Fusion Experiments with Human Tumour Cells

J. F. Watkins

Dept. of Medical Microbiology
The Welsh National School of Medicine
Cardiff, Wales

This paper reports some results of experiments in which cells from human tumours have been fused with mouse cells by Sendai virus inactivated by ultraviolet irradiation (1, 2, 3). The reasons for wanting to perform these experiments are as follows:

1. If malignant change in human tumour cells is determined by the product of a specific gene locus, it may be possible, by hybridisation with mouse cells, to generate a mouse tumour containing only one human chromosome, since human chromosomes are usually lost from mouse x human hybrid cells.
2. If the human tumour cells contain an oncogenic virus, one or more of the following phenomena may occur as a result of fusion:
   a) Maintenance of part of the tumour cell genome as part of the genome of a continuously growing hybrid line may lead to spontaneous induction of virus.
   b) If the virus is xenotropic the hybrid environment may be permissive, allowing full expression of the virus genome.
   c) A mouse tumor may be produced in which all human chromosomes have been lost, but malignancy is maintained by translocation of an integrated human viral genome to the mouse genome.
3. Because of possible rearrangement of antigenic determinants on the surface of hybrid cells, such cells may have applications in attempts at immunotherapy.

Tumour material is obtained within a few hours of surgical removal. The tissue is coarsely minced and incubated in pronase for one to two hours. The cell suspension is centrifuged and resuspended in culture medium. The suspension consists of clumps of tumour cells mixed with single cells many of which are dead. The clumps at first attach loosely or not at all to the plastic surface of the culture bottle, whereas fibroblasts attach readily. In this way some separation of tumour cells and fibroblasts can be achieved. Depending on the conditions the clumps often start to flatten within a few days, and then appear as colonies of up to a few hundred cells, within which small numbers of cells are seen in mitosis. After a month or two the colonies are not much larger than their original size and they gradually degenerate. This pattern of events has occurred in material from carcinoma of colon, rectum, stomach, bladder, thyroid, parathyroid, and breast. Fusion experiments are carried out either immediately after pronase treatment or after a day or two in culture, before the clumps have attached to the bottle. A suspension of tumour clumps is mixed with mouse cells and inactivated Sendai virus; the agglutinated cells are incubated in plastic bottles for periods up to several months, and examined repeatedly for evidence of hybrid cell formation.

Thirteen tumours (carcinoma of bladder, parathyroid, breast, colon, and vulva, and glioma and melanoma) have been fused with primary kidney cells from inbred CBA mice. During periods of incubation of up to six months, with one possible exception, there was no evidence of hybrid cells growing more rapidly than the mouse kidney cells, although from time to time single hybrid metaphases were seen. CBA mice injected with stamples of the cultures have so far not developed tumours. The reason for this failure may be technical, or it may be more fundamental. In a fusion experiment in which melanoma cells were fused with CBA kidney cells the heterokaryons did not follow the expected course. The nuclei remained separate, with no evidence of synchronous mitosis, for up to nine days.

In one of the above experiments, eleven weeks after fusion of cells from a carcinoma of the colon, a single rapidly growing colony of transformed appearance was noticed. This colony, which has grown up into a line disignated 33FA1, has some interesting characteristics. It shows mixed haemadsorption with a rabbit antiserum against human fibroblasts; this antiserum does not react with primary CBA mouse kidney cells. A rabbit antiserum raised against 33FA1 reacts with human fibroblasts as well as CBA mouse kidney cells. The cells are subtetraploid, with 60 to 80 telocentric and acrocentric chromosomes. Chromosomal examinations of the line are still in progress. So far it looks as though a D-group chromosome, and possibly a G-group chromosome may be present. The cells have been examined by Professor Bodmer for the presence of β_2-microglobulin, the gene for which he and his colleagues have assigned to chromosome 15 (Goodfellow, *et al.* (1975). Nature, 254:267). No evidence of β_2 microglobulin was found. If a human D-group chromosome is present, therefore, it will probably be 13 or 14.

