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SHORT COMMUNICATION

# SURFACE CHARACTERISTICS OF SV40 TRANSFORMED TRANSPLANTABLE AND NON-TRANSPLANTABLE MOUSE CELLS

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Among the characteristics of malignant tumor cells, the most important for malignancy is the transplantability of the cells in host animals. Kit et al. (1969) established a tumor line in BALB/c mice (Texas Inbred Mouse Co., Houston, Texas), which is constantly transplantable and which was derived from non-transplantable SV40 transformed BALB/c mouse kidney cells (mKS-A). Usually it is very difficult to transplant SV40 transformed mouse cells into mice without some pretreatment of the host animals, such as X-ray irradiation or thymectomy (Aaronson and Todaro, 1968; Takemoto et al., 1968). The infection of the host animal by RNA-containing mouse leukemia virus could have been the cause of conversion of mKS-A cells from a nontransplantable to a transplantable form. But this possibility seems unlikely for the following reasons; (1) the transplantability of the mKS-A tumor remained unchanged and was hereditarily stable, (2) non-transplantable mKS-A cells carry type C virus particles (Kit et al., 1971) and (3) the original mKS-A cells which were not transplantable in BALB/c mice have remained non-transplantable.

The passage history of the mKS-A tumor cell lines is shown in Table 1. Tumor cell lines are designated as mKS-A TU-1, mKS-A TU-2, ... and mKS-A TU-7 according to their passage levels. Since this tumor line contains SV40 specific intranuclear T-antigen and infectious SV40 virus could be recovered from these tumor cells after fusing them with African green monkey kidney cells (CV-1) in the presence of UV-inactivated HVJ (Sendai virus), it is evident that mKS-A TU-7 is derived from the original non-transplantable mKS-A cells.

The transplantable nature of mKS-A and mKS-A TU-7 was confirmed by the experiments shown in Table 2. From the results in this table, it is obvious that the transplantabilities of the two cell lines are not affected by the age of the host mice.

We only examined one non-transplantable SV40 transformed mouse kidney cell line and one transplantable tumor line derived from the former. However, it seems important to compare the characteristics of these two to elucidate the nature of the transplantability of transformed cells. It is conceivable that the difference in transplantability is the result of a difference of the cell surface. There are many reports of changes in the cell surface after viral transformation or lytic infection with polyoma virus (Burger, 1969; Inbar and Sachs, 1969; Duksin et al., 1970; Benjamin and Burger, 1970; Eckhart et al., 1971). In this work we

compared the surface characteristics of these two cell lines using influenza virus and concanavalin A.

Influenza viruses can be adsorbed to the surface of a great variety of cells and it seems likely that the extent to which it is adsorbed may reflect the character of the surface membrane. As influenza virus A/Okuda/57 (H2N2) was used at an *in ovo* passage number of 285. Allantoic fluid containing influenza virus was diluted with Eagle's MEM to give a virus concentration of 512 hemagglutination units/ml. mKS-A and mKS-A TU-7 cells were harvested at the subconfluent stage using EDTA ($5.4\times 10\ M^{-4}$) solution in PBS and were suspended in Eagle's MEM without serum. Equal volumes of virus suspension and cell suspension ($7.5\times 10^6$ cells/ml) were mixed in a test tube and kept in an ice-bath with gentle shaking for 30 min. Then the mixture was centrifuged and virus in the supernatant was measured by its effect in hemagglutination of chick red blood cells (0.5%). This adsorption procedure was repeated twice. Fig. 1 represents the result of adsorption of A/Okuda/57 (H2N2) influenza virus onto EDTA treated

