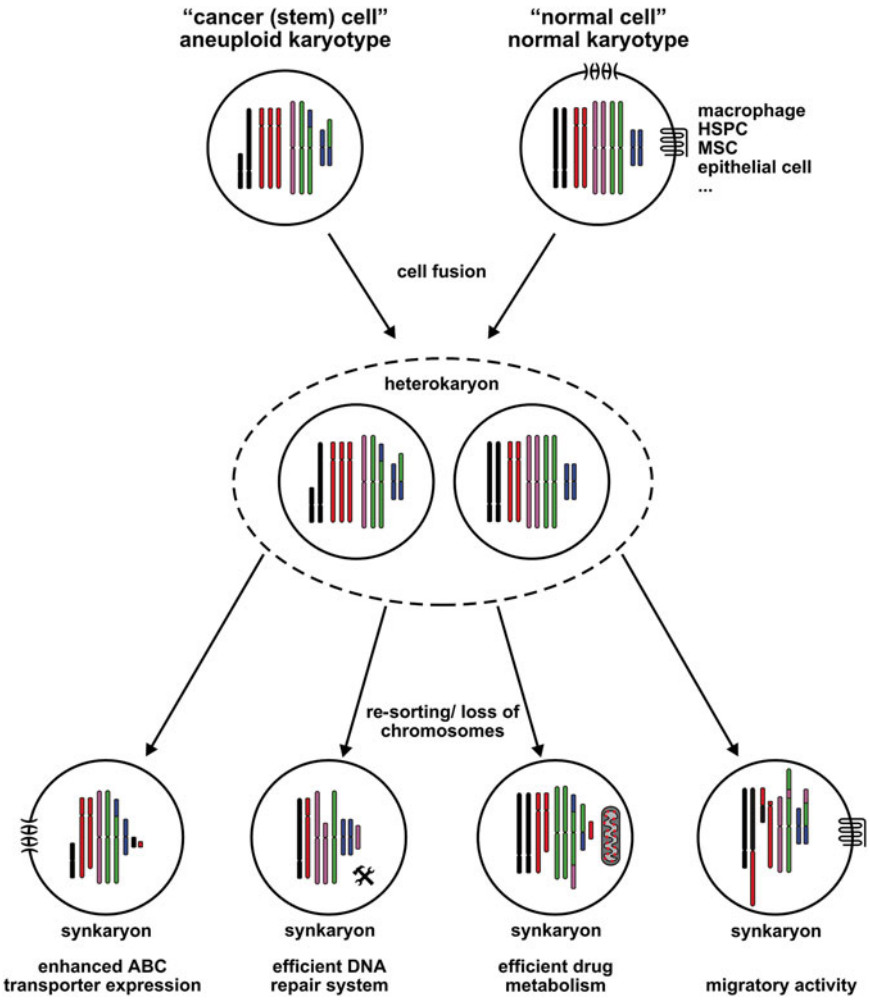


Fig. 1 Schematic scheme of cell fusion, heterokaryon-to-synkaryon transition and the origin of unique hybrid cells. Fusion of a cancer (stem) cell and a normal cell, like a macrophage or an epithelial cell, first results in heterokaryon formation: a hybrid cell with two distinct nuclei. During heterokaryon-to-synkaryon transition both nuclei will fuse. This process is accompanied with an unpredictable re-sorting and loss of chromosomes ultimately resulting in the evolution of discrete unique hybrid cells, which could exhibit novel properties

neuron fate [30]. These findings of Blau et al. reveal the occurrence of genetic alterations after fusion of distinct cell types without directly exchanging genetic material through heterokaryon-to-synkaryon transition.

However, the formation of synkaryons is strongly associated with chromosomal rearrangements, like deletions, translocations or loss of whole chromosomes [48, 55, 56] (Fig. 1) and chromosomal abnormalities can nearly be found in all solid tumors and also in hematologic cancers [57, 58]. Furthermore, especially in the early stages of several forms of cancer the phenomenon of whole chromosome set multiplication (polyploidy), particularly

