# RESTRICTED LATERAL DIFFUSION OF CONCANAVALIN A RECEPTORS OF DIFFERENT MALIGNANT CELLS OF THE NERVOUS SYSTEM

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## 1. Introduction

It is well known [1] that the properties of many surface components of malignant cells are different from their non-malignant counterparts (composition, enzymes, cellular transport etc.). One of the most characteristic properties of malignant cells is the absence of contact inhibition. This should be dependent on the state of the surface receptors, including their mobility [1]. A number of measurements of this mobility, as well as a number of theories, were based on data concerning the formation of caps and patches [2]. This type of lateral mobility, however, could not reflect the lateral diffusion of the receptors. For example, the concanavalin A (Con A) receptors of malignant C1 1D fibroblasts which collected into caps did not demonstrate any detectable ($< 10^{-12}$ $cm^2$ $s^{-1}$) lateral diffusion [3].

In the present study we used a direct method to measure lateral diffusion. This technique allows the measurement of the return of fluorescence to a spot bleached on an otherwise uniformly labelled cell surface [4–7]. Previous results furnished by this method showed that the lateral diffusion of Con A receptors of malignant fibroblasts is essentially slower that that of the same receptors of their non-malignant counterparts [3]. In the present investigation we utilized malignant cells originating from various types of neural cells to measure the lateral diffusion of Con A receptors. We found that this diffusion in the malignant cells was much slower that in their non-malignant counterparts described elsewhere [8]. This fact could perhaps reflect an essential difference between all malignant and primary cells.

## 2. Materials and methods

Concanavalin A, prepared by chromatography on Sephadex (Pharmacia) was conjugated with fluorescein isothiocyanate (FITC) (Fluka) as described [3,8]. Part of this material was succinylated [9]. Sedimentation analysis showed that our preparations were homogeneous. Cells were labelled by bathing with FITC–Con A or succinyl–Con A at 21–23°C for 1–2 min at 0.5–1 mg/ml concentration in phosphate-buffered saline. Three types of neural malignant cells were used: a neuroblastoma line, clone S 20 isolated from a spontaneous tumor in the mouse [10]; the $C_6$ glial line isolated from a rat brain tumor appearing after a number of injections of nitrosomethyl urea [11]; and the VF strain cloned from SV 40 transformed cells which originated from the hypothalamus of a 14 day-old mouse fetus [12]. The cells of the VF strain are rich in glycerol-3-phosphate dehydrogenase, which is also known to be present in large quantities in the cells of the $C_6$ glial line (see [12]). The mobility of Con A receptors was measured at various times after plating. The cells were grown on coverslips in Petri dishes with F10 medium for the $C_6$ and VF lines and with MEM medium for the S 20 line. All media were supplemented with 10% fetal calf serum, glucose (6 mg/ml) and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin).

The cells were observed using phase contrast microscopy with transmitted light. Fluorescence measurements were effected with MPV 2 equipment. The incident light source was a 75 W tungsten lamp with appropriate filters in Ploem OPAK. A 5 μm spot



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on the cell surface was photo-bleached by first passing the beam of a HBO 100 lamp through a diaphragm, then by collimating the light with a X 100 (1.30) objective. The time of photo-bleaching was 1.5–4 s, temperature of measurement was 21–23°C.

## 3. Results and discussion

Typical data with fluorescein labelled Con A are shown in fig.1. With NB S-20 neuroblastoma cells recovery of fluorescence intensity was observed. Using these data we calculated the diffusion constant with the theory developed by Axelrod et al. [7] using the initial slope (fig.1) of the recovery curves [13]. The tracings of the recovery curves were close to a straight line for at least the initial 30 s. The value of $D$ determined in this way is the average of all fluorescent labelled molecules, including those which are entirely immobilized [13]. The diffusion constant for the Con A receptors of the neuroblastoma cells having round or non-complicated shapes (9 cells after 7 h plating) was found to be $(1.5 \pm 0.5) \times 10^{-11}$ $cm^2\ s^{-1}$ (± SE). For morphologically differentiated cells with different culture densities, however, the lateral mobility was found to be increased to $(3.0 \pm 0.5) \times 10^{-11}$ $cm^2\ s^{-1}$ (33 cells).

Little or no recovery was observed with 19 $C_6$ cells and 80 VF cells regardless of cell density or the presence of processes, whether the cell body or the process itself was bleached. Data obtained using succinyl–Con A did not show the presence of detectable recovery (8 $C_6$ cells). In all these cases the diffusion constant was estimated to be $D < 10^{-11}$ $cm^2\ s^{-1}$.

