Cell fusion: A hidden enemy?

The recent findings that cell fusion may be involved in stem cell differentiation (Medvinsky and Smith, 2003) raise a possibility that cell fusion has undiscovered functions, some of which can perhaps be found by revisiting the old ideas that cell fusion can promote disease, especially cancer.

Cell fusion is a process in which two or more cells become one by merging their plasma membranes. The ability of a cell to fuse to other cells is referred to as fusogenicity. The progeny of cell fusion are known as hybrids. Perhaps the best-known hybrids are hybridomas, which are made by fusing myeloma cells with lymphocytes to produce monoclonal antibodies. Although cells can be easily fused in the laboratory using readily available chemicals, cell fusion in live organisms appears to be a complex, poorly understood, multistep process that involves cell-cell recognition, cell adhesion, and membrane fusion (reviewed in Hernandez et al., 1996).

Cell fusion is a part of normal development and tissue homeostasis

Our life begins with fusion of our parents' gametes. A pregnancy depends on normal functioning of the placental barrier, the main part of which is the syncytiotrophoblast, a gigantic syncytium (a cell resulting from fusion of numerous cells) whose surface area can reach 10 square meters (reviewed in Benirschke, 1998). As the embryo develops, its muscles are formed by fusion of myoblasts into syncytia through a multistep process that involves products of multiple genes (reviewed in Taylor, 2002). In the adult body, the maintenance of the bones is controlled in part by osteoclasts, which are multinuclear cells formed by the fusion of mononuclear progenitors. Macroscopic foreign objects, such as a splinter or an implanted device, are encapsulated by foreign body giant cells (FBGC) that attempt to dissolve the intruder and are thought to be formed by fusion of mononuclear precursors (reviewed in Anderson, 2000). Langhans cells, a variation of FBGC, are found in tuberculosis patients at the sites of local inflammation.

The observations that embryonic stem cells may differentiate into multiple cell types through cell fusion suggested a new role of cell fusion in mammalian development (Terada et al., 2002; Vassilopoulos et al., 2003; Wang et al., 2003; Ying et al., 2002), although alternative explanations for these observations have not been ruled out (McKay, 2002). Studies in the nematode *C. elegans* (reviewed in Shemer and Podbilewicz, 2000; Mohler et al., 2002) provide evidence that cell fusion can be a major part of development, at least in invertebrates. About one-third of the cells that are born in this organism are subsequently fused into 44 syncytia in a reproducible and stereotypic way. Remarkably, even the side of a cell that will fuse is predetermined.

In summary, cell fusion of normal somatic cells is a tightly controlled process that is restricted to only a few cell types in humans, and results in terminally differentiated multinuclear cells incapable of proliferation. Intriguingly, tumor cells appear to violate strict rules of cell fusion.

Cell fusion and cancer

The idea that cell fusion contributes to cancer progression was introduced almost 100 years ago with a proposal that malignancy is a consequence of hybridization between leukocytes and somatic cells (reviewed in Rachkovsky et al., 1998). Sixty years later, this idea was expanded by proposing that hybridization of tumor cells with lymphocytes results in metastatic cells (Mekler, 1971), and that cell fusion promotes the phenotypic and genotypic diversity of tumors (Warner, 1975). Several lines of evidence support at least some of these notions.

Many tumor cells are fusogenic

The propensity of various tumor cells to fuse spontaneously in tissue culture has been a known, though unexplained, phenomenon for many decades. In fact, this property of tumor cells was used to make somatic cell hybrids before fusion with polyethylene glycol (PEG) or viruses had been developed (Barski and Cornefert, 1962). Some tumor cell lines are so fusogenic that they fuse spontaneously more efficiently than in the presence of PEG, a difference explainable by the toxicity of this agent (Wakeling et al., 1994). The ability to fuse is not limited to a particular cell or tumor type and can occur between tumor cells as well as between a tumor and a normal cell (reviewed in Larizza and Schirrmacher, 1984). Tumor cell fusion is not limited to tissue culture. Human tumor cells injected into hamsters produced metastases formed by hybrids of human and hamster cells (Goldenberg et al., 1974). In another study, treatment of chimeric mice with a carcinogen resulted in tumors, in which about 1% of cells had marker alleles from both parental strains, an observation explainable by cell fusion (Fortuna et al., 1990). Interestingly, despite these observations, cell fusion is rarely, if at all, considered in contemporary studies that use experimental models of cancer.

