Short Communication

CORRELATION OF IN VITRO TRANSFORMATION WITH IN VIVO TUMORIGENICITY IN 10T½ MOUSE CELLS EXPOSED TO UV LIGHT

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It has been shown that mammalian cells in culture can be transformed to a malignant phenotype by treatment with carcinogenic agents. Such in vitro transformation assays have been extremely useful, both for the testing of potential carcinogens and for the elucidation of cellular mechanisms of oncogenesis (Di-Paolo, 1974; Heidelberger, 1973). Differing endpoints have been used as the criteria for transformation. These include changes in morphology (DiPaolo et al., 1969; Reznikoff et al., 1973) and the loss of anchorage dependence for growth (Bouck & DiMayorca, 1976; Styles, 1977). Ultimately, however, the validity of any such in vitro system can only be justified by demonstrating a direct correspondence with the behaviour of the same cells in vivo. In other words, what are regarded as normal cells must not be tumorigenic in an appropriate host animal, whilst cells that are judged to be transformed in vitro must be (Shields, 1976). Until in vitro transformation can be compared to in vivo tumorigenesis in terms of molecular mechanism, this would probably remain as the most operational criterion.

The C3H mouse embryo-derived 10T½ cell line has been one of the most widely used in vitro transformation systems. We have shown that exposure of these cells to 254 nm ultraviolet light (UV) alone will induce both transformed cells and ouabain-resistant mutants, thus providing a single system in which mutagenesis and transformation can be studied in parallel (Chan & Little, 1976, 1978). In this communication, we report that scoring UV-induced transformants in this system by a morphological criterion indeed correlates closely with both the acquisition of anchorage independence for growth in vitro and tumorigenicity in vivo.

The methodology for inducing transformants by 254 nm UV has been described elsewhere (Chan & Little, 1976). Cells from transformed foci were cloned by the steel-cylinder method and grown up into sufficient quantities for the experiments. The normal 10T½ cells used as controls were cells that have never been exposed to UV; they were in Passages 14–16.

To test for anchorage independence for growth, melted agar (1-2%) containing 0.4% bacto-peptone was mixed at 45°C with an equal volume of Eagle's basal medium made to twice the normal concentration and supplemented with 20% heat-inactivated foetal calf serum (Flow Laboratories). Aliquots of 4 ml of this mixture were deposited on 60 mm plastic tissue-culture dishes (Falcon) as the base layer and cooled to room temperature. Separate 4ml aliquots of the same mixture were mixed with equal volumes of a test cell suspension prepared to a concentration of 5 × 10⁴ cell/ml in normal medium (i.e., the concentration with 10% serum). This mixed cell suspension, now containing 2.5 × 10⁴ cell/ml, 0.3% agar and 0.1% bacto-peptone, was then plated in 4 ml
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Fig. — Cellular morphology at the periphery of a Type II focus (left) and a Type III focus (right). The bottom frames are higher magnifications of the peripheries shown in the upper frames.
aliquots on top of the base layer. The plates were incubated for 2 weeks in a 5% CO₂ humidified incubator held at 37°C and then examined both macroscopically and microscopically for colony formation.

To test for tumorigenicity, an appropriate number of test cells suspended in 1 ml of Earle's balanced salt solution were injected s.c. at the nape of the neck into C3H/HENMTV⁻ mice obtained from the Frederick Cancer Research Center, Frederick, Maryland, U.S.A. These mice were syngeneic with the animals from which the 10T½ cell line was originally derived.

The protocol of the transformation experiments allows cells that have lost the property of contact inhibition of growth to produce dense foci of piled-up cells on top of a background monolayer. From several experiments, we randomly selected 8 independent foci for cloning. All 8 foci showed extreme piling-up of cells, classified as Type II or Type III according to the original classification proposed by Reznikoff et al. (1973). The criss-crossing cell morphology is seen only at the periphery of Type III foci. These criss-cross cells, usually smaller than normal cells, give a Type III focus a diffused edge, whereas the interface between a Type II focus and the background monolayer is abrupt. The Figure shows the periphery of typical Type II and Type III foci that have been fixed and stained with crystal violet. On the basis of this morphological criterion, the 8 clones were scored as Types II or III (Table I).

