Cell fusion and plasticity

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Abstract

Cell plasticity is a central issue in stem cell biology. In many recent discussions, observation of cell fusion has been seen as a confounding factor which calls into question published results concerning cell plasticity of, particularly, adult stem cells. An examination of the voluminous literature of "somatic cell fusion" suggests the relatively frequent occurrence of "spontaneous" cell fusion and shows that the complicated cellular phenotypes which it can give rise to have long been recognized. Here, a brief overview of this field is presented, with emphasis on studies of special relevance to current work on cell plasticity.

Introduction

Cell plasticity is a central issue in stem cell biology. Mammalian cloning has proven to be a reproducible technique in multiple species, clearly indicating that mammalian somatic nuclei, even from fully differentiated cells, can be reprogrammed into a totipotent status when placed in enucleated oocytes (Wilmut et al., 2002). Moreover, recent publications claim that somatic stem cells can convert into developmentally-unrelated cell types both *in vivo* and *ex vivo* without the manipulations of nuclear transfer (Eglitis and Mezey, 1997; Ferrari et al., 1998; Gussoni et al., 1999; Petersen et al., 1999; Brazelton et al., 2000; Lagasse et al., 2000; Mezey et al., 2000; Theise et al., 2000; Jackson et al., 2001; Krause et al., 2001; Orlic et al., 2001; Shimizu et al., 2001; Jiang et al., 2002; Korbli et al., 2002; Sata et al., 2002). These exciting possibilities of 'transdifferentiation' have become of great interest in recent stem cell research for multiple reasons. First, they call into question traditional concepts of cell lineage and development. Second, they raise the possibility that relatively accessible stem cells, for example hematopoietic stem cells, could be used to repair organs from which stem cells are not easily isolated, such as the brain. Finally, the possib-

ility of directed differentiation or transdifferentiation of adult stem cells raises the possibility that the use of human embryonic stem (ES) cells, which raises contentious ethical and political issues, could be avoided. Despite recommendations of caution by several experienced investigators (see for example, Weissman et al. (2001) and Lemischka (2002)), these reports have inspired speculation that adult stem cells could become nearly any cell type simply by incubation in a suitable culture environment or by transplantation into appropriate sites in the body. However, two research papers (Terada et al., 2002; Ying et al., 2002) now cast doubt on many claims of transdifferentiation by proposing an alternative hypothesis. In these reports, either bone marrow cells or brain cells, genetically marked with green fluorescent protein (GFP), spontaneously fused to ES cells in mixed cultures, resulting in GFP-positive hybrid cells having the pluripotent trait of ES cells. Indeed, generation of the GFP-positive pluripotent cells could have been misinterpreted as dedifferentiation of somatic cells, as described in many recent 'transdifferentiation' reports, if detailed genetic analysis had not been performed. With these new reports, it was realized that many previous studies had failed to provide adequate genetic or other convincing evidence of cell identity to sub-

stantiate claims of transdifferentiation. Some recent discussions suggest that spontaneous fusion and the characteristics of the resultant cells are perhaps more surprising than 'transdifferentiation' (see for example, McKay (2002) and Tsai et al. (2002)). In fact, an examination of the voluminous literature of 'somatic cell fusion' suggests otherwise – the relatively frequent occurrence of 'spontaneous' cell fusion and the complicated cellular phenotypes which it can give rise to have long been recognized. Here, a brief overview of this field is presented, with emphasis on studies of special relevance to current work on cell plasticity.

'Spontaneous' cell fusion

Cell fusion is a natural occurrence in multicellular organisms. Some notable examples in animals are fusion of egg and sperm during fertilization, the formation of multinucleated myotubes by fusion of myoblasts and the generation of osteoclasts by fusion of mononuclear phagocytic precursor cells. Cell fusion may also be observed under pathological conditions induced by cell injury, certain viral or bacterial infections and during malignant cell growth (Ringertz and Savage, 1976; Hernandez et al., 1996). Understanding of the molecular mechanisms involved in plasma cell membrane fusion has advanced dramatically in recent years, as discussed elsewhere (Vignery, 2000; White and Rose, 2001; Taylor, 2002).

As observed by the earliest developers of cell culture techniques, cell fusion also occurs when animal cells are placed *in vitro*, even between cell types not normally observed to fuse in the intact animal. Such events may be induced by the artificial conditions of the *in vitro* environment; however, that they may be, at least in some cases, also reflective of natural events in the animal can not be excluded.