the 4n state (tetraploidy), frequently occurs [59, 60]. Since the tetraploid state is proposed as an unstable intermediate that can cause aneuploidy and subsequently cancer, e.g., through loss of tumor suppressor genes [61–63], one can suggest cell fusion processes as possible initiator of the "diploidy–tetraploidy–aneuploidy" pathway in tumor progression. Duncan and his group reported in 2009 that fusion-derived hepatocytes can generate daughter cells containing only one-half DNA content and partly extensive aneuploidy after investigating liver tissue restoration by cell fusion in bone marrow transplanted mice. These observations were contrary to all their expectations, because one might rather expect at least the tetraploid state among fusion-derived hybrids. To confirm ploidy reduction of initially tetraploidy or polyploidy fusion-derived hybrids and exclude other mechanisms like transdifferentiation or horizontal gene transfer, they further intensively examined this phenomenon by tracing marker segregation in hybrid cells. Ploidy reduction events were ultimately associated with a high degree of marker/chromosome loss in a non-random fashion as well as independent marker segregation and could result in genetically heterogeneous diploid daughter hepatocytes. The authors proposed ploidy reduction as a feasible mechanism increasing liver diversity, but it could also generate a subset of hepatocytes lacking proper growth control and harboring oncogenic potential, what might contribute to tumorigenesis [64].

Boveri already proposed aneuploidy as a cause for cancer more than a century ago, after observing a correlation between chromosomal aneuploidy and cancer [65]. Since then, the significance of chromosomal aberrances has been discussed many times either simply as outgrowth of malignant transformations [66] or as a matter of fact as driving force of tumorigenesis [67]. There are several origins by which a cell might gain or lose chromosomes. Holland and Cleveland reviewed in 2012 defects of mitotic checkpoint signaling, chromosome cohesion defects, multipolar mitotic spindles as well as hyperstable kinetochore–microtubule attachments as possible pathways leading to an aneuploid karyotype during mitosis [68]. Anyway, Boveris' observations could equally be underpinned by the cell fusion theory, since heterokaryon-to-synkaryon transition as consequence of cell fusion is concomitant with chromosomal rearrangements [48]. Thus, cell fusion could result in a higher degree of aneuploidy and foster genomic instability, thereby generating genetic diversity and giving rise to unique hybrid cells. These assumptions hypothesize cell fusion neither as the origin nor as a consequence of aneuploidy in cancer cells, but rather as a driving force of chromosomal abnormalities and ultimately tumor progression. Li and colleagues proposed aneuploidy itself as destabilizing force and initiator for autocatalytic karyotype evolution, caused by the impairment of chromosomal proteins, abnormal numbers or structures of spindle apparatus proteins and

centrosomes [69]. This process could subsequently result in lethal, preneoplastic and ultimately neoplastic karyotypes [70, 71].

Surviving aneuploid cells harbor not only increased genomic instability [72], they can also be associated with a strongly decreased proliferation potential and cellular fitness, due to the proteotoxic stress or altered cell metabolism [73–75]. Fibroblasts isolated from human patients with trisomy 21 proliferate more slowly than diploid control cells [74]. Similar deleterious effects on cell growth could be found in yeast strains containing one or more additional chromosomes [73, 76]. Most of the aneuploid yeast strains exhibited a growth defect during G1 phase concomitant with G1 cyclin accumulation [75, 76]. Moreover, extra copies of single chromosomes in budding yeast strains led to increased chromosome loss, mitotic recombination, and defective DNA repair mechanism [72].

Recently, Silk et al. reported that while on the one hand a moderate elevated level of whole-chromosome missegregation resulting from a reduction in centromere-bound motor protein CENP-E could result in an enhanced rate of tumor induction, on the other side increasing the rate of chromosome missegregation beyond a certain threshold rather suppresses tumor growth by causing cell death. These data where based on experiments exacerbating chromosome missegregation in CENP-E$^{(+/-)}$ mice by reducing levels of the mitotic checkpoint component Mad2, too. Furthermore, Silk et al. proposed that the additional contexts in which increased chromosomal missegregation resulting from reduced CENP-E suppresses tumor formation have already a preexisting, elevated basal rate of CIN that is exacerbated by reduction of CENP-E. Consequently, they proposed a treatment with CENP-E inhibitors useful in CIN tumors of breast, lung and colon [77].