TABLE 1. *Characteristics of BALB/c tumor*

| | Passage level | SV40 T-Ag | Transplantability in mice | Virus in cell extracts | Rescue of virus |
|---|---|---|---|---|---|
| mKS-A TU-1 | $TC_{71}M_1$ | + | not tested | − | + |
| mKS-A TU-2 | $TC_{71}M_2$ | + | not tested | − | + |
| mKS-A TU-3 | $TC_{71}M_8$ | + | + | − | + |
| mKS-A TU-4 | $TC_{71}M_{10}$ | + | + | − | + |
| mKS-A TU-5 | $TC_{71}M_{12}$ | + | + | − | + |
| mKS-A TU-6 | $TC_{71}M_{12}TC_8M_2$ | not tested | not tested | not tested | not tested |
| mKS-A TU-7 | $TC_{71}M_{12}TC_8M_5$ | + | + | − | + |

*TC and M indicate passages in tissue culture and in mice, respectively. The presence of virus in cell extracts was checked by inoculation of supernatants of cell extracts (after freeze-thawing, sonication and centrifugation) onto African green monkey kidney (CV-1) cell monolayers. Rescue of virus was done as described elsewhere (Dubbs and Kit, 1968).*

TABLE 2. *Effect of age of host on tumor production by inoculation of mKS-A or mKS-A TU-7 cells*

| Cell | Passage level | Number of cells inoculated | Age of mice | Days after inoculation | |
|---|---|---|---|---|---|
| | | | | 14 | 28 |
| mKS-A | 69 | $2.5\times 10^4$ | 4 days | 0/6[a] | 0/2 |
| | 69 | $6.5\times 10^4$ | 4 | 0/5 | 0/5 |
| | 69 | $6.5\times 10^4$ | 6 | 0/8 | 0/6 |
| | 69 | $6.5\times 10^5$ | adult | 1/3 | 0/3 |
| | 65 | $4.0\times 10^6$ | adult | 0/6 | 0/6 |
| mKS-A TU-7 | 11 | $2.5\times 10^4$ | 3 days | 6/6 | 6/6 |
| | 11 | $2.5\times 10^5$ | 7 | 6/6 | 5/5 |
| | 11 | $2.5\times 10^6$ | adult | 3/3 | 2/2 |

*a* (number of tumors)/(number of mice inoculated)
*Tumor cells were injected into animals subcutaneously in the scapular region.*

mKS-A or mKS-A TU-7 cells. The result shows that there is a difference in the capacities of adsorption of A/Okuda/57 (H2N2) virus onto these EDTA-treated cells.

Concanavalin A (Con. A) was purchased from Miles Laboratories, Inc. as a solution (25 mg/ml) in saturated saline. Before use, it was diluted 1: 30 with Eagle's MEM and dialysed against 100 volumes of Eagle's MEM for 5 hr at 4 C. Subconfluent cell cultures were used for tests of agglutinability. Cultures were rinsed thoroughly with phosphate buffered saline (PBS). Then the cells were loosened from the vessels using PBS containing $5.4\times10^{-4}$ M EDTA and dispersed by gentle pipetting. Cells were washed with Eagle's MEM by low speed centrifugation and resuspended in Eagle's MEM at a concentration of $4\times10^{6}$ cells/ml. All dilutions were made using Eagle's MEM. Equal amounts (0.025 ml) of cell suspension and Con. A were mixed in the well of a concavity slide and gently shaken for 10 to 20 min at 27 C. Then the extent of cell agglutination was checked under the microscope. The symbols 0, +, ++, +++ and ++++ correspond to 0, 0–25, 25–50, 50–75 and 75–100% clumping of cells. Table 3 shows the agglutinability of these cell lines by Con. A. mKS-A TU-7 is more agglutinable than mKS-A, irrespective of its in vitro passage history. Arndt-Jovin and Berg (1971) reported that transformed and non-transformed cells have the same number of specific binding sites for Con. A, and they suggested that the difference in the agglutination of these cells by Con. A may be due to steric hindrance, allosteric conversion or ionic interaction. It is conceivable that the difference in the extent of agglutinability of the cells by Con. A reflects a difference in the surface characteristics of these cells and that, in the case of virally transformed cells, the surface characteristics are, to some extent, controlled by viral genomes integrated into the host cell chromosomes (Eckhart et al., 1971). One possible explanation of the difference in the agglutinabilities of mKS-A and mKS-A TU-7 by Con. A is a difference in the grade of expression of the SV40 virus gene function(s), but other explanations, such as mere selection of cells by in vivo passage, have also to be taken into account.