As described in Ringertz and Savage's classic review (1976) of the literature of cell fusion, W. H. Lewis (1927) described the occurrence of 'spontaneous' cell fusion as a mechanism for generating multinucleated cells from both tumor and normal tissue cells placed *in vitro*. The formation of hybrid cell lines resulting from such spontaneous fusion events was not proven until more than thirty years later, when Barski and colleagues (1961, 1962) isolated such cells from mixed cultures of two different sarcoma-forming mouse cell lines and showed that they possessed the karyotype and other marker properties expected of syngaryons (cells having chromosomes derived from

both parents in a single nucleus). Cell fusion was soon confirmed using a variety of different cell types. It is noteworthy that the first use of a genetic selection technique for isolating hybrid cells, Littlefield's (1964) refinement and application of the 'HAT' (hypoxanthine-aminopterin-thymidine) selection method of Szybalski et al. (1962), was used to isolate hybrid cells formed 'spontaneously' in mixed cultures of A9(HGPRT⁻) and B82(TK⁻) mouse cells. Davidson and Ephrussi (1965) soon adapted this method to isolate hybrids formed spontaneously in mixed cultures of A9 cells and normal mouse cells. A9 parental cells died in the HAT medium. Normal mouse cells formed a monolayer of cells and soon stopped growing. Hybrids could survive in the HAT medium; in addition they had acquired from the A9 parent the property of vigorous growth in culture and had lost the property of 'contact inhibition' of growth exhibited by the normal cells.

These early studies illustrate some important concepts of relevance to recent reports of spontaneous cell fusion in culture. First is the observation that hybrid cells, though burdened with a double complement of chromosomes, may, because of the combination of traits they acquire, sometimes grow more rapidly than one or even both parental cell types. In fact, the term 'hybrid vigor', borrowed from the jargon of plant and animal breeders, has been used to describe this trait (see Ringertz and Savage, 1976). Those experienced in tissue culture applications will appreciate that cells with even a slight growth advantage can, in a reasonably short amount of time, become a major component of a mixed cell culture. Secondly, the studies demonstrate that cell fusion is a relatively common event in mixed cell cultures, occurring with cells of many species and phenotypes. The comprehensive review of these early studies by Ringertz and Savage (1976) led to the conclusion that 'spontaneous' fusion of cells in culture occurs with a frequency between one in one hundred and one in one million cells, depending on cell type and culture conditions. These authors also pointed out that 'spontaneous' events in biology are 'those for which a cause is not known', an important observation still relevant today.

Cell fusion and plasticity

Development of methods for inducing cell fusion at higher frequencies, first using viruses, most notably Sendai virus (Okada, 1958; Harris and Watkins, 1965; Okada and Murayama, 1965) and then chemicals such

as polyethylene glycol (Pontecorvo, 1976), led to the use of cell hybrids in a variety of studies. An early application of the method was the mapping of genes to chromosomes (Ruddle et al., 1971; Ruddle and Kucherlapati, 1974). Weiss and Green (1967) formed hybrids from human and mouse cells and demonstrated that the synkaryons tended to lose human chromosomes upon prolonged growth. Extensive studies of cell hybrids formed from cells derived from a variety of organisms led to the observation that in such cross-species hybrids, the chromosomes of one species were preferentially lost – human chromosomes are generally discarded in mouse/human cell hybrids (Weiss and Green, 1967; Migeon and Miller, 1968) but retained in human/mosquito cell hybrids (Zepp et al., 1971). Perhaps of most relevance to recent studies, however, is the fact that *intraspecific* hybrids, especially those formed from *normal* cells rather than cell lines, show a much greater chromosomal stability than interspecies hybrids (Engel et al., 1969; Bengtsson et al., 1975). In summary, cell hybrids formed in culture or *in vivo*, though relatively rare and having a high chromosome number, may persist and even grow vigorously while expressing a mixed set of traits. As described below, the set of phenotypic traits expressed by such hybrid cells is hard to predict, though the literature of the era of ‘somatic cell fusion’ does lead to some generalizations.

Many early cell fusion experiments were directed toward gaining insight into the mechanisms of cell differentiation and gene expression – could the program of genes expressed by a differentiated cell be fundamentally altered? No doubt many of these studies were inspired by dramatic advances in the field of nuclear transplantation using cells derived from lower eukaryotic organisms, most notably those reported by Gurdon and collaborators with *Xenopus laevis* (Fischberg et al., 1958; Gurdon, 1962; Gurdon and Melton, 1981). In brief, these remarkable studies demonstrated that animals could be formed by transplanting the nucleus of a differentiated cell into an enucleated egg. Although adult cell nuclei generally were capable of inducing only partial development, it was shown that the nuclei from larval intestinal epithelial cells were capable of producing fertile adult animals. These early studies, and others performed with *Rana pipiens* (Briggs and King, 1952, 1960), clearly indicated that the nuclei of at least some differentiated cells retained the competence to direct the formation of an entire animal, that is, were genetically totipotent. Since these early studies, the techniques of

somatic cell nuclear transfer have been adapted to a wide variety of mammalian species, including sheep, cattle, mice, pigs, goats rabbits and cats (reviewed by Wilmut et al., 2002).

