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CHANGES IN THE NUCLEAR MEMBRANE SURFACE FOLLOWING TRANSFORMATION OF CHICK EMBRYO FIBROBLASTS BY ROUS SARCOMA VIRUS: THE EFFECT OF CAMPTOTHECIN

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Introduction

The net electronegative charge of the surface plasma membrane has been of interest for a number of years,[1] and changes in cell surface properties following oncogenic transformation or drug action (for a review, see ref. 2) have been demonstrated. Primary chick embryo fibroblasts (CEF) transformed by Rous sarcoma virus (RSV-CEF) have been shown to have an elevated net negative electrophoretic mobility (compared to normal CEF) related to cell transformation but not to virus infection.[3]

Recently attention has been focused on the surface properties of subcellular organelles. The electrokinetic properties of rat liver mitochondria,[4] nuclei,[5] and lysosomes[6] have been reported. The present paper gives evidence that SR-RSV-CEF nuclei, isolated in sucrose, $CaCl_2$, have elevated electrophoretic mobilities when compared to untransformed CEF nuclei and that the difference is due to increased N-acetylneuraminic acid (NANA) on the surface of SR-RSV-CEF nuclei. This method has potential for comparing drug effects on the surface of transformed and normal nuclei. Preliminary results on the effect of camptothecin which inhibits nuclear but not mitochondrial[8] nucleic acid synthesis are presented.

Materials and Methods

The virus used was the Schmidt-Ruppin strain of subgroup A (SR-RSV), kindly supplied by Dr. H. Hanafusa.[8] Primary cultures of chick embryo cells derived from a leukosis-free flock which were tested for uniform susceptibility to RSV-subgroup A viruses were infected with the virus and incubated at 37°C in forced air-CO_2 incubators. The cultures were divided on the second day after infection and used for biochemical studies on the fourth day, when over 90% of the cells appeared visibly transformed by SR-RSV. Uninfected cells for controls were handled in an identical manner. Cells were prepared and maintained in F-12 medium and virus assays were carried out as previously described.[9] All cells were harvested at the point when the control CEF and infected cells had formed an almost complete monolayer on the surface of the petri dish, so that the effects of cell contact would be the same in all cultures.

Since great differences in the surface properties of nuclei occur depending on the mode of preparation,[5] the procedure for preparing sucrose, $CaCl_2$ nuclei is described. The procedure is essentially that devised by Dounce and co-workers without use of lead or detergent,[10] as previously described.[5] The cells were harvested in 0.44 M sucrose, 3.3 mM $CaCl_2$ by gentle scraping with a rubber policeman. Approximately 300 mg of cells (wet weight) in 0.44 M sucrose, 3.3 mM $CaCl_2$ was homogenized for 15 strokes in a tight Dounce homogenizer. The homogenate was centrifuged at 700 x g for 20 min. The supernatant was discarded and the pellet resuspended by 10 strokes with a loose Dounce homogenizer in 20 volumes of 2.2 M sucrose, 3.3 mM $CaCl_2$ and centrifuged at 64,000 x g for 10 min. Only the lower white portion of the pellet, which formed a streak, was used in these studies. The nuclei were washed three times with either saline sorbitol or physiological saline prior to mobility measurements or enzyme treatment.

Measurements were made at 25 ± 0.1°C in a horizontal cylindrical chamber of small volume (10 ml) equipped with reversible blacked platinum electrodes.[1,4-6] The mobilities of the particles were calculated in μm/s/V/cm;

each value was obtained by timing the movement of at least 20 nuclei with reversal of polarity after each measurement. The alignment of the apparatus was checked by the method of Heard and Seaman.[1] Determinations of the mobility of washed human erythrocytes were made in a solution of 0.0145 M NaCl, 4.5% sorbitol, 0.6 mM $NaHCO_3$, which is termed saline-sorbitol. Normal blood for this purpose was obtained from healthy donors of the phenotype A Rh^+, taken into EDTA, and immediately washed, and electrophoretic mobilities were determined. Heard and Seaman[1] reported a value for the electrophoretic mobility of human erythrocytes of -2.78 ± 0.08 μm/s/V/cm, while in the present experiments a value of -2.81 ± 0.02 μm/s/V/cm was found in saline-sorbitol. A minimum of three independent experiments was performed for each electrophoretic mobility determination, and all values are the means of at least 100 readings ± S.D. In all cases homogeneous populations were encountered for the particles studied.

Protein was determined by the method of Lowry et al.[11] using bovine serum albumin as standard.

Camptothecin (100 μg/ml) treatment of whole cells was for one hour at 37°C. It has been demonstrated[7] that this concentration of the drug will inhibit nuclear DNA and RNA synthesis. After drug treatment the cells were centrifuged and the nuclei isolated.

In other experiments, isolated nuclei were treated with neuraminidase or hyaluronidase purchased from Worthington Biochemical Corporation, as previously described.[5] Approximately 1.0 mg of nuclei (as protein) was treated with 1.0 ml of various concentrations of enzyme in physiological saline. The pH was adjusted to 6.5-7.0, and the tubes were incubated at 37°C for 20 min. To terminate the enzyme activity on the nuclear surface, cold saline-sorbitol was added, and the nuclei were centrifuged for 10 min at 700 x g. The nuclei were washed twice with saline-sorbitol prior to dilution for observation of electrophoretic mobility.