Because the evidence for cell fusion in human cancers is indirect, it is not as compelling as that for experimental tumors. One observation is that premature chromosome condensation (PCC) is observed in tumor cells (Atkin, 1979; Kovacs, 1985; Williams et al., 1976). PCC is a typical result of a fusion between cells in different stages of the cell cycle, although PCC can be also induced in mononuclear cells, for example by caffeine. Cell fusion could also explain the origin of multinuclear tumor cells in which nuclei undergo asynchronous DNA synthesis or mitosis, even though alternative explanations of this phenomenon are as plausible (Sheehy et al., 1974). It is unclear whether the scarcity of reports on cell fusion in human tumors is due to rarity of this event, to a difficulty to detect it, or to insufficient interest in this subject. The last possibility should not be underestimated. For example, the number of reports on apoptosis that were published during the last ten years is about 100 times higher than that published in the preceding twenty. With all likelihood, this difference is due to a change in the subject's popularity rather than a change in incidence or role of apoptosis in studied organisms.

Hybrids can be more malignant than the parental cells

Importantly, while physiological fusion of normal somatic cells produces nonproliferating differentiated multinuclear cells, fusion of tumor cells results in proliferating hybrids. The survival rate of these hybrids in tissue culture can be as high as 1% (Miller et al., 1988; Wakeling et al., 1994). Considering that the rate of cell fusion in experimental tumors was also estimated at 1% (Fortuna et al., 1989), a 1 cm^3 tumor of about 10^9 cells may harbor 10^5 proliferating hybrid cells. The question is, can any of these cells be more malignant than their parents?

In fact, one of the early reports on spontaneous cell fusion came from an observation that cultures containing a mixture of two mouse cell lines were occasionally overgrown by a new cell line, whose karyotype was the sum of the parental karyotypes

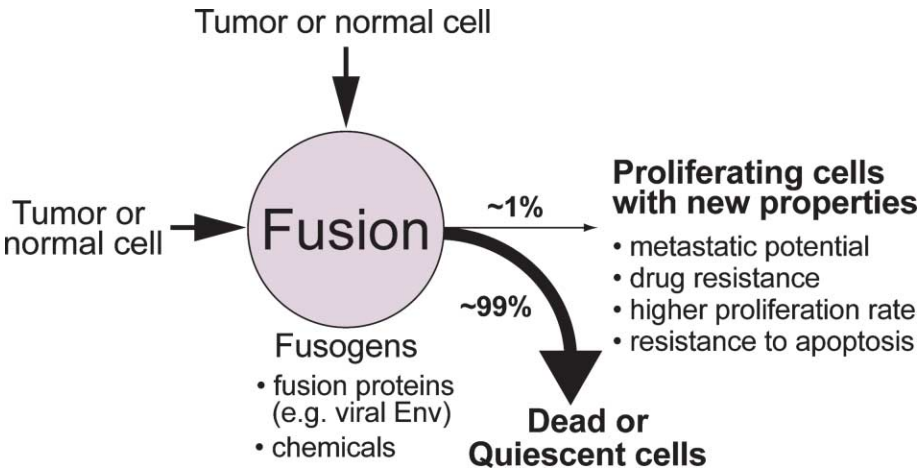

Figure 1. Promiscuous cell fusion as a generator of cell diversiy.

Physiological fusion of normal cells in the body is tightly controlled. However, cellular or viral fusion proteins, as well as environmental factors, may induce cell fusion of bot tumor and normal cells. Although the majority of the resulting cells will die or be quiescent, a small fraction will be able to proliferate. Some of the proliferating cells may have unwanted properties, such as increased malignancy.

(Barski and Cornefert, 1962). This observation indicated that the new cell line is a hybrid, and that hybrids can grow faster than their parents. The subsequent studies indicated that, at least in experimental systems, hybrids can be more drug-resistant and more metastatic than the parental cells (Figure 1).