The technique of induced somatic cell fusion *in vitro* permitted analysis of fundamental questions of developmental biology in higher animals. In the earliest studies, cells expressing traits characteristic of two differentiated states were fused and the progeny synkaryon cell lines were then analyzed for the traits. In some studies, individual heterokaryons (cells containing two different types of nuclei in a mixed cytoplasm) were analyzed. With development of the technique of cytochalasin-induced cell enucleation (Carter, 1967; Prescott et al., 1972; Wright and Hayflick, 1972), some refinements of the basic protocol became possible. In ‘cybridization’ experiments, a cytoplasm (a cell’s cytoplasmic component surrounded by a plasma membrane) was fused to a whole cell of another type (Poste and Reeve 1971, 1972; Bunn et al., 1974). In ‘cell reconstruction’ (Veomett et al., 1974) or ‘nuclear transplantation’ (Lucas and Kates, 1976), a cytoplasm from one cell type was fused to a karyoplast (a nucleus surrounded by a thin shell of cytoplasm and a plasma membrane) of another cell type. Cell hybrids, cybrids and reconstituted cells were all used to address the basic concept of cell plasticity – could a cell be re-programmed to express genes characteristic of another differentiated state?

The earliest studies of differentiated traits in hybrid cells entailed the study of pigmentation (melanin synthesis) in cells formed from hamster melanoma and mouse fibroblast cells (Davidson et al., 1966, 1968). In these studies, pigmentation was lost or ‘extinguished’ in hybrid cells. Shortly after these reports, Weiss and collaborators began an extensive study of hybrids formed between rat hepatoma and mouse non-liver cells (see for example, Schneider and Weiss (1971), Fougere and Weiss (1978), and Mevel-Ninio and Weiss (1981)). Although they too initially saw extinction of traits associated with the hepatic phenotype, further analysis revealed that, upon loss of mouse chromosomes, rat liver traits could be re-expressed. Furthermore, some of the hybrid cell lines went on to express mouse liver-specific products. These phenomena of gene ‘extinction’, ‘re-expression’, and ‘cross-activation’ were subsequently seen in a wide variety of cell fusion studies, as described in the reviews cited above and elsewhere (Lucas, 1983; Blau and Blakely, 1999). In general, techniques of cybridization and cell reconstruction substantiated the results of whole cell

fusion, except that the phenomena observed were generally transient in nature. For example, fusion of a mouse fibroblast cytoplasm to a rat hepatoma cell 'extinguished' albumin synthesis, but for no more than 48 hr (Kahn et al., 1981a). Transient activation of liver-specific functions by non-liver nuclei after exposure to hepatoma cytoplasm was also described, but this phenomenon may have been due at least in part to selective pressure exerted in the cell culture system used (Gopalakrishnan and Anderson, 1979; Kahn et al., 1981b).

In their discussion 'Cell Commitment and Differentiation', Maclean and Hall (1987) singled out three experimental systems from the literature of somatic cell fusion which may be most pertinent to the topic of nuclear re-programming and cell plasticity. In the first, mouse teratoma cells were fused to Friend erythroleukemia cells, and globin genes in the teratoma nuclei were induced (McBurney et al., 1978). In the second, mouse fibroblast nuclei were placed into cytoplasts from rat hepatoma cells and transient expression of the liver-specific mouse tyrosine aminotransferase was observed (Lipsich et al., 1979). In the third system, Blau and collaborators (1983, 1985) produced heterokaryons from mouse muscle cells and a variety of human cell types. The cells survived for many days as non-dividing multi-nucleated heterokaryons. The synthesis of several human muscle specific proteins was definitively demonstrated. The use of 'transient heterokaryons' was subsequently used to show the partial reprogramming of cells to other differentiated states, including erythroid (Baron and Maniatis, 1986), hepatic (Spear and Tilghman, 1990) and pancreatic (Wu et al., 1991) lineages. A partial reprogramming of erythroid cell nuclei was also seen when they were transplanted into enucleated cells (Bruno et al., 1981). Although not as comprehensive as the results of the amphibian nuclear transplantation studies, these results nonetheless argued for a remarkable degree of genome plasticity in adult somatic cells.