In this context, an interesting fusion study between normal cells and distinct cancer cell types, concerning the proliferative and tumorigenic behavior of generated hybrids, was undertaken by Harris and Klein already in the early 1970'. First, they observed that the fusion of a cancer cell with a normal cell, what resulted in hybrids containing approximately the sum of chromosome numbers of parental cell lines, seemed to suppress the highly malignant character of the tumor cell as measured by lower proliferation potential and heavily reduced capability to produce tumors in mice. But after a few generations of continuing cultivation of hybrid cells, they began to lose chromosomes by the time resulting in aneuploid cells which regained the ability to proliferate progressively and give rise to tumors in vivo. In addition, fusion-derived hybrids which showed already after primary fusion a rapid and substantial loss of chromosomes were highly tumorigenic, too, indicating that fusion events between normal cells and cancer cells generate malignant hybrids with much higher frequency [78–80].

The data are partly controversial concerning the proliferative and tumorigenic behavior of aneuploid (hybrid) cells. However, fact is that aneuploidy means enormous burden for the cell metabolism and can lead to reduced cell growth and genomic instability. But what on the one hand seems to have a negative impact on cells could on the other site harbor the main advantage of cell fusion. Hybrid cells arisen through cell fusion between a tumor cell and a tumor cell or a normal cell undergoing radical genomic changes while heterokaryon-to-synkaryon transition, could adopt a diversified phenotype and thereby promoting tumor heterogeneity.

5 Aneuploidy—A Consequence of Cell Fusion?

The phenotype of hybrid cells arisen in a tumor context through cell fusion processes is not predictable. It may be assumed that the majority of cells (about 99 %) will dye or become quiescent after merging, and it is considered that only about 1 % will contribute to tumor progression [58]. Beneficial attributes due to chromosomal aberrances could be one selection mechanism, how cancer cells could survive in the selective pressure of the tumor microenvironment and thereby promoting tumor progression. Moreover, it could explain the extended heterogeneity often found in tumor cell populations [81, 82] (Fig. 1 and 2) and provide cancer cells with an increased variability to adapt in foreign tissues [83–85].

The several possibilities of phenotypic outcomes after cell fusion of cancer cells and normal cells were nicely pointed up by Rachkovsky et al. in melanoma–macrophages hybrids. Some hybrid clones showed a decreased capacity to induce metastasis formation and some few hybrids completely lost the metastasis formation capacity, even though the majority of the hybrids possessed an increased migratory behavior in comparison to the parental cells [16]. These findings and the sharp distinction concerning the melanin content of emerging hybrids independent of the reduced or enhanced migratory potential display very well, how hybrid cell clones can differ among each other. Evidence of chromosomal translocations in cell fusion-derived cells and cancer context had been provided by Jacobsen et al. in 2006. Besides carrying a mixture of both human and mouse chromosomes, human–mouse chromosomal translocations were also found in about 8 % of the hybrids, which has arisen from cell fusion between malignant human breast cancer cells and mouse stromal cells [86].

But besides chromosomal content exchange, Dittmar et al. reported that the fusion of human breast cancer cells with human breast epithelial cells containing stem cell characteristics, can give rise to stable hybrid cell clones containing nearly the double MCN ranging from 78 ± 11 (M13MDA435-1 hybrid clone) to 88 ± 13 (M13MDA435-3 hybrid clone) as compared to the

