Alterations in Surface Glycoproteins and Level of Sialyltransferase Activity of Human Embryo Kidney Cells Infected with Oncogenic Adenovirus Type 12

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AOI, Y. and YOKOTA, M. *Alterations in Surface Glycoproteins and Level of Sialyltransferase Activity of Human Embryo Kidney Cells Infected with Oncogenic Adenovirus Type 12.* Tohoku J. exp. Med., 1978, **125** (2), 177-183 — Human embryo kidney (HEK) cells infected with adenovirus type 12 and its cytotoxic mutants (*Cyt*) synthesized different glycoproteins respectively on the cell surface at the early stage of infection. Furthermore, a less and a highly tumorigenic *Cyt* mutants synthesized different glycoproteins. The level of sialyltransferase activity in the microsomes of HEK cells was increased when cells were infected with adenovirus type 12. It was confirmed that there was a positive correlation between the sialyltransferase activity in the microsomes and tumorigenicity. — oncogenic adenovirus type 12; microsomal sialyltransferase activity; tumorigenicity

It is generally accepted that cell surface alterations are the primary biochemical events leading to the loss of growth regulation in virus transformed cells (Tooze 1973). However, what surface alterations are directly related to the development of tumor in vivo still remains to be clarified.

Several approaches have been adopted to find a correlation between the cell surface changes and tumorigenicity (Glick et al. 1973; Glick et al. 1974; Onodera et al. 1976). One of the most important problems may be to isolate the tumor specific transplantation antigen (TSTA) and to elucidate its chemical structure.

Human adenovirus type 12 (H12) is highly oncogenic in hamsters. However, most H12 *Cyt* mutants are less tumorigenic in hamsters and less efficient in transformation of cells (Takemori et al. 1968; Takemori et al. 1969). These mutants, which do not show the typical cytopathic effect in adenovirus-infected cells, were named on the basis of their cytotoxic effect.

The comparison of less tumorigenic H12 *Cyt* mutants with highly tumorigenic H12 wild type (WT) showed that they differed only in their capacity to induce cell surface changes, such as lectin-agglutinability (Yamamoto et al. 1972; Salzberg and Raskas 1972) and TSTA (Yamamoto et al. 1972). This close correlation between tumorigenicity and the capacity to induce cell surface changes

provides an efficient system which is useful for analysis of the relationship between transformation and membrane alteration.

In the previous paper (Aoi 1977), the author suggested that the level of sialic acid content in the plasma membrane may be under the control of the microsomal sialyltransferase activity. There may be a link between sialic acid content in the plasma membrane and the transformed phenotype in the cell lines.

This paper describes a comparative study of glycoproteins in the plasma membrane and the sialyltransferase activity in the microsomes of H12WT-infected and *Cyt* mutant-infected cells. The results also suggest a close correlation between tumorigenicity and alteration in the glycoproteins of the plasma membrane.

MATERIALS AND METHODS

Secondary cultures of human embryo kidney (HEK) cells and H12WT, *Cyt* 31 and *Cyt* 66 mutants were used. These mutants were named by N. Takemori according to the proposal made by Ginsberg et al. (1973). Most *Cyt* mutants are less tumorigenic in hamsters with an exception of *Cyt* 66, which is highly tumorigenic (Takemori et al. 1968) and not defective in inducing cell surface change (Yamamoto et al. 1972). The procedures for cell culture, virus production and virus assay were described previously (Yamamoto et al. 1972).

The plasma membrane and microsomes were prepared according to a modified method of Ray (Ray 1970). The membrane isolation procedure is summarized in Fig. 1. Protein determination was carried out by the method of Lowry (Lowry et al. 1951), using bovine serum albumin as a standard. The activity of sialyltransferase in the microsomes was measured according to a modified method of Grimes (Grimes 1970). Successful application of this method to the microsomes used in this experiment was reported previously (Aoi 1978).

Cells were plated on 65 mm Falcon Petri dishes at a concentration of 4×10^5 cells/dish and grown in Eagle's minimum essential medium (MEM) supplemented with 10% calf serum. When the cells formed a monolayer, the medium was discarded and the cells were infected with H12WT, *Cyt* 66 and *Cyt* 31 at 10 PFU/cell, respectively. Fifteen hr after infection (before the onset of viral DNA synthesis), the cells were labeled with 2.5 μ Ci/ml of 3 H-glucosamine (D-6- 3 H]-glucosamine, specific activity 25 μ Ci/mmol, the Radiochemical Centre) for four hr. After being pulse labeled with radioactive sugar as described above, the cells were harvested with rubber policeman (without the use of trypsin) and then centrifuged at $200 \times g$ for 10 min.

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out by the method of Fairbanks (Fairbanks et al. 1971). Polyacrylamide gels (7.5%) were run with a constant current of 5 mA/gel until cytochrome c migrated to the bottom of the gel. The following proteins were used as markers for the molecular weight estimation of sample peaks: human globulin H chain (160,000) (I), bovine serum albumin (68,000) (II), chymotrypsinogen (25,500) (III) and cytochrome c (11,700) (IV). They were indicated as (I), (II), (III) and (IV) in the figure. The gels were cut with a gel slicer which gave slices approximately 1.0 mm width. The slices were swollen overnight at 55° with 0.3 ml of H₂O₂. To each vial were added 10 ml of dioxane scintillation fluid and the radioactivity in each sample was counted by Beckman (SL-200) liquid scintillation counter.