Cell fusion can contribute to drug resistance

Cell fusion can consolidate resistance to various drugs by combining genes responsible for resistance to various agents. For example, fusion of tumor cells that were resistant to 5-fluorouracil with tumor cells resistant to methotrexate produced hybrids that were resistant to both drugs (Miller et al., 1989). An unexpected observation was that the hybrids were resistant to mephalan, a drug to which both parental lines were sensitive. This observation emphasized the difficulty in predicting the diversity that cell fusion can create. Our own experiments indicated that fusion of drug-sensitive transformed cells to primary cells results in heterokaryons that are resistant to apoptosis (Duelli and Lazebnik, 2000). Although this effect lasted only a few days (our unpublished data), it is not unreasonable to speculate that even a temporary resistance may allow a small fraction of tumor cells to survive a session of chemotherapy.

Cell fusion can promote the ability to metastasize

The ability to metastasize is, arguably, the deadliest property of cancers. A proposed link between cell fusion and metastasis can be described as the "wolf in sheep's clothing" model. The model suggests that a tumor cell becomes metastatic by fusion to normal cells that travel throughout the body freely, such as lymphocytes or macrophages. Several studies support this idea (reviewed in Pawelek, 2000; Rachkovsky et al., 1998). One study found that a parental cell line, when injected in a mouse, produced hundreds of colonies in the liver, while a derivative line produced only a few (Kerbel et al., 1983). Surprisingly, when a colony formed by the derivative line was injected into a new host, it produced as many colonies as did the parental cell line. The analysis of histocompatibility markers and karyotypes of the host mice, the injected cells, and the resulting metastases led to the conclusion that the increase in the metastatic potential was due to a fusion with the host cells. This conclusion was supported by the finding that the high metastatic potential can be restored in the derivative cells by fusing them with mouse bone marrow cells before injection.

Other studies discovered that cell fusion can determine the tissues into which tumor cells metastasize. For example, fusion of nonmetastatic mouse plasmacytoma or myeloma cells with lymphocytes or splenic dendritic cells not only resulted in metastatic hybrids, but the target tissues of metastases varied with the type of normal cells used as well (De Baetselier et al., 1984). One can only wonder whether implications of this finding are considered in developing cancer vaccines, which involves injecting patients with hybrids between tumor cells and normal dendritic cells. Because tissues that are common sites of metastases are normally rich in macrophages, the "wolf in sheep's clothing" model argues that tumor cells gravitate to these sites because, as macrophage hybrids, they acquire macrophage's tropism (Munzarova and Kovarik, 1987).

Another possibility is that tumor cells can acquire the "sheep's clothing" by fusing to a normal cell of a tissue, thus producing a tumor cell that can grow in the new environment. This mechanism is not unlike that proposed for the stem cell differentiation, where a stem cell differentiates by fusing to a resident cell from a host tissue (Medvinsky and Smith, 2003).

Cell fusion can increase tumor cell diversity

Perhaps the main property of cancer cells that makes them malignant is the ability to produce diverse progeny. Indeed, as an oncology textbook states, "…by the time of initial diagnosis, cancers consist of multiple subpopulations of cells with diverse genetic, biochemical, immunologic, and biologic characteristics" (Fidler, 1997). How this diversity emerges and how it is maintained is not clear. The evidence that cell fusion can contribute to tumor heterogeneity came from using cell fusion as a tool for somatic cell genetics, in particular to investigate whether malignancy is a dominant or recessive trait. Because hybrids between a highly and a weakly malignant tumor cell line were highly malignant, the initial conclusion was that malignancy is a dominant trait (Barski and Cornefert, 1962). However, the subsequent studies found that hybrids between tumor cells and normal cells were not tumorigenic (Harris, 1971; Stanbridge, 1976). The controversy was resolved by realizing that hybrids become tumorigenic if they lose certain "normal" chromosomes, an observation that eventually led to the discovery of tumor suppressor genes (the history of the search is reviewed in Anderson and Stanbridge, 1993; Harris, 1993).

A less appreciated outcome of these studies was the conclusion that chromosomal aberrations are hallmarks of hybrids. These aberrations include chromosome nondisjunction, mitotic recombination, translocations, deletions, insertions, and inversions, a list that is remarkably similar to that observed in tumor cells (reviewed in Larizza and Schirrmacher, 1984). Although how exactly cell fusion causes these abnormalities is not clear, the result is hardly surprising considering that cell fusion produces cells with a sum of parental chromosomes and more than

two centrosomes.

