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Alpha-2-Macroglobulin in Normal and Malignant Human Cells*

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Abstract—*Using the indirect immunofluorescence technique we have studied the cellular distribution of the protease inhibitor alpha-2-macroglobulin* (α_2M) *in normal and malignant cultured human fibroblasts. Normal fibroblasts take up from the culture medium and accumulate into the cytoplasm large amounts of bovine* α_2M. *We did not detect any human* α_2M *synthesized by the fibroblasts. SV40 transformed human fibroblasts and two cell lines originating from a human fibrosarcoma (HT1080) and from a human rhabdomyosarcoma (RD-2) showed a reduced amount of intracellular* α_2M. *The* α_2M, *controlling the activity of cellular proteases, could play an important role in normal cellular behaviour.*

INTRODUCTION

PROTEOLYTIC enzymes alter the growth characteristics and surface properties of normal cells. In some cases, a mild proteolytic enzyme treatment of confluent untransformed fibroblasts can result in initiation of cell division [1, 2]. Furthermore, a variety of transformed cultured cells and tumors have elevated protease activities [3–7]. For these reasons, the cellular mechanisms that regulate proteolytic enzymes could play an important role in the control of the normal growth morphology, *in vitro* as well as *in vivo*. Alpha-2-macroglobbulin (α_2M) is a major plasma protein and functions as an inhibitor of a wide variety of proteolytic enzymes [8, 9]. The presence of α_2M in cultured fibroblasts has been recently shown [10–14]. Mosher and Wing [10] and Mosher *et. al.* [12] have reported that different types of normal human fibroblasts synthesize and secrete this protein. On the contrary, Pastan *et al.* [11], using cultured NRK rat fibroblasts, and Van Leuven *et al.* [14], using human fibroblasts, were unable to detect any synthesis of α_2M. However, the same authors have shown that these cells take up α_2M from the fetal bovine serum in the culture medium [11, 14].

Using the indirect immunofluorescence technique we have studied the cellular distribution of α_2M in normal and in SV40 transformed human fibroblasts and in two cell lines originating from a human fibrosarcoma and from a human rhabdomyosarcoma.

MATERIALS AND METHODS

Cells

All cell lines were grown at 37°C in Minimal Essential Medium supplemented with 10% fetal bovine serum (Flow Laboratories, Irvine, Scotland). Normal human skin fibroblasts were prepared from explants of healthy human adults as described by Martin [15].

HT-1080 [16] and RD-2 [17] cells were obtained from Drs. Azzarone and Varnier (Istituto di Microbiologia, University of Genoa, Italy). GM54VA, SV40 transformed human fibroblasts, were obtained from Dr. C. Croce (Wistar Institute, Philadelphia). All the cells used release a large amount of fibronectin into the medium ([18] and L. Zardi, unpublished results) which could be considered a fibroblastic characteristic [19].

Immunofluorescence

For immunofluorescence studies the cells were replated by trypsinization on glass cover slips in 40 mm Petri dishes and were used 2 days after plating. Cell fixing was carried out

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as follows: cells were washed three times with PBS at room temperature, drained and fixed for 30 min with methanol at 20°C. After rinsing in PBS the first antiserum was added and the cells were incubated for 30 min in a moist chamber at 37°C. The cells were then thoroughly washed with PBS and incubated for another 30 min with fluorescein conjugated to goat antiserum to rabbit IgG (Behringwerke, Marburg-Lahn, W. Germany) diluted 1/20 with PBS. After rinsing thoroughly in PBS the cells were washed gently and briefly in water and were mounted in 90% glycerol diluted in PBS. Cells were examined by phase contrast and epifluorescent illumination in a Leitz Orthoplan microscope with an I.2 filter block using 100× oil immersion objective.

Rabbit antiserum to human α_2M was purchased from Behringwerke (Marburg-Lahn, W. Germany) and diluted 1/40 in PBS.

Alpha-2-macroglobulin was purified from plasma by the procedure described by Roberts *et al.* [20].

Immunoabsorption was accomplished by incubating 1 ml of 1/30 rabbit anti-human α_2M with 300 μg of purified α_2M for 24 hr at 4°C.