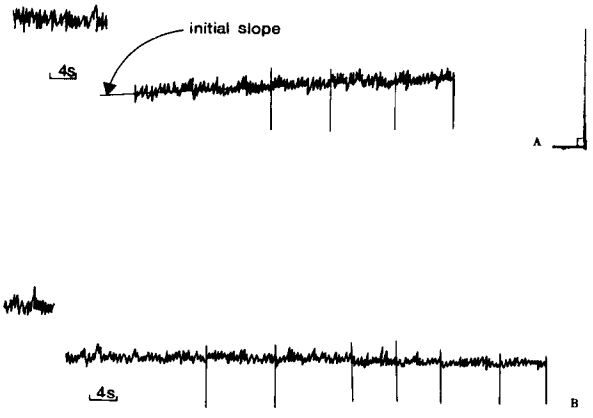


Fig.1. Recorder trace of the fluorescence intensity of a bleached spot on the fluorescein–Con A labelled malignant cells. (A) Clone NB S 20. (B) Clone $C_6$. The recording on the left is the intensity of the spot before bleaching. Measurements were done continuously for the first 25 s and then discretely every minute.

Various types of malignant neural cells have been utilized here. We have reported elsewhere [8] that Con A receptors of glial cells derived from dissociated embryonic brain cells moved with a diffusion constant $D = (4.0 \pm 0.5) \times 10^{-11}$ $cm^2\ s^{-1}$ and that the Con A receptors of the homologous neurons had a diffusion constant $D = (11–14) \times 10^{-11}$ $cm^2\ s^{-1}$ (table 1). Comparison between our present data and those on

Table 1
Values of the lateral diffusion of concanavalin A receptors of the several types of normal cells and their malignant counterparts

| | Normal | Malignant |
|---|---|---|
| Fibroblasts | $\simeq 10^{-11}$ [a] | $< 10^{-12}$ (C1 1D, VA-2)[a] |
| Myoblasts | $(0.8–3) \times 10^{-11}$ [b] | |
| Glia | $4 \times 10^{-11}$ (74)[c] | $< 10^{-11}$ ($C_6$) (27) |
| Neurons | $(11–14) \times 10^{-11}$ (30)[c] | $(1.5–3) \times 10^{-11}$ (NB S20) (42) |

[a] Data from [3]
[b] Data from [14]
[c] Data from [8]

Coefficients ($cm^2\ s^{-1}$). Temperature of measurements was 21–23°C. Numbers in parentheses refer to the number of measured cells.

non-malignant neural cells therefore shows that the lateral diffusion of nervous system tumor cells is consistantly and profoundly slower than the mobility of the Con A receptors of their primary counterparts. Similar differences were obtained between malignant mouse fibroblasts, clone 1D, and their non-malignant counterparts, C57BL/10 [3] and also between malignant human fibroblasts, clone VA-2, and their non-malignant counterparts WI 38 (Zagyansky and Edidin, unpublished). It appears that this difference in the mobility of Con A receptors could reflect some essential properties of the cell surface.

The membrane-associated cytoskeletal systems seem to be involved in the mechanism of the receptor mobility [2,15]. On the other hand it is well known that these systems are drastically altered by transformation, which results in a large decrease in the quantity of microfilaments [1] (of course many other alterations are provoked by transformation [1]). It is thus attractive to relate the decrease of the mobility of Con A receptors following transformation to massive alteration of the cytoskeletal systems. It is interesting to note that, as the neurons of the primary culture contain more mobile Con A receptors than the glial cells, the NB S-20 neuroblastoma cells contain more mobile Con A receptors than cells from clone $C_6$.

We can also notice other similar correlations:

(1) When non-malignant fibroblasts reach confluence the lateral diffusion of Con A receptors increases [3] and the development of the intensive microfilament–microtubule system also increases [16].

(2) The maturation of neurons is accompanied by an increase in the mobility of the Con A receptors [8], as well as by an increase in the quantity of microfilaments in their axons [17]. Thus the value of $D$ of the Con A receptors on the neurons, which is 3 times greater than that of the glial cells [8], could be correlated with the fact that neurons have a more highly developed contractile system [18]. Of course the mechanism of receptor mobility could be very complicated and direct proof of the proposed models is still lacking [2].

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