CULTURE CONDITIONS SELECTIVE FOR GROWTH OF TUMOUR CELLS

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The aim was to set up in vivo - in vitro models for human colon and lung tumours, in which tumour cells could be transplanted as well in nu/nu, BALB/c mice (nudes) as in monolayer culture.
Data were presented about the fate of 23 human lung tumours explanted subcutaneously into nu/nu mice (BALB/c). Squamous cell ca, adenoca and undifferentiated tumours gave takes respectively in 7 out of 8, 7/9, 1/5. Retransplantable tumours were obtained from 3 squamous cell ca and 2 adenoca, so far. One tumour could not be characterized as such, the explants showed no take.
Fragments of two human colon tumours (HCT-2 and HCT-3) both adenocarcinomas gave rise to transplantable tumours in nudes. Parts of the second passage were used for retransplantation in nudes and for starting the cultures. These cultures (CHCT-2 and CHCT-3, respectively) were started as small pieces or as cell suspensions after trypsinization. They showed various cell types, probably derived from connective tissue of mouse as well as tumour cells of human origin. The main layer consisted of fibroblastic cells in which small groups of epithelial like cells, that eventually formed clones. Furthermore, especially in the beginning, there were many floating cells and cell clusters, partly colouring with trypan blue and evidently dead. With short trypsinization a technique effectively used to separate rat bladder tumour cells from surrounding fibroblasts, it was tried to select also in these cultures the epithelial like cells. These treatments lead to some selection, but the cultures remained a mixture of cell types. The explanation for this could be that the tumour cells were removed during the early period of culturing, e.g. by refreshing the medium and that in fact the epithelial like cells were derived from endothelial mouse cells. To investigate this, a number of tests was performed. After s.c. injection of 10^6 cells into nudes, the CHCT-2 cells induced tumours, the CHCT-3 cells did not. Unlike the starting material, the histology of the human colon tumours and the HCT-2 passage in nudes was characterized as adenocarcinoma, these tumours were undifferentiated sarcomas.
According to the TG and B banding technique both cell lines had a mouse karyotype. CHCT-2 being diploid; CHCT-3 hypotetraploid containing 78 chromosomes. No human chromosomes were found.
EM pictures showed no convincing characteristics of epitheloid cells; C particles were found in CHCT-3 cells.
In conclusion the CHCT-2 and CHCT-3 cell lines developed into mouse cultures, no human tumour cells could be detected. That the connective tissue cells of the mouse transformed into sarcoma cells is probably due to the culture technique used, being a high cell inoculum combined with passaging from the stationary phase. In the meantime cultures were started with pieces or cell suspensions derived from human lung tumours (undifferentiated squamous cell carcinomas). The anchorage of the cells was first poor. Some fibroblasts settled, most cells remained as single cells or aggregates in suspension. After some weeks the aggregates attached to the surface and on top of the fibroblastic layer. To avoid the loss of tumour cells, the following measures were taken.

A. During all medium changes the culture medium was centrifuged first and the cells were returned to the cultures along with the fresh medium.
B. To overcome the overgrowth of the fibroblasts, part of the cultures got a special treatment.
   1. Cis-hydroxy-l-prolin (100 µg/ml) was added to inhibit the growth of the fibroblastic cells (Whei-Young Kao)

2. The serum concentration was lowered from the normally used 10% to respectively 5 and 2.5%, based on the concept that tumour cells are less sensitive for low serum concentrations than non-malignant cells are.
3. Treatment with with short trypsinizations. Slides are presented of phase contrast pictures of human lung tumour cultures after the various treatments and times.

The results can be described as follows.

A. Normal culture conditions (Hanks Eagle's medium + 10% newborn calf serum pen and strep). Not-attached cells returned to culture. The fibroblasts settled first and started growing. After some time the aggregates of epithelial cells (probably tumour cells) attached and gave rise to islands. These islands were surrounded by fibroblastic cells, which hampered the outgrowth of the epithelial cells. During trypsinization the fibroblastic cells detached first as was shown by growing the detached cells in new flasks. However, the remaining fibroblasts were activated more than the epithelial cells, of which only a few large groups could survive these treatments.

B1. Cis-hydroxy-1-prolin (100 µg/ml) blocked the growth of fibroblastic cells. But longer treatment - over 4 days - also harmed the condition of the fibroblastic cells and to less extent the epithelial cells, as was clearly shown by changing the medium back to normal.

2. Serum concentrations of 2.5 and 5 percent had a selective effect on cell growth in favour of the epithelial tumour cells. The 5% serum concentration was preferred because the growth of the tumour cells was better, although part of the fibroblastic cells were active under these conditions too.

3. Short time trypsinization can be used for selection (see A) or for stepwise detachment of cells that are fastened for a long time to the surface.

In summary, the development of established cell lines derived from human colon and lung carcinomas can be misleading and asks for special precautions and controls. These precautions are : not to remove floating cells and cell aggregates from the culture, especially not during the first passages. To choose culture conditions in favour of growth of tumour over non-malignant cells.

a. by using serum concentrations below 6%, 4-5% will probably be the best.
b. to emply with carcinomas short treatment(s) of 3-6 days with cis-hydroxy-1-proline, endconcentration 100 µg/ml.
c. to use short time (minutes) trypsinization separating the cells that first detach, if the fibroblastic cells hamper the growth of tumour cells.

To characterize the cells in culture, as far as possible. Human lung tumours gave takes 15 out of 23 in nu-nu mice (BALB/c); 5 were retransplantable, so far.

REFERENCE

Whei-Young Kao, W. Nature 266: 63-64 (1977).

DISCUSSION

Dendy : Cis-hydroxy-1-proline must not contact too long the cells. How long is this ?

Klein : A week seems to be the best period.

Mareel : How long after transplantation into the nudes do you find your takes ?

Klein : In general, we find takes after ± 70 days, when we do the first transplant. In further passages the "takes" arise faster.

Mareel : Is the stroma in the take from the mouse or from the original mammary tumour ?

Klein : I think it's from the mouse.

Leighton : Did you check this tumour in an organ culture system ?

Klein : I didn't try, but I suppose it is the same.

Freshney : When you trypsinize your cells during passage, I think your tumour cells are very sensitive to this method. What is your impression ?

Klein : That is correct. We try to trypsinize as short as possible (1 min.) and add the medium back. In these instances the fibroblasts retract better than the epitheloid cells. This technique can be repeated several times.

Coordinating Committee for Human Tumour

Investigation

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(C.T.O.C.)

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Contents :