RESULTS

By indirect immunofluorescence, using normal human skin fibroblasts and rabbit antibodies to human α_2M we observed numerous bright granular structures particularly in the perinuclear area (Fig. 1). Since antibodies to human α_2M cross-react with bovine α_2M, we grew cells for four generations in rabbit serum, to distinguish whether the stained structures were due to human α_2M synthesized by the fibroblasts or to bovine α_2M taken up from the medium. Under these conditions the cells did not show any fluorescence.

We obtained identical results using normal human skin fibroblasts obtained from four different subjects and MRC5 embryonic human fibroblasts.

Figure two shows fluorescent micrographs of GM54VA, SV40 transformed human fibroblasts, stained with antibodies to α_2M. About 30% of these cells did not present any fluorescence. The number of structures stained was greatly reduced with respect to that in the normal fibroblasts.

In HT1080 human fibrosarcoma and in RD-2 human rhadbomyosarcoma cells only about 5–7% of the cells showed very few granular structures stained by antibodies to α_2M (Fig. 3). The results are summarized in Table 1.

DISCUSSION

Using rabbit antibodies to human α_2M and normal human fibroblasts we have observed a large number of stained granular structures. Absorption of antisera with human α_2M removed completely the fluorescence indicating that the stained structures really contain α_2M. The fact that we did not observe any fluorescence in human fibroblasts grown for four generations in rabbit serum, indicates that the intracellular α_2M observed in cells grown in 10% fetal bovine serum is the result of an uptake of α_2M from the bovine serum in the culture medium.

The above results, absence of detectable amount of α_2M synthesized by cultured

Table 1. α_2M *in normal and malignant human cells*

Cell line	Labelled cells (%)†	Intensity of fluorescence in the labelled cells
Human skin fibroblast*	100	+ + +
MRC5	100	+ + +
GM54 VA	70	+ +
HT1080	7–5	+
RD-2	5	+

MRC5, normal human fibroblasts; GM54 VA, SV 40 transformed human fibroblasts; 1080 human cells from a fibrosarcoma; RD-2 human cells from a rhabdomyosarcoma.

*We have obtained identical results from human skin fibroblasts from four different subjects.

†Percentages were obtained by counting 500 cells for each cell line.