The aberrant chromosome segregation associated with cell fusion was proposed to explain aneuploidy as a hallmark of cancer cells. Although aneuplody is a feature of nearly all of more than 20,000 solid tumors analyzed in humans (Heim and Mitelman, 1995; Mertens et al., 1997), and it was even proposed to be a cause rather than a consequence of cancer (reviewed in Duesberg and Rasnick, 2000), how and why tumor cells become aneuploid is not clear. The observation that tumor cells are prone to cell fusion, the fact that fusion results in polyploid cells, and the finding that cell fusion is followed by abnormal chromosome segregation, together argue that if cell fusion is a part of cancer progression, then tumor cells *should* eventually become aneuploid. However, this explanation of aneuploidy by no means excludes other mechanisms, such as mutations that cause abnormal mitosis.

Changes in epigenetic regulation that follow cell fusion are another factor that can contribute to tumor diversity (reviewed in Kikyo and Wolffe, 2000).These changes appear to be unpredictable, and gene expression can be selectively silenced, activated, or unchanged (Ringertz and Savage, 1976). Considering that the human body has about 200 cell types that are different because of their gene expression pattern, it is easy to imagine how cell fusion can produce monsters.

In summary, cell fusion can be an engine of genomic and epigenetic variability that has a potential to make cells with new properties at a rate exceeding that achievable by random mutagenesis.

Why are tumor cells fusogenic?

There is no definitive answer to this question, which is not surprising considering that the phenomenon lost whatever popularity it had before modern experimental approaches capable of dissecting its mechanism were developed. Several factors were proposed to explain formation of hybrids in tumors: viruses (De Baetselier et al., 1984), cholesterol crystals (Kerschmann et al., 1995), and the natural fusogenicity of macrophages (Munzarova and Kovarik, 1987).

The ability of viruses to induce cell fusion is mediated by the viral proteins that mediate entry of enveloped viruses into the cell. These proteins are members of the fusion protein family, which includes both viral and cellular proteins (reviewed in Martin and Ruysschaert, 2000). The fusion proteins are identified by a hydrophobic motif, known as the fusion peptide, which is required for the fusogenicity of these proteins.

Perhaps any fusion protein or any other agent that induces cell fusion should be investigated for its carcinogenicity. For example, HIV is known to induce syncytia, which suggests that in addition to its known role in disease (Fais et al., 1997; Orenstein, 2001), it might be useful to investigate whether this ability contributes to malignancies associated with this virus, such as Kaposi's sarcoma.

Remarkably, the genes that encode human endogenous retroviruses (HERV) or their individual proteins, including the fusion proteins, comprise at least eight percent of the human genome (Griffiths, 2001). In fact, syncytin, the fusion protein that is thought to mediate formation of the syncytiotrophoblast and is specifically expressed in placenta, is the envelope fusion protein of HERV-W (Mi et al., 2000). This finding indicates that expression of HERV proteins can determine cell fate, and, incidentally, gives support to the hypothesis that placental mammals evolved because our ancestor had the germ line infected with a retrovirus (Harris, 1998).

The sheer abundance of HERV genes in the human genome provides an ample opportunity for their deregulated expression. Indeed, HERV particles were found in both normal and tumor tissues (Bieda et al., 2001). Production of HERV has been associated with multiple sclerosis (Blond et al., 1999) and cancer, although whether this association is causative is unclear, in part because HERV particles do not appear to be infectious. Perhaps a causal link between HERVs and these diseases can be found by investigating whether these particles induce cell fusion. Cell death is a predominant outcome of cell fusion, which might explain how expression of HERV can lead to multiple sclerosis, while the ability of some cells to survive cell fusion can contribute to cancer, as we discussed.

Overall, at least in experimental systems, cell fusion has been linked to several fundamental features of cancer, even though molecular mechanisms that cause this fusion remain obscure. Although one can argue that this link is a peculiarity of experimental tumors, the hypothesis that cell fusion contributes to cancer in humans seems to be equally plausible. Considering that modern tools of experimental biology have not been applied to studies of cell fusion in tumors, there is an abundance of possibilities that are ready to be explored.

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