The cells of this line were carrying a virus which could be labelled with tritiated uridine and which banded on sucrose density gradient equilibrium centrifugation at a density of 1.23, which is the same density as Sendai virus. However, electron microscopy of phosphotungstate stained preparations of the virus, showed particles of diameter 85nm to 150nm, which is smaller than paramyxoviruses (150–300nm). Haemadsorption tests on the cells have been repeatedly negative, and after two successive passages of the virus in the allantoic cavity of hens' eggs no haemagglutinating activity could be detected. A rabbit antiserum prepared against the cells, and their carried virus, did not inhibit haemagglutination by Sendai virus. The virus is cytopathogenic for primary CBA mouse kidney cells. Electron microscopy of sections of detached cells in the 33FA1 cultures showed particles of the same size as those visualised by phosphotungstate staining. Similar particles have now been seen after repeated electron microscopic examination of the original tumour. We are continuing investigations on this virus to determine whether it is, after all, Sendai virus, or a defective variant of Sendai virus, which has persisted since the initial fusion. It could also be a virus from the gut, fortuitously present in the tumour, or a mouse virus contaminating the CBA mouse kidney cells. Whatever the virus proves to be, this line at least illustrates some of the problems of interpretation which can arise as a result of this experimental approach.

Primary mouse kidney cells, like most human tumour cells, divide slowly in culture, so perhaps it is not surprising that a hybrid between them should divide slowly. Some fusion experiments were therefore done with 3T3 cells (mouse fibro-

blasts). Many hybrid metaphase spreads, in company with 3T3 cell metaphase spreads, were seen in a fusion experiment with thyroid carcinoma cells, but it proved impossible to separate a hybrid clone from the background of 3T3 cells. 10^7 cells of the whole culture were injected into nude mice by Dr. Stiles, in Dr. Sato's laboratory. So far, after two months, no tumours have developed. A visible tumour in nude mice contains about 10^8 cells. If a single malignant cell was present in the original inoculum it must therefore have a generation time, if it is dividing at all, of more than two or three days.

In order to overcome some of the technical problems referred to above, CBA mouse kidney cells were transformed with SV40 virus. One of the transformed clones was mutagenised, and a mutant clone selected which was resistant to thioguanine. This mutant does not incorporate hypoxanthine, and therefore dies in medium containing azaserine and hypoxanthine in which wild-type cells grow readily. Two fusion experiments using this clone, one with cells from carcinoma of the rectum, the other with a primary culture of melanoma cells, have both produced hybrid colonies within a few weeks. Recent fusion cultures made with carcinoma of the colon and carcinoma of the breast also contain actively growing cells with some morphological characteristics of hybrid cells. The fact that SV40 virus is present in the hybrid cells may create further problems of interpretation as the work develops. These problems may not be too great, as the SV40-transformed CBA mouse kidney cells have so far failed to produce tumours in CBA mice.

It is therefore possible to make hybrid lines from human tumour primary suspensions fused with a continuous mouse line. The question of whether a particular hybrid is derived from tumour cells, or from normal cells associated with the tumour is difficult to answer. The only guidance can come from the properties of the hybrid in vitro and in vivo. For example, if the original tumour is producing a hormone, then hormone production by the hybrid would be evidence favouring the view that the human parental cell was a tumour cell, especially if the tumour material was derived from a metastatic deposit. Malignancy of a hybrid line in nude mice would also be consistent with a tumour cell parent. Chromosomal abnormalities in the original tumour would also help interpretation. These problems do not arise, of course, in the rare cases where a hybrid can be made by fusion of a cell line derived from the human tumor, provided that, as with melanoma cultures, the line has undoubtedly developed from malignant cells.

Summary

Some preliminary results are reported of etperiments aimed at establishing hybrid cell systems for the study of the somatic cell genetics of human tomour cells.

References

1. H. Harris and J. F. Watkins, Hybrid cells derived from mouse and man: artificial heterokaryons of mammalian cells from different species. Nature, 1965, *205*: 640.
2. J. F. Watkins, Cell fusion for virus studies and the production of hybrid cell

lines, in 'Methods in Virology', volume V, ed. Koprowski & Maramorosch, Academic Press, New York, 1971, pp. 1–31.
3. J. F. Watkins, Cell fusion in the study of tumour cells, International Review of Experimental Pathology, volume 10, Academic Press, New York. 1971. pp. 115–139.