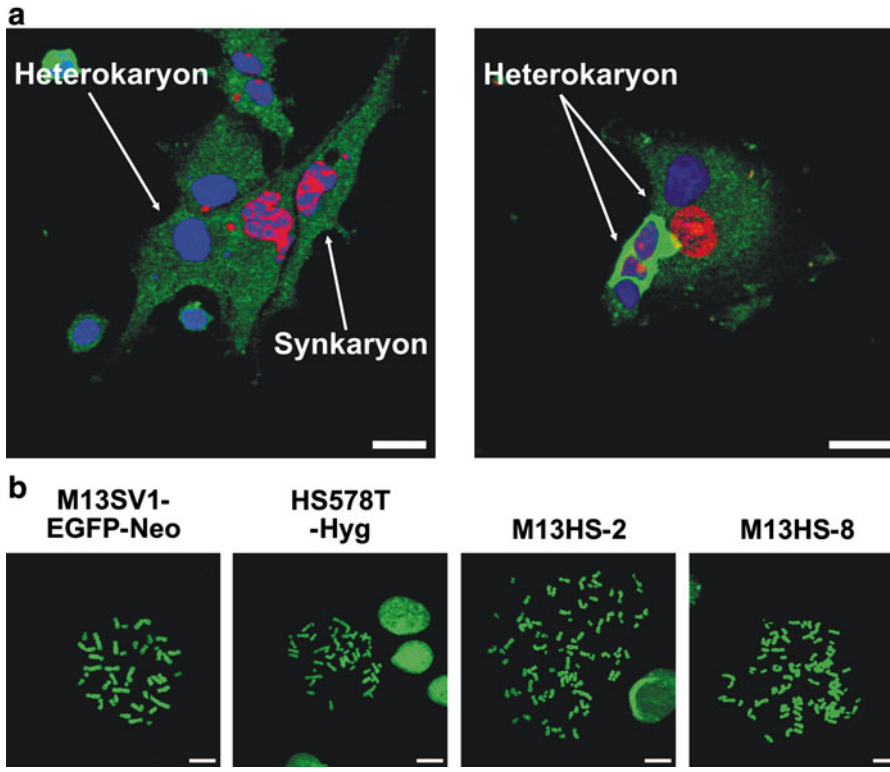


Fig. 2 Spontaneous fusion between HS578T breast cancer cells and M13SV1-EGFP-Neo breast epithelial cells. (**a**) M13Sv1-EGFP-Neo and HS578T breast cancer cells, which were previously fed with BrdU, were co-cultivated for 48 h. Subsequently, cells were fixed with 4 % paraformaldehyde and stained for EGFP (*green*) and BrdU (*red*) and DNA (Syto60, *blue*). Heterokaryons are characterized by being positive for EGFP and containing at least two nuclei: a tumor cell derived BrdU positive and an epithelial derived BrdU negative. Synkaryons are characterized by EGFP expression and only one BrdU positive nucleus. (**b**) Hybrid cells derived from HS578T breast cancer cells and M13SV1-EGFP-Neo breast epithelial cells exhibit an increased mean chromosomal number. Metaphase chromosomes of HS578T and M13SV1-EGFP-neo parental cells and hybrid cell clones M13HS-2 and M13HS-8 were stained with Sytox60. Shown are representative data. Bar = 20 μm

parental cell lines (Fig. 2). In addition to it, these hybrid cells owned jointly a unique gene expression profile, an elevated proliferative activity, as well as an altered migratory behavior, albeit not all stable human breast cancer–breast stem cell hybrids revealed an increased resistance to chemotherapeutic drugs [51, 87]. In contrast, the same group found a MCN of rather half the sum of parental cells in stable hybrid cell clones from a coculture of murine BMDCs and the murine mammary carcinoma cell line 67NR-Hyg. However, the mBMDC–67NR-Hyg hybrids also possessed an altered migratory behavior as well as an enhanced drug resistance [88].

Interestingly, multipolar metaphases and unequal divisions with lagging DNA content could not only be observed in breast cancer–MSC hybrid cell cultures but also during growth of tumor

xenografts in vivo as related by Rappa et al. in 2008 [50]. Moreover, multipolar mitoses were also observed in homotypic hybrids of human parental breast carcinoma cell lines MA11 and MDA-MB-231, albeit in a lower frequency than in breast cancer–MSC hybrids [50].

In addition to structural or numerical chromosome aberrations, what could be traced back to either a loss of chromosomes during heterokaryon-to-synkaryon transition [48] or due to mitotic ploidy reduction [64, 89], Goldenberg et al. reported about unidentifiable chromosomes found in human cancer cell–hamster cell hybrids [90]. Studies of Andersen and colleagues demonstrated that bone-resorbing osteoclasts from myeloma patients contained nuclei with chromosomal translocations of myeloma B-cell clone origin in over 30 % of the osteoclast population. The transfer of malignant DNA to osteoclasts reflects a highly efficient mechanism how myeloma cells could directly affect and promote bone destruction by generating active bone resorbing hybrid cells. These observations further suggest cell fusion as driving force of malignancy through nuclear reprogramming in osteoclast–myeloma clone hybrids [91]. Furthermore, Lu and Kang revealed that in vitro or in vivo spontaneous cell fusion of two bone- and lung-tropic sublines of the MDA-MB-231 human breast cancer cell line can give rise to stable hybrid cells acquiring dual metastatic organotropism. The synkaryonic hybrids assimilated organ-specific metastasis gene signatures from both parental cells and were genetically and phenotypically stable over long-term passages [92].