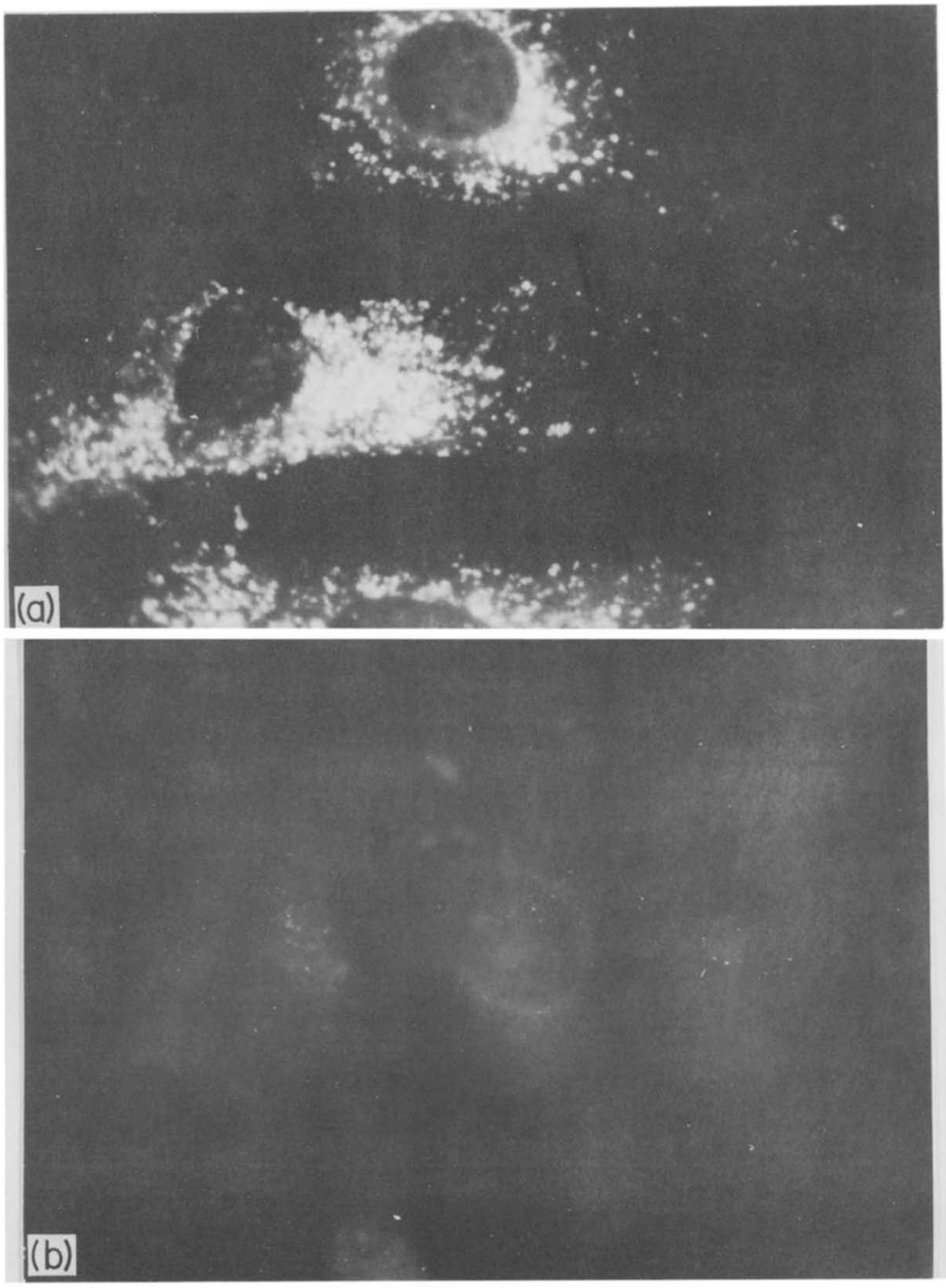

Fig. 1. *Immunofluorescent photographs of normal human skin fibroblasts stained with antibodies to* α_2M *before* (*a*) *and after* (*b*) *adsorption with human* α_2M. 1500 ×.

