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Viral mediated cell fusion

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Viral fusion – the making, or breaking, of a tumour?

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Understanding exactly how normal cells acquire the full range of oncogenic mutations required to convert them into fully malignant cells remains a critical issue of debate (Figure 1). One extreme postulates that random mutations to proto-oncogenes accumulate with time (Figure 1a), whereas the other extreme proposes that transformation depends upon the acquisition of aneuploidy through a series of aberrant mitoses (Figure 1b). Almost certainly, both mechanisms are, in reality, operative.

Where do viruses fit into these pathways? Viral infection can be directly mutagenic[1] but can also promote chromosomal rearrangement, damage and replication through expression of viral genes, which push the target cell into repeated cycles of division.[2] However, as attractive as viruses are as major carcinogens, the lack of even remnants of viral genomes in most human tumors means that, curiously, only a minority of human cancers can be firmly assigned a viral aetiology. An intriguing paper by Duelli et al.[3] now offers a possible alternative mechanism that might allow us to apportion blame to viruses as tumour inducers even when we can no longer detect them in the final disease.

Duelli and colleagues observed, to their surprise, that normal human fibroblasts could spontaneously fuse with human fibroblasts expressing the adenoviral E1A gene in culture. By applying double drug selection, they recovered dikaryon hybrids (never larger, multinucleated syncytia), which survived for at least 20 days in culture. Only one of the partner cell lines induced fusion, those cells did not fuse themselves, and fusogenicity could be transferred via conditioned medium. This fusogenic activity colocalized with particles of 100–200 nm and

with cellular proteins CD9 and CD81, which typically label sub-cellular vesicles, named exosomes.[4] Polypeptides were also identified in the exosome/fusogen fraction from a D-type primate retrovirus called Mason–Pfizer monkey virus (MPMV). CD9 and CD81 colocalized with MPMV gp20 envelope, MPMV capsid p27 and reverse transcriptase activity. Interestingly, productive infection of target cells with MPMV was not necessary for fusion by the MPMV/exosome preparations. Therefore, hybrids do not necessarily bear the hallmarks of viral infection. The authors concluded that, in some way, MPMV is released intimately associated with exosome-like particles and that the presence of this virus confers fusogenicity upon these exosomes.[3]

Although normal cell lines could be fused by the MPMV/exosome preparations, the resulting hybrids did not proliferate. In contrast,

hybrids between partners expressing both E1A and a mutant RAS oncoprotein proliferated in vitro and grew in soft agar, whereas neither of the parental lines were able to do so.

Therefore, the authors propose that virus/exosome-mediated fusion of individual cells could be an important initiator of oncogenic transformation in vivo. Thus, fusion between two cells, each one harbouring a separate mutation in different proto-oncogenes, could bring together a novel constellation of cooperating transforming mutations to drive the evolution of the fully transformed phenotype in the resultant hybrid (Figure 2a). Alternatively, under rare conditions, the constellation of retained, stable chromosomes following fusion (aneuploidy) may be compatible not only with survival of the dikaryon, but also with a growth advantage that allows proliferation (Figure 2b). Additionally, in one fusion, both mutation accumulation and chromosomal instability could generate a more aggressively transformed hybrid progeny (Figure 2c). Either way, fusogenic viruses may be carcinogens in a manner not previously associated with their known transforming activities. Moreover, the product of such fusions may also not be accompanied by detectable viral infection.

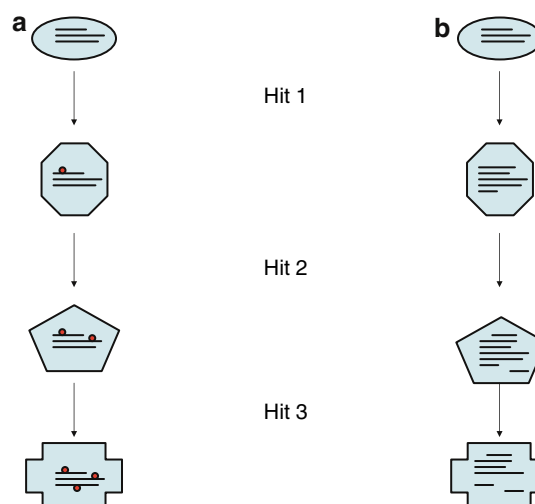The studies reported by Duelli et al. stop a long way short of