RESULTS

The isolation procedure of the plasma membrane according to the method of Ray (Ray 1970) except that the following steps were reformed to improve the yield: after centrifugation at $5,000 \times g$ for 10 min, the precipitates were taken up

Fig. 1. Isolation procedure of the plasma membrane.

in a small volume of distilled water (1–2 ml), and then this suspension was layered on the 37% sucrose solution (w/w).

SDS-PAGE patterns of glycoproteins in the plasma membrane newly synthesized by HEK cells are shown in Fig. 2(a). Major components were observed in fraction No. 10 and in fractions Nos. 18–19. Cells infected with H12WT newly synthesized fraction No. 10 glycoprotein, but there was no glycoprotein peak at fractions Nos. 18–19 (Fig. 2(b)). On the other hand, cells infected with either *Cyt* 31 or *Cyt* 66 produced a different pattern from that of mock infected or WT-infected cells. The major peak (fraction No. 10) was not detected in either case (Fig. 2 (c), (d)).

The newly synthesized glycoproteins in the virus infected HEK cells were different in the SDS-PAGE patterns. The microsomal sialyltransferase activity involved in the elongation of the terminal carbohydrate (sialic acid) of glycoproteins in the plasma membrane was examined. Cells infected with H12WT and *Cyt* mutants were harvested at 19 hr after infection and microsomes were prepared. The transfer of CMP-[¹⁴C]-sialic acid to desialized fetuin was measured (Table 1). The activity of sialyltransferase increased by 230% in WT-infected HEK cells than that of uninfected cells. The level of sialyltransferase in *Cyt* 66 (highly tumorigenic) infected cells was 1.23 times higher than that of *Cyt* 31 (less tumorigenic) infected cells.

Fig. 2. SDS-PAGE patterns of ^3H -glucosamine labeled glycoproteins in the plasma membrane.
 (a) mock-infected cells. (b) H12WT-infected cells. (c) *Cyt* 31-infected cells. (d) *Cyt* 66-infected cells.

DISCUSSION

Concerning the molecular defects of *Cyt* 31 (less tumorigenic) mutant, two interesting facts have been reported (Yamamoto et al. 1972). First, cells infected with H12WT or *Cyt* 66 (highly tumorigenic) demonstrated marked lectin agglutinability at 24 hr to 32 hr after infection. In contrast, cells infected with *Cyt* 31 mutant showed much less marked lectin agglutinability during the infection process. This observation, indicating that *Cyt* 31 mutant does not induce surface alterations, is in contrast to the marked surface changes induced by H12WT or *Cyt* 66. Secondly, *Cyt* 31 does not induce transplantation immunity in hamsters. However, the correlation between the surface alterations induced by infection in vitro and the lack of induction of TSTA in vivo still remains to be clarified.

TABLE 1. *Level of sialyltransferase activity in the microsomal fraction after infection of human adenovirus 12 and Cyt mutants*

	Sialyltransferase activity
Mock infection	5920±350*
H12WT (oncogenic)	13777±794
Cyt 66 (oncogenic)	10890±830
Cyt 31 (less oncogenic)	8870±521

* Results are expressed as the mean±s.e. of 3 to 5 determinations, and as cpm/mg of microsomal protein.

The study in this paper was performed to find out biochemical mechanisms for the phenomena described above. It was found that the newly synthesized glycoproteins and the level of sialyltransferase activity in the microsomes were characteristic to the cells infected with each mutant. It is worthy of note that the level of sialyltransferase activity was raised by oncogenic virus infection and that the degree of activation was different depending on the infected mutants. These results seemed to indicate that the level of sialyltransferase activity in the microsomes is related with the tumorigenicity.

Sialic acid content in the plasma membrane has been reported to correspond well with the microsomal sialyltransferase activity (Aoi 1978). Sialic acid in the cell surface glycoproteins has been reported to be involved in the determination of MN antigenicity (Springer and Ansell 1958) and tumor antigen (Sanford 1967; Currie and Bagshawe 1968; Simons et al. 1971; Bekesi et al. 1971). Moreover, the presence or absence of sialic acid on the surface has a considerable effect on the fluidity of the plasma membrane, e.g., Con A agglutinability (Novogradsky and Katchalski 1973; Vaheri et al. 1972; Kemp 1968). In this context, it is considered that sialic acid content in the plasma membrane of HEK cells seemed to be raised by infection of those viruses and altered surface glycoproteins.

On the other hand, it has been known that adenovirus types 1, 2 and 5, although classified as non-oncogenic, do transform rodent cells in vitro (Freeman et al. 1967; McAllister et al. 1969; Williams and Ustacebeli 1971) and transformed rat cells are non-tumorigenic (Freeman et al. 1967) unless the animals are immunosuppressed (Gallimore 1972). Whether cells transformed by non-oncogenic adenovirus are related to the level of sialyltransferase activity in the microsomes, is not explored at this moment. Research along this problem is now in progress.

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