Conversions of electrophoretic mobilities to other electrokinetic parameters were made using the Helmholtz-Smoluchowski equation and the generalized Gouy equation for a uni-divalent ionic system, as described by Heard and Seaman.[1]

Results and Discussion

The electrophoretic mobilities of the nuclei were indicative of a homogeneous population. Only normal distributions, as opposed to bimodal or trimodal, were encountered. Isolation and purification of the nuclei by other procedures involving the use of detergents[10] or a pH of 5 or lower (commonly referred to as citric acid nuclei) were avoided since they are thought to alter the nuclear surface by completely removing the outer nuclear membrane.[10]

Table 1 demonstrates that (a) the CEF cell nuclei have a much higher net negative electrophoretic mobility than rat liver sucrose, $CaCl_2$ nuclei (cf. ref. 5); (b) transformation of the CEF cells by SR-RSV causes a significant elevation in the net electrophoretic mobility of the isolated sucrose, $CaCl_2$ nuclei, and (c) both the CEF and SR-RSV-CEF sucrose, $CaCl_2$ nuclei have large numbers of electrons per particle surface.

After neuraminidase treatment (under conditions which remove all nuclear surface susceptible NANA), the electrophoretic mobilities of SR-RSV-CEF and CEF nuclei are the same (Table 2). Hyaluronidase-treated nuclei had significantly decreased mobilities, but the mobility of the isolated SR-RSV-CEF nuclei was higher than that of the CEF nuclei after hyaluronidase treatment. It is therefore unlikely that the elevated negative charge of the transformed nuclei is due to increased hyaluronic acid. Since incubation of isolated nuclei with denatured neuraminidase or hyaluronidase did not affect their electrophoretic mobility, the decreased mobilities of the nuclei after enzyme treatment cannot be attributed to nonspecific enzyme absorption. Thus, upon transformation by the SR-RSV the nuclear membrane of the CEF cell is altered, having an increased electrophoretic mobility which is due to increased neuraminidase-susceptible NANA. This finding is in agreement with the data of Kaneko *et al.*[12] which

Table 1. Electrophoretic mobility of CEF and RSV-CEF nuclei and other electrokinetic parameters

Particle	Mobility (μm/s/V/cm)	Zeta potential (mV)	Surface charge (e.s.u./cm^2)	No. of electrons (millions per particle surface)
CEF nuclei (sucrose, $CaCl_2$)	-2.44 ± 0.04	32.2	2.72×10^3	35.0
SR-RSV-CEF nuclei (sucrose, $CaCl_2$)	-2.63 ± 0.04	34.4	2.92×10^3	37.4

Data for mobilities are means of 6 independent experiments ± 1 S.D. Total no. of observations was in each instance greater than 20; i.e. mobilities of over 120 particles were measured in 6 independent experiments. Data are for saline-sorbitol of ionic strength 0.0145 at 25°C at 0.6 mM $NaHCO_3$, 4.5% sorbitol, pH 7.2 ± 0.1. Experiments were performed as given in the text.

Table 2. Electrophoretic mobility of CEF and SR-RSV-CEF sucrose, $CaCl_2$ nuclei after enzyme or drug treatment

Particle	Mobility (μm/s/V/cm)			
	Neuraminidase treated	Hyaluronidase treated	Camptothecin treated cells*	Denatured enzymes
CEF nuclei	-2.36 ± 0.07	-2.38 ± 0.04	-2.44	-2.44
SR-RSV-CEF nuclei	-2.38 ± 0.02	-2.48 ± 0.05	-2.63	-2.64

Values are means ± 1 S.D. of 120 observations for neuraminidase treatment and 60 observations for hyaluronidase treatment. All data are for measurements in saline-sorbitol of ionic strength 0.0145 g-ions/l at 25°C at 0.6 mM $NaHCO_3$, 4.5% sorbitol, pH 7.2 ± 0.1. Neuraminidase treatment was at a concentration of 40 μg/mg nuclear protein; hyaluronidase, 50 μg/mg particle protein. Treatment was at 37°C for 20 min. For incubation with denatured neuraminidase and hyaluronidase, the enzymes were first boiled for 5 min.

*Cells were incubated with 100 μg camptothecin/ml as described in Methods.

indicated that rat ascites hepatoma (AH 108A) nuclei bound ten times as much Ricinus communis agglutinin as did normal rat liver nuclei. Furthermore, Keshgegian and Glick[13] have described differences in nuclear glycopeptides from normal and virus-transformed cells. Camptothecin had no effect on the electrophoretic mobility of the "normal" or transformed nuclei. Since the drug inhibits nucleic acid but not protein or glycoprotein synthesis,[7] this finding indicates that the elevated electrophoretic mobility of the transformed nuclei is not influenced by inhibition of nucleic acid synthesis by the drug.

The results of these experiments demonstrate that, like the surface plasma membrane,[2] the surface of a subcellular particle (namely, the nucleus) is altered in oncogenically transformed cells. This suggests that the viral genome alters host cell synthesis and degradation of not only plasma membrane components but also nuclear membrane and possibly other subcellular particle surfaces.

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