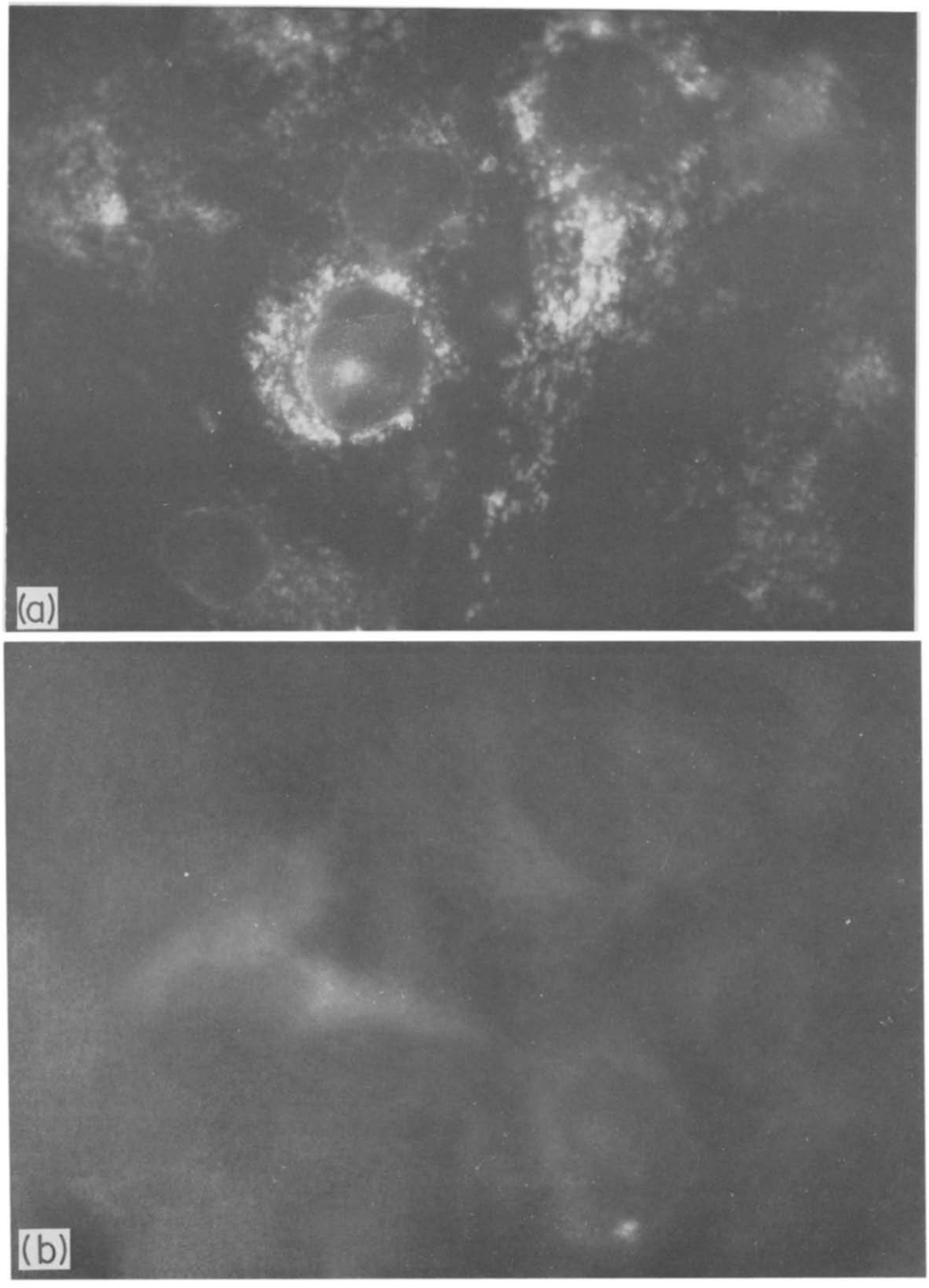

Fig. 2. Immunofluorescent photographs of GM54VA, SV40 transformed human fibroblasts, stained with antibodies to α_2M *before* (*a*) *and after* (*b*) *adsorption with human* α_2M. 1500×.