Taken together, all these reported data give evidence for cell fusion processes between cancer cells and normal cells and show that the initially disadvantage of aneuploidy in hybrid cells can be concomitant with the main opportunity of originating extensive phenotypic heterogeneity through coincidental chromosomal rearrangements. The loss and/or resorting of chromosomes as well as structural genomic changes can give rise to hybrid cell clones with diversified phenotypes either exhibiting tumor progressive behavior like enhanced migratory activity, or at the same time provide cells with antitumor properties. However, these data also shine a light on how unpredictable and moreover incalculable the phenotype of emerging hybrid cells can be.

6 Cell Fusion and Metastasis

Until now tumor cell dissemination from primary solid cancers and invasion to distant organs is the ultimate cause of death in about 90 % of cancer patients, because it is the most uncontrollable and the least understood state of tumor progression [93]. Metastatic spread is a complex multistep process including cancer cell

migration and invasion, intravasation and circulation in the blood stream, extravasation and ultimately adaption to the new microenvironment for being capable to build a secondary tumor [94, 95]. For all these steps tumor cells must extensively modulate their phenotype to obtain enhanced migratory activity, navigate of themselves through new environments and gain the ability to understand various stimuli of cell–cell interactions. It had been proposed that tumor cells need to undergo epithelial-to-mesenchymal transition (EMT)—a process of transformation in which they lose epithelial characteristics and achieve a more invasive stem cell-like shape to disseminate in the blood stream [96, 97]. Albeit most EMT data are based on in vitro studies, there is also evidence for well-differentiated epithelial cells in metastatic lesions that fail to express molecular EMT markers [98], suggesting that tumor cells do not desperately need to undergo EMT to adopt a metastatic phenotype in vivo [99]. However, cell fusion of a tumor cell with a cell harboring an mesenchymal cell shape with enhanced migratory potential, like macrophages or other tumor infiltrating leucocytes, could be one possible mechanism how metastatic transformation of cancer cells could occur enabling them to separate from the primary tumor mass [100, 101].

It has been shown several times that macrophages are highly fusogenic cells that do fuse with distinct carcinoma cells in vitro [102–104] and thereby could adopt increased motility. Fusion experiments of mouse Cloudman S91 melanoma cells with macrophages led to hybrid cells revealing an increased migratory activity in vitro and tended to become highly melanotic [16, 105–107]. Moreover, in vivo evidence of macrophage fusion with transformed intestinal tissue epithelial cells was provided by Powell et al. They used a mouse parabiosis model to introduce green fluorescent protein (GFP)-expressing circulating blood cells into an intestinal tumor-bearing $Apc^{Min/+}$;ROSA26 mouse, finding that fusion between mainly macrophages, but in a less frequent rate also B- and T-lymphocytes, and tumor epithelium occurs during the natural progress of tumorigenesis. Comparing of the transcriptome profiles showed that hybrids shared a transcriptome identity with both parental macrophages and epithelial cells, in addition to a set of unique transcripts including a number of genes known to be modulated in metastasis [40].

But an altered proliferative as well as migratory activity of cancer cells can also be acquired through cell fusion of cancer cells with distinct normal cells, like BMDCs or even breast epithelial cells as Dittmar and coworkers reported in several studies. Interestingly, hybrids derived from spontaneous cell fusion events between murine BMDCs and 67NR-Hyg mouse mammary carcinoma cells as well as hybrids of human breast epithelial cells containing stem cell characteristics and cells of the human breast cancer cell line MDA-MB-435-Hyg responded in cell migration assays

with a markedly increased locomotory behavior to growth factors like EGF or the chemokine SDF-1α, whereas stimulation of parental cells resulted in unchanged or even decreased migratory activity [51, 88, 108]. Moreover, data of Berndt et al. nicely demonstrated how cell fusion of non-responding human breast epithelial cells containing stem cell characteristics and human breast cancer cells can give rise to a CCL21 sensitive hybrid cell line responding with enhanced migration in vitro. Since the CCL21/CCR7 axis has been associated with lymph node metastasis of different primary carcinomas, these data are in view with the hypothesis that cell fusion processes could lead to elevated migratory activity of cancer cells and thereby enhancing tumor progression [109].