Figure 1 Mechanisms of cellular transformation. Normal cells (oval) may be converted to fully malignant tumour cells (crosses) through several intermediate phenotypes (octagons and pentagons) either by the sequential accumulation of mutations in crtitical proto-oncogenes during repeated cell divisions (**a**) or by the acquisition of abnormal numbers and structures of chromosomes (aneuploidy) through a series of aberrant mitoses (**b**). These models are certainly not mutually exclusive.
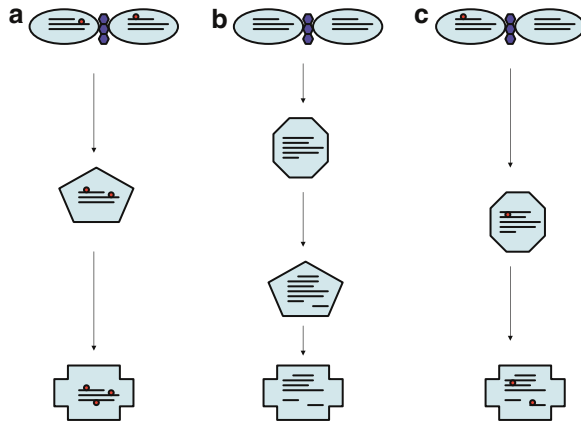
Figure 2 Exosome/virus-mediated fusion could accelerate the process of cellular transformation. (**a**) Fusion between two cells, each one harbouring a separate mutation in different proto-oncogenes, could create a hybrid with cooperating transforming mutations that accelerate the chance of additional hits to create the fully transformed phenotype. (**b**) Fusion between two cells will generate aneuploidy, which may, under certain conditions, confer a growth advantage on the hybrid. (**c**) A combination of (**a**) and (**b**) could also generate aggressively transformed hybrid progeny.
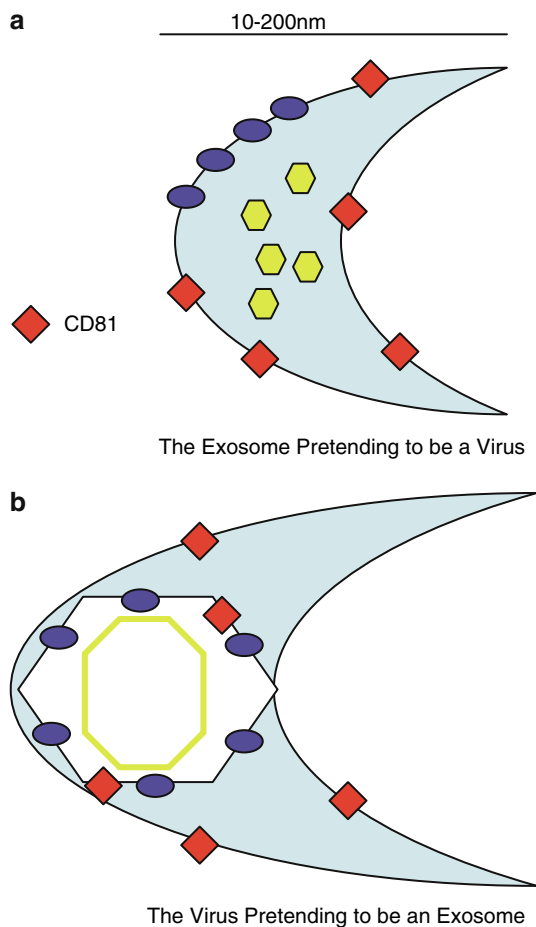

Figure 3 When is an exosome not an exosome? It is unclear as to exactly how the virus proteins and exosomes coexist to form a fusion-competent entity. (**a**) Fusion activity resides in a fraction containing typically cup-shaped exosome vesicles of 100–200 nm, suggesting that the MPMV proteins (such as the MPMV ENV (blue ovals) and capsid (yellow)) are incorporated into exosomes to create virus-like particles, which are, nonetheless, exosomal. (**b**) Alternatively, exosomal proteins (CD9, CD81) (red diamonds) might be incorporated directly into MPMV viral particles, conferring upon particles, which are essentially viral, an exosomal-like appearance.

proving the hypothesis that virus-mediated cell fusion between cells causes cancer. However, they do raise several fascinating possibilities, that will require further, often rather intricate, experiments to confirm or refute.

First, no information is yet available about the ability of these hybrids to form tumors *in vivo*. Their survival was heavily selected for by a dual drug selection regimen *in vitro*, which will drive formation of viable hybrids with a vengeance, but it is not clear where such extreme selective pressures would come from *in vivo*. Therefore, the true oncogenic potential of these dikaryon hybrids is unclear. Further experiments on their ability to form tumours in mice will be highly informative. Moreover, further detailed karyotypic and molecular analysis of the hybrids (which chromosomes are retained, damaged or re-assorted, and which genes continue to be expressed relative to the fusion partners) will be essential to prove a role for exosome/virus-mediated cell fusion as a legitimate mediator of oncogenic transformation.

