Effect of Cytochalasin B on Malingant Cells

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Introduction

By contrast to their untransformed parents, mouse cells transformed by SV40 are killed by cytochalasin B (Kelly and Sambrook, 1973), a drug which prevents cytoplasmic division and inhibits movement in animal cells (Carter, 1967). When exposed to cytochalasin B, normal cells become binucleated but further division is blocked and upon removal of the drug, the binucleated cells undergo cellular division and retain their viability even after a prolonged treatment. In transformed cells, nuclear division and cellular division are no longer coupled precesses. When cytoplasmic division is blocked by cytochalasin B, most cells become multinucleated and do no recover after removal of the drug.

We have used the ability of cytochalasin B to discriminate normal from transformed cells as a basis for selecting drug resistant variants from the SVT2 line of SV40-transformed mouse cells. These cells which had regained a normal response to the drug have been shown to have an altered expression of virus specific functions and contain less integrated viral DNA sequences than the parental cells. (Kelly and Sambrook, 1974)

The cytochalasin B resistant variants were shown to have unexpected growth properties *in vivo*: by contrast to their transformed parents, they failed to give rise to tumors when injected into mice of the same Balb/c strain, although they were tumorigenic in immunodeficient 'nude' mice. Normal mice, previously injected with the cytochalasin B resistant cells did not develop tumors when challenged later with the transformed parent cells. These results suggested that the variant cells were capable of eliciting an immune rejection response and therefore might be useful in an approach to cancer immunotherapy (Sato *et al*, submitted for publication).

We report in this paper that Lewis lung carcinoma cells respond to cytochalasin B in a manner similar to that of SV40-transformed cells and are efficiently killed by the drug.

Effect of cytochalasin B on lewis lung carcinoma cells

Cells from the Lewis lung carcinoma have been examined for their susceptibility to cytochalasin B. They were compared to a differentiated cell line, PDC1, which has been derived from the mouse teratocarcinoma and shown to have normal growth properties (Boon et al, 1974). Cells growing in culture were treated with cytochalasin B at a concentration of 2 and 5 $\mu g.ml^{-1}$. After 4 days, the medium

was replaced with medium to allow the spreading of the cells and examination of the nuclei. Most PCD1 were binucleated, whereas most Lewis lung carcinoma cells had more than 2 nuclei per cell with often as many as 8 to 12 nuclei per cell. The viability of the cells was determined by plating efficiency. As shown in table I the viability of the Lewis lung carcinoma cells is markedly reduced by a 4 days exposure to the drug. At both concentrations used, only 1 in 10^4 cells appeared capable of forming a colony. PCD1 cells were only little affected by the same treatment.

Table I: Efficiency of plating of Lewis Lung Carcinoma and PCDl Cells

Incubation in cytochalasin B		
(ug. ml^{-1})	LLC	PCD1
0	55	100
2	0.0001	25
5	0.0001	20

Table I: LLC cells were derived from a lung metastasis of the Lewis Lung Carcinoma, after two passages in culture and were obtained from Dr. R. Fauve. PCDl cells were obtained from Dr. T. Boon.
Cells were grown in plastic Petri dishes in Dulbecco's modified Eagle medium containing 10 % fetal calf serum, at 37° and 12 % CO_2. Cells were seeded at a density of 10^4 cells/cm^2 and cytochalasin B dissolved in DMSO was added 12 h. later at the concentration indicated. After 4 days the medium was removed and cells were allowed to spread in fresh medium. They were resuspended 6 h. later and plated at various dilutions. The efficiency of plating is expressed in % of cells forming colonies after two weeks.

We are presently investigating the effect of cytochalasin B on the *in vivo* growth of the Lewis lung carcinoma cells injected into syngeneic C57B1/6 mice. Preliminary results indicate that the appearance of local tumor and metastasis is retarded when animals are injected with the drug.

Discussion

When treated in culture with cytochalasin B, cells from the Lewis lung carcinoma become multinucleated and their viability is drastically reduced, a behaviour previously reported for SV40-transformed mouse cells (Kelly and Sambrook 1973). Other studies on cells from different species transformed by various DNA tumor viruses (Wright and Hayflick, 1972; Hirano and Kurimura, 1974), and studies on cytochalasin B resistant variants of SV40-transformed mouse cells (Kelly and Sambrook, 1974) have suggested that the response to cytochalasin B is a consequence of the expression in transformed cells of a specific viral function. It is of interest that cells from the Lewis lung carcinoma, a spontaneous tumor of the mouse, exhibit a similar response to the drug.

Because of its ability to discriminate between normal and transformed cells, cytochalasin B is a potential chemotherapeutic agent. In addition to its direct kill-

ing effect on malignant cells, the drug may also be useful through the selection of variant cells capable of eliciting an immune rejection reaction of the tumor cells.

We are investigating these possibilities on the Lewis lung carcinoma. We have shown that the cells are very efficiently killed *in vitro* by cytochalasin B and preliminary experiments indicate that the drug may also be active *in vivo*. Further studies are needed to determine optimal conditions of action *in vivo* and whether or not cytochalasin B acts directly on the tumor cells in a manner similar to that observed in culture. We have recently isolated a cytochalasin B resistant cell line from the lung carcinoma cells (unpublished results). If these cells indeed present the property described for the resistant variants of SV40-transformed mouse cells to immunise animals against the parental tumor cells, they will provide a useful tool in an approach to immunotherapy.

References

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