In addition to in vitro studies the migratory behavior of hybrids has also been traced in vivo. Murine Cloudman S91 melanoma–macrophage hybrids not only revealed an increased migratory activity in vitro but showed a more aggressive metastatic behavior in vivo as well [16, 104]. Goldenberg and his group reported the transfer of human genes to normal xenogeneic host cells via heterokaryon-to-synkaryon formation after long-term xenogeneic passage of human Hodgkin lymphoma cells transplanted into hamster cheek pouches. Beside the association of malignancy and the survival of functional human genes, the xenografts were highly metastatic in the animal host [110]. Recent data of Li and coworkers revealed that hybrids from fusion of human low metastatic HepG2 cells × rat MSCs exhibited beside elevated expression of EMT markers, matrix metalloproteinase 2 and 9 activities and aneuploidy, an enhanced in vitro invasion and migration. Furthermore, hybrids generated increased numbers of lung and liver metastases, indicating an elevated metastatic potential also after in vivo xenograft transplantation [111].

Characterization of human MSCs and human MDA-MB-231 breast carcinoma cell hybrid xenografts showed a highly increased metastatic phenotype associated with >10-fold elevated expression of aldehyde dehydrogenase (ALDH) 1A3 isoform, the main ALDH isoform in breast cancer stem cells [112] as well as overexpression of mesenchymal genes like secreted protein, acidic, cysteine-rich (SPARC) in comparison to the parental MDA-MB-231 cells or xenografts [50]. Interestingly, upregulation of SPARC was also observed by Chakraborty in melanoma–macrophage hybrids [15]. Since overexpression of SPARC has often been associated with the most aggressive and highly metastatic tumors concomitant with poor prognosis [113] elevated SPARC expression levels in tumor cell–MSC hybrids may suggest cell fusion as possible mechanism to acquire such mesenchymal markers.

Recently, the first evidence of in vivo human cancer fusion has been given by Lazova et al. Carrying out a genotype analysis of a metastatic melanoma lesion in the brain that had arisen following allogeneic bone-marrow transplantation using forensic short

tandem repeat length-polymorphisms and sex chromosomes to distinguish donor and patient genomes, revealed evidence for both donor and patient alleles in tumor cells throughout the tumor [114]. These data are strongly indicating that a BMDC–tumor cell fusion event had initiated the generation of this tumor and that the abundance of such events in vivo could harbor a substantial impact on understanding metastatic spread.

In summary, several independent studies demonstrated that cell fusion between tumor cells and normal cells can very likely give rise to hybrids exhibiting increased locomotory activity. Understanding and targeting the underlying molecular mechanism leading to metastatic spread could be one of the most important challenges to obstruct further tumor development.

7 Drug Resistance and Cancer Stem Cells

While metastatic spreading and establishment into foreign tissues is one important property of tumor progression, the development of drug resistance mechanisms of cancer cells before or while chemotherapeutic treatment is not less precariously. Some studies provide evidence that cell fusion could give rise to drug resistant hybrid cells even if parental cells show sensitiveness for applied therapeutics [115]. So did Miller et al. already in 1989, when demonstrating that cell fusion of 5-fluorouracil resistant 44FTO with methotrexate resistant 168FAR tumor cells produced hybrids resistant to both drugs and in addition even more resistant to mephalan than either parental cell line [115]. Hybrids of mouse MSC–67NR-Hyg where shown to harbor a markedly enhanced resistance towards doxorubicin, 17-DMAG, etoposide and paclitaxel, which was associated with increased Abcb1a/Abcb1b expression levels. Involvement of certain Abc multidrug transporter was functionally confirmed by blocking Abcb1a/Abcb1b with the inhibitor verapamil, what completely overrode the hybrid cell resistance towards doxorubicin. However, resistance towards 17-DMAG, etoposide and paclitaxel was not more than partly decreased in hybrids indicating the involvement of additional multidrug reporters or the requirement of further drug resistance mechanisms in cancer cell hybrids [88]. In contrast, analysis of gene expression profiles correlated to doxorubicin drug resistance in human MCF-7 breast cancer cell derived hybrids, showed increased levels of ABCB1 and ABCB5 expression in fused cells. In addition, Yang observed that low doses of doxorubicin had a positive effect on the fusion rates of breast cancer cells in vitro, indicating that a chemotherapeutic drug by itself could favor cell fusion events [116]. This phenomenon could be traced back to the upregulated ABCB5 expression in MCF-7 cells after doxorubicin treatment, since the ABCB5 expression could directly be involved

in cell fusion processes [117]. However, dose-dependent upregulation of specific multidrug resistance transporters while exposure to chemotherapeutics seems to be a common mechanism of certain cancer cells circumventing ultimately cell destruction [118].