In addition to some interesting philosophical issues (such as whether the fusion-inducing vesicles are simply viruses masquerading as exosomes (Figure 3), or vice versa), from a gene therapy perspective these findings are most interesting because the authors use them to challenge the use of fusogenic viro/gene therapy for the treatment of tumours. Several groups have shown that expression of hyperfusogenic forms of viral envelope genes (fusogenic membrane glycoproteins, FMG) causes tumour cells to fuse into large, multinucleated syncytia.[5,6] Eventually, syncytia become metabolically untenable and undergo cell death through apoptosis[7] or metabolic exhaustion and mitotic catastrophe.[8] FMG-mediated tumour cell fusion is both efficient for local tumour cell killing and also primes antitumour immunity.[8] An extension of FMG gene delivery has been the use of fusogenic viruses, and clinical trials of measles virus intratumoral therapy have already started.[9] In this respect, the proliferating hybrids that Duelli observed by MPMV/exosome-mediated fusions were small di- or trikaryons but never large syncytia. So what decides the fate of fusion and its resultant
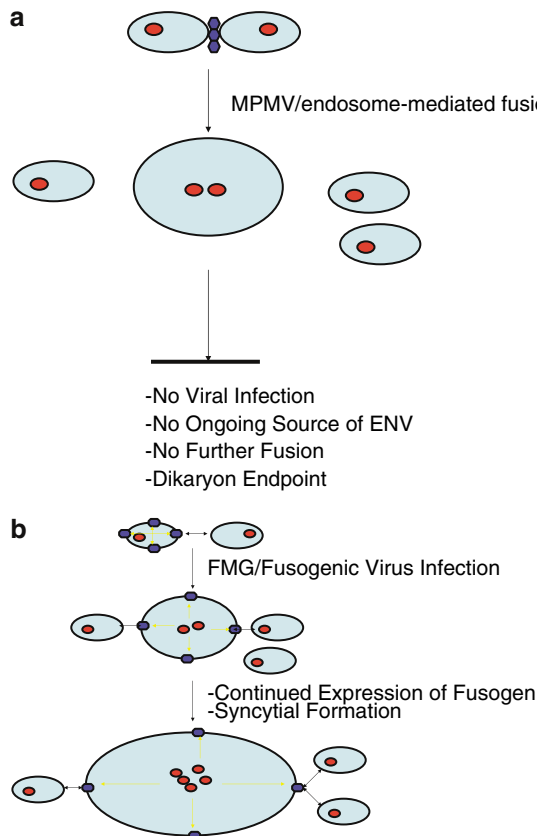
Figure 4 Determing the fate of fusion – size matters. (**a**) Fusion mediated by MPMV/ exosome vesicles probably requires juxtaposition of several envelope-containing vesicles between any two cells, making the available concentration of fusogenic envelopes both very low and time limited in its supply. Therefore, a limited quantity of fusogen and no ongoing supply from within limits the size of the hybrids to di- and trikaryons). (**b**) Following infection with a replicating fusogenic virus, or transduction with an fusogenic membrane glycoprotein (FMG)-containing vector, ongoing expression of the fusogen from within the developing hybrids (yellow arrows representing transcription/translation of the FMG genes) drives continuous recruitment of bystander cells into large heterokaryons to generate the syncytia, which will ultimately die.

hybrids? It seems likely that the concentration, and availability, of the fusogen is likely to be critical (Figure 4). Following infection with a replicating fusogenic virus, *ongoing* expression of the fusogen drives continuous fusion with multiple cells (to generate the syncytia, which are ultimately doomed to die) (Figure 4b). In contrast, in the case of fusion by MPMV/exosomes, there is only a small and finite amount of fusogen available (Figure 4a). Fusion mediated by MPMV/exosome vesicles probably requires juxtaposition of several envelope-containing vesicles between any two cells, making the available concentration of fusogenic envelopes both very low and time limited in its supply. These kinetic and quantitative issues probably explain the limited extent of fusion (di-, trikaryons) seen by Duelli *et al*.[3] compared to the storm-

ing syncytia seen in fusogenic gene and virotherapy.[5]

The disturbing specter raised by Duelli *et al*. is whether fusogenic gene/virotherapies run the risk of generating more aggressive, or even completely new, tumours? We know that fusing tumour cells release large numbers of exosomes.[8] If such vesicles also incorporate fusogenic envelopes from the vectors, could they cause fusion between two tumour cells, with different sets of transforming mutations, to make a small hybrid with a more aggressive phenotype leading to a tumour within a tumour? Or could these vesicles even fuse a normal cell of one type (maybe infiltrating the tumour or in the stroma) with a tumour cell to create a proliferating hybrid that forms a brand new tumour type? Certainly, no such new tumours have been observed in any of the animal

studies where significant tumour cures have been reported using fusogenic viro/gene therapy strategies (although it may be that such models have not been optimally established to detect such events). Nonetheless, these outcomes are theoretically possible. For such possibilities to be seriously considered as a risk in replicating, fusogenic virotherapies, follow-up studies will need to show that (1) such fusion events really can generate precursors of tumours, (2) that the dynamics of FMG, or fusing virus, expression are also compatible with exosome/virus-mediated fusion where the fusogen is limiting and syncytia are avoided and (3) that unexplained or novel tumours are really generated *in vivo* in animals treated with such therapies.

Importantly, these results raise the possibility that viral (MPMV)–exosome-mediated fusion may bring together heterologous cells to create new hybrid offspring with unforeseen and possibly dangerous consequences. Where such fusions bring together compatible genetic re-assortments that allow the fusion to be productive, rather than destructive, it is possible that precursors of tumours may be born. As infection by the virus is not necessarily required for fusion, there may be no trace of the viral role in the process – a sort of fusion hit-and-run. Perhaps, therefore, viral infections lead to many more initiating events on the road to cancer formation than we have been able to give them credit for. Such a hypothesis will be very difficult to prove, but the findings by Duelli and colleagues certainly give us fusion for thought. ■

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