Of special interest here were other cell fusion studies which used as one fusion partner teratocarcinoma cells, embryonic stem (ES) cells or embryonic germ (EG) cells. Initial studies appeared to indicate that fusion to a transformed somatic cell line suppressed the differentiation capacity of teratoma cells (Finch and Ephrussi, 1967; Jami et al., 1973). The apparent suppression of pluripotency in cell hybrids was substantiated by McBurney and Strut (1979) using embryonic carcinoma cells and mouse 3T3 fibroblasts; in contrast, *retention* of pluripotency was reported

in fusions between embryonic carcinoma cells and thymocytes (Miller and Ruddle, 1976) and also in hybrids with erythroleukemia cells, if the embryonic carcinoma cells used were a tetraploid line, indicating a 'gene dosage' effect (McBurney, 1977). Iwakura and colleagues (1985) suggested that mouse embryonic carcinoma cells could be induced to differentiate into various morphological types by fusion to enucleated rat myoblasts and argued for the presence of cytoplasmic regulators of gene expression which could direct expression in the embryonic carcinoma cell nuclei. As noted above, McBurney and colleagues (1978) had shown that teratoma cell nuclei could apparently be induced to express globin genes after fusion to erythroleukemia cells. Also, reactivation of the inactive X chromosome was demonstrated when thymocytes were fused with embryonic carcinoma cells (Takagi et al., 1983). Later experiments with normal stem and germ cells clearly suggested dominance of the pluripotent cell genome, at least with respect to the properties studied (reviewed by Surani, 2001). For example, Tada et al. (2001) have shown reactivation of the Oct-4 gene, which is normally expressed only in totipotent and pluripotent cells (Yeom et al., 1996; Pesce et al., 1998), in the somatic nucleus of hybrids between stem cells and thymocytes. Embryonic germ cells cause similar changes in somatic cell nuclei and, in addition, induce alterations in the status of imprinted alleles (Tada et al., 1997). Furthermore, both types of hybrid cells, those of somatic cells with ES or EG cells, retain their pluripotent capacity when placed into blastocysts (Tada et al., 1997, 2001).

Taken together, the results of somatic cell fusion experiments indicate the existence of transferable factors which can suppress or induce gene expression. Similar studies focused on cell cycle progression rather than gene expression likewise suggest the existence of factors which could induce premature entry into phases of the cell cycle (Rao and Johnson, 1970). In a dramatic example of this latter phenomenon, Lipsich et al. (1978) showed that dormant, terminally differentiated nuclei from chicken erythrocytes could be reactivated when placed in enucleated mouse cytoplasts; however when placed in cytoplasts isolated from cells synchronized in the S-phase of the cell cycle, the reactivating nuclei also began synthesizing DNA. Mercer and Schlegel (1982) showed that fusion with cytoplasts prepared from S-phase cells could also induce quiescent fibroblasts to re-enter the cell cycle and begin DNA replication. These results are of interest here since it is clear that many specialized, i.e.,

fully differentiated cells in the body are withdrawn from the cell cycle, and clearly need to be returned to a proliferative state if they are to support development of an embryo or if they are to serve as the source of cell cultures for therapeutic applications.

Molecular analyses of gene expression and cell cycle progression now lend plausible explanations for many of the phenomenon seen in cell fusion experiments. For example, expression of cell cycle stimulatory proteins such as cyclin E can prematurely induce cells to enter S-phase (Resnitzky et al., 1994; Wimmel et al., 1994). Similarly, with respect to inducible gene expression, individual transcription factors which can have dramatic effects on the program of genes expressed have been identified; for example, ectopic expression of members of the MRF (muscle regulatory factors) family of transcription factors can induce activation of muscle-specific genes in non-muscle cell types (Davis et al., 1987; Choi et al., 1990; Russo et al., 1998). In retrospect, it seems obvious that such potent trans-acting factors were transferred from one cell type to another by the techniques of somatic cell fusion.

Concluding remarks

In summary, early results obtained by nuclear transplantation with amphibians combined with somatic cell fusion experiments provided evidence for the potential pluripotency of somatic cell nuclei and for their capacity to be re-programmed. As such, they provided a background of both information and inspiration for subsequent work which resulted in dramatic successes in mammalian cloning (reviewed by Wilmut et al., 2002). Recent observations on the spontaneous fusion of somatic cells and ES cells resulting in pluripotent hybrid cells (Ying et al., 2002; Terada et al., 2002) indicates that these early studies may now be of more than historical interest. In the discussion above, several traits of hybrid cells which may have relevance to current studies were emphasized.

In many recent discussions, observation of cell fusion has been seen as a confounding factor which calls into question published results concerning stem cell activities. These observations may be seen in another light as an opportunity for developing new methods and approaches for intractable clinical problems. For example, could cell fusion *in vivo* be used as a means to deliver genes to cells which are damaged or to organs in need of cell replenishment? Could cells ex-

pressing a particular phenotype be constructed *in vitro* by cell fusion and then be targeted to a particular site in the body? It is predicted that further studies on cell fusion and genome plasticity, designed with knowledge of the extremely large and diverse literature touched upon only briefly here, should lead to new and important insights and eventual therapies.

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