Enhanced resistance towards therapeutic drugs has also been shown for cancer stem cells (CSC). CSCs are a small subpopulation in tumors cells containing a high capacity to rebuild parental tumors upon transplantation. It is also assumed that CSCs exhibit common stem cell characteristics like self-renewal, differentiation in several lineages, and tissue restoration [119, 120]. Moreover, CSCs are not only proposed to induce primary tumors or metastasis in distinct organs but also to give rise to cancer recurrence after therapy treatment [121–125]. These assumptions are based on the ability to survive cancer treatment most likely because of the upregulation of multidrug resistance transporters, the slow cell cycle activity and the apparently highly efficient DNA damage checkpoint [126–129]. However, it had also been shown that the nature of CSC is variable in different cancer types and that CSCs are not necessarily more resistant or slower proliferating than the rest of the tumor cell population [130–133]. Thus, one challenge to overcome in cancer treatment strategies is defining the characterization of these cell subpopulations in the tumor mass. Several studies have shown that cell fusion occurs in solid cancers in vivo between tumor cells and tumor cells or tumor cells and other cells of the tumor environment [40, 41, 86, 90, 134]. Furthermore, there is evidence to suggest that hybrid cells, derived from such cell fusion events, could exhibit altered properties including an increased tumor formation [135], an enhanced resistance to undergo apoptosis [136] as well as an enhanced drug resistance [51, 58, 137].

According to all these given data, it is not far to seek that Bjerkvig and coworkers proposed the cell fusion hypothesis as origin of CSCs [48]. Later the hypothesis was extended and newly defined by Dittmar et al., supposing that cell fusion could originate recurrence CSCs, a cell type defined through initiation of recurrences exhibiting an "oncogenic resistance" phenotype [87, 125, 138]. These theory is mainly based on the observation that tumor relapse can be associated with resistance to first-line therapy as well as a more aggressive phenotype than the primary tumor, which is in marked contrast to the original CSC hypothesis [138, 139]. If a CSC survives the targeting cancer therapy you would rather expect a regrowing tumor phenotypically similar to the original tumor, but this is not merely the case what indicates that the recurrence can only be induced by a new CSC type (rCSC).

Furthermore, Dittmar et al. proposed the rCSC could initial originate by cell fusion while tumor treatment by itself. Therapies often cause massive tumor tissue destruction and ultimately extensive inflammatory responses resulting in the recruitment of

immunocompetent cells like macrophages, or other cell types known to be involved in tissue restoration by cell fusion like MSCs and HSPCs [1, 33, 34, 36]. Published data of Rizvi et al. proposed even long-lived intestinal progenitor or stem cells as fusion partners for recruited BMDCs into the intestine of BMDC transplanted mice [41].

Although an increased incidence of cell fusion processes while cancer treatment cannot be ruled out since inflammation is a positive trigger for cell fusion [29, 30, 38], there are only few studies concerning cell fusion of tumor cells with tumor cells or normal cells or even stem cells leading to hybrids exhibiting stem cell like characteristics. Recently published data of Ding and colleagues presaged some CSC-like characteristics in generated hybrids. Spontaneous cell fusion of breast cancer cells and into T2 macrophage lineage differentiated U937 cells ($U937D_2$) showed a significantly decreased proliferative ability using the CCK-8 assay compared to parental MDA-MB-231 and MCF-7 cell lines, respectively. The hybrid proliferative index averaged no more than 21.5 % and 34.6 % of the parental cells. Moreover, hybrids gained the ability to form more spheres with larger sizes after being plated in non-adherent mammosphere culture conditions for 1 week. In addition, albeit only the MCF-7 and $U937D_2$ hybrids revealed a more invasive phenotype, both hybrid cell clones showed an enhanced migratory activity in vitro and were highly tumorigenic after injecting in mice [140].

These data not only illustrate cell-to-cell fusion as a common process in health but also provide evidence for an involvement in tumor pathogenesis. Due to the many cell types qualified for cell fusion combined with the not predictable phenotype of cancer hybrid cells mainly caused by aneuploidy and chromosomal instability, hybrids could acquire new features. Hybrids exhibiting stem cell characteristics, a higher motility as well as a better adaptability being presumably aneuploid could ultimately adopt better to foreign tissue, thereby promoting tumor progression. Overall, the outcome of possible cell fusion processes triggered by inflammation or even by therapeutic treatment must not be neglected and moreover, further approaches are required to scrutinize the precise details of cell–cell fusion mechanisms in cancer progression to receive the ability of developing new targeting strategies.

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