When the 8 clones were tested in soft agar, 5 were clonogenic and 3 were not. Table I indicates which clones after 2 weeks of incubation produced colonies which were either macroscopically visible as spheroids or microscopically visible as cell aggregates, both presumably progenies of single cells. Columns 3 and 4 in this table indicate a correlation between Type III morphology and the ability to grow in soft agar. Type II cells, on the other hand, required attachment to solid substrate for growth, as did normal cells.

The tumorigenicity test with 4 of these clones indicated that Type III cells also form tumours in vivo. These results are shown in Table II. When 2×10⁶ cells were injected, there was a 100% tumour take. The tumours became discernible by palpation about one month after injection. By 3 months, they had either killed the host or caused gross deformity of the neck region. When inocula of less than 2×10⁶ Type III cells were injected, the tumour yield was less than 100%. This agrees with the results of the coinjection experiments of Stiles et al. (1976) in that the number of cells in the inoculum must be large enough to provide a local microenvironment that is suitable for the growth of the transformed cells. The one line derived from a Type II focus failed to cause tumours even at inocula of 2×10⁶ cells, consistent with the failure of Type II cells to grow in soft agar (Table I). Likewise,

TABLE I.—Morphological type and ability to grow in soft agar of 10T½ clones

| Clone | Inducing UV dose (ergs/mm²) | Morphological type | Agar growth |
|-------|----------------------------|--------------------|-------------|
| TU1   | 200                        | III                | +           |
| TU2   | 200                        | III                | +           |
| TU3   | 200                        | III                | +           |
| TU4   | 300                        | III                | —           |
| TU5   | 100                        | II                 | —           |
| TU6   | 100                        | III                | +           |
| TU7   | 100                        | III                | +           |
| TU8   | 100                        | II                 | —           |
| Normal| 0                          |                    | —           |

TABLE II.—Tumorigenicity of 10T½ clones. Tumour yield is expressed as the ratio of number of tumour-bearing animals to number injected

| Clone | Morphological type | Cells injected (×10⁶) | Tumour yield |
|-------|--------------------|----------------------|--------------|
| TU1   | III                | 2                    | 4/4          |
| TU2   | III                | 2                    | 2/2          |
| TU3   | III                | 2                    | 2/2          |
| TU4   | III                | 2                    | 1/3          |
| Normal| II                 | 2                    | 0/3          |
|       |                    | 2                    | 0/4          |
no tumours arose in animals injected with normal cells.

There is therefore good agreement between the 3 endpoints, morphological change, growth without anchorage and tumorigenicity. On the basis of these results, we conclude that the Type III foci induced by UV in 10T\(\frac{1}{2}\) cells are indeed malignant transformants. These data also show that Type II cells, though having lost the property of contact inhibition of growth, have not acquired full malignant potential as shown by criss-cross morphology and the abilities to grow without anchorage and to form tumours in the host. These results suggest that the transformation of a normal cell to a fully malignant one is a multi-step process with the loss of contact inhibition of growth being an earlier step than morphological change, anchorage independence and tumorigenicity. It is not clear, however, whether the acquisition of these transformed traits follows a mechanistically defined sequence or not, since we have not been able to isolate clones according to an endpoint other than morphology, and test for their behaviour with respect to the other characters. Barrett et al. (1977), working with hamster embryo cells treated with benzo(a)pyrene, seem to favour the view of a defined order of acquisition of the transformed traits. In any event, it seems clear that cells which have been initiated in the transformation process can be arrested at points before the process is complete. This might be the basis for demarcating the oncogenic process into the 2 phases of initiation and promotion (Berenblum, 1975; Sivak, 1978).

Finally we propose that, in using the 10T\(\frac{1}{2}\) system as a quantitative assay for transformation by UV, only Type III foci should be scored as transformants.

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