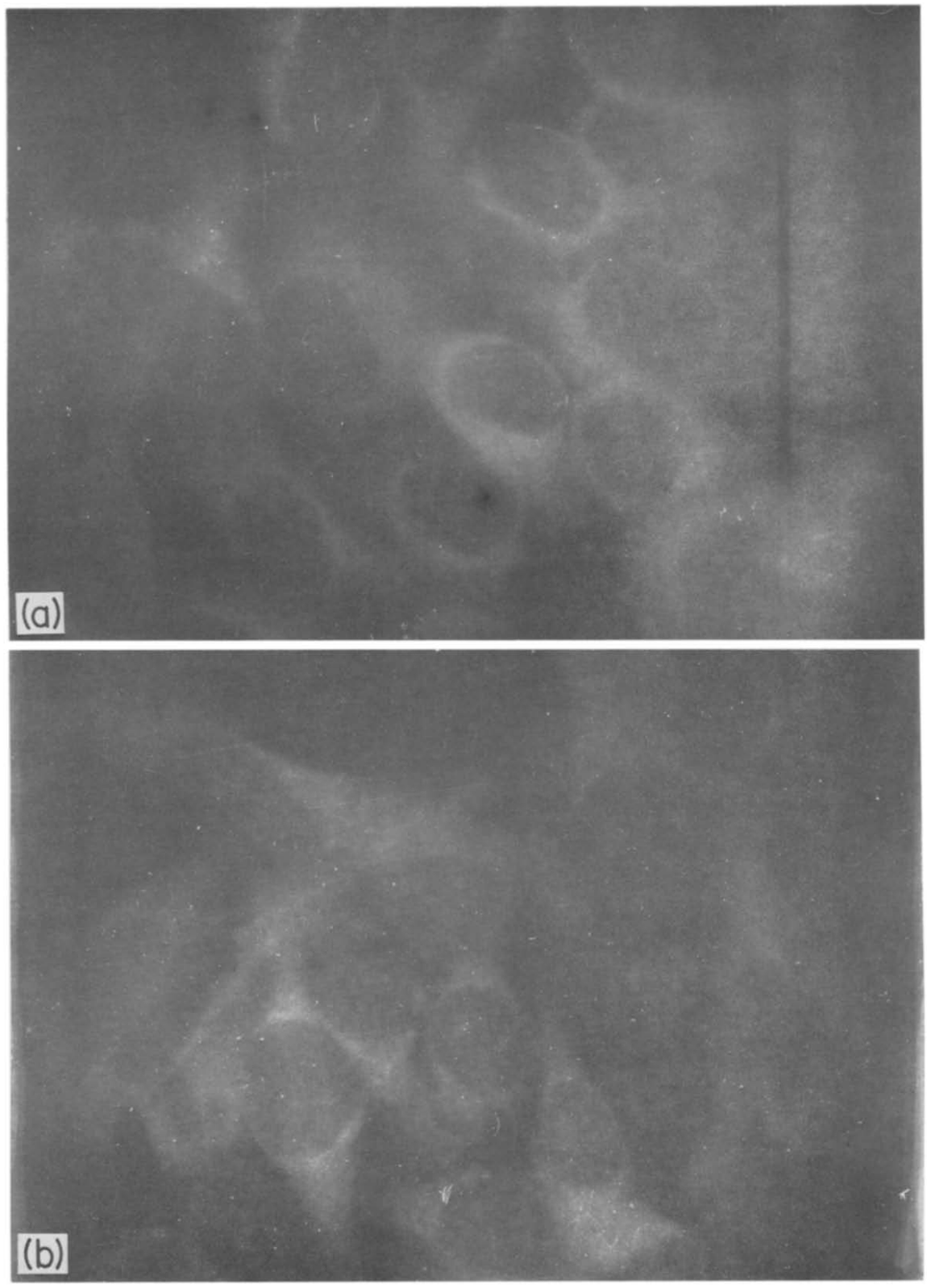

Fig. 3. *Immunofluorescent photographs of HT*1080 *fibrosarcoma human cells, stained with antibodies to* α_2M *before* (*a*) *and after* (*b*) *adsorption with human* α_2M. 1500 ×.

human fibroblasts and uptake of α_2M from the culture medium, agree with previous results by Pastan *et al.* [11] on mouse and rat fibroblasts and by Van Leuven *et al.* [14] on human fibroblasts. Similarly, Mosher *et al.* [12], who by using (35.S) L-methionine report synthesis of α_2M by human cultured fibroblasts, did not observe intracellular human α_2M with the immunofluorescence technique. This suggests that human fibroblasts synthesize a very small amount of α_2M that is not detectable with the immunofluorescence technique.

The reduced capacity of SV40 transformed and malignant cells (HT1080 and RD-2) to accumulate α_2M could be either due to a reduced uptake or to an accelerated catabolism. A very recent report by Van Leuven *et al.* [14] suggests that the reduced accumulation of α_2M in transformed cells is very likely due to a reduced uptake rather than an accelerated catabolism.

This difference could not be due to the phases of the cell cycle since in normal human cells we observe a large amount of α_2M in all the cell cycle phases (Zardi, unpublished).

Previously Pastan *et al.* [11] reported a reduced amount of α_2M in rat and mouse cells transformed by Moloney sarcoma virus but not in other transformed mouse or rat cells. The fact that no difference was observed between normal and transformed rodent cells could have low relevance because rodent cells are known to be genetically labile and to readily create spontaneous variants [21]. A more extensive examination of the amount of α_2M in transformed human cells is in progress also using a new procedure for fixation of cells [13].

However, a reduced presence of α_2M could lead to an abnormal activity of intracellular proteolytic enzymes with consequent alterations in cellular components and behaviour. Our preliminary results indicate (Zardi *et al.*, in preparation) a correlation between the amount of intracellular α_2M and presence of fibronectin, a major cell surface protein that is responsible for maintaining normal cell morphology, cytoskeleton and adhesion ([22] and references therein).

All these observations indicate that the role of α_2M in maintaining normal growth and morphology requires more investigation.

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