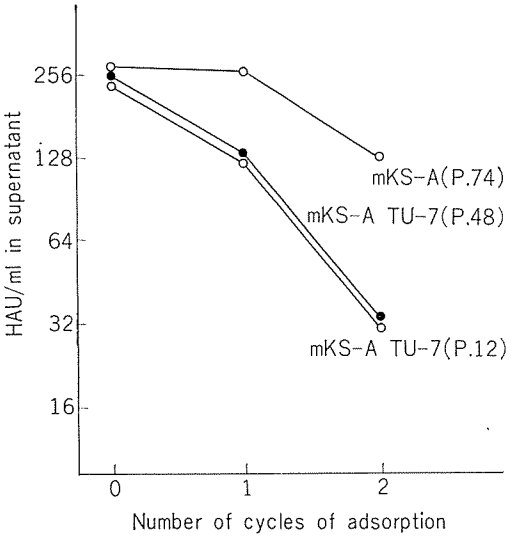


FIGURE 1. *Adsorption of influenza virus onto mKS-A and mKS-A TU-7.*
*The concentration of cells used for adsorption was 7.5 $\times$ $10^{6}$ cells/ml.*

Transplantability, or the extent of progression, must be determined by many factors. In this work we only examined the surface characteristics of the cells by comparing the properties of non-transplantable mKS-A and

TABLE 3. *Agglutinability of mKS-A and mKS-A TU-7 cells by Concanavalin A (Con. A)*

| Cell line | Passage level in vivo | Concentration of Con. A ($\mu$g/ml) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 5 | 25 | 50 | 500 |
| mKS-A | 71 | 0 | 0 | + | + | ++ |
| mKS-A TU-7 | 7 | 0 | + | + | ++ | +++ |
| mKS-A TU-7 | 45 | 0 | + | ++ | +++ | +++ |

*The procedures for this experiment are described in the text. The concentration of Con. A is expressed as the final concentration.*

transplantable mKS-A TU-7. The capacity to adsorb influenza virus, or the situation of receptor sites for virus, seems to represent other characteristics of the cell surface than the binding sites for Con. A or wheat germ agglutinin.

It is well known that sialic acid residues on the cell surface are important in adsorption of influenza virus, even if they do not represent the actual receptor sites for the virus. Usually virally transformed cells contain fewer sialic acid residues than non-transformed cells (Makita and Seyama, 1971). We did not estimate the content of sialic acid residues on the surface of mKS-A TU-7 cells, but from the capacity to adsorb influenza virus, the surface of mKS-A TU-7 cells may have more sialic acid residues than that of mKS-A, or the distribution of sialic acid residues on the surface of these two strains may be very different. If so, it is possible that sialic acid residues may reduce the immunogenicity of the cells by giving them an electrostatic force repulsing immunocompetent lymphocytes. Sialic acid residues may also cover transplantation antigens on the cell surface rendering the cells more transplantable. mKS-A grew as tumors in mice after immunosuppression of the mice by whole body irradiation with $^{60}Co$ (unpublished data). So, the highly immunogenic nature of mKS-A could be one cause of its non-transplantability in mice. The immunogenicity of cells is mainly due to the nature of the cell surface, so the reason for the difficulty in transplanting SV40 transformed cells in mice may be explained by examining the surface characteristics of these cells.

Another factor to be considered is the presence or absence of type C particles in transformed cells. Kit et al. (1971) reported the presence of $^{3}H$-uridine particles in both mKS-A cells and mKS-A TU-5 cells, and they could not find any relationship between these particles and the oncogenicity of mKS-A tumors.

Further studies are needed to clarify whether these differences between transplantable and non-transplantable cell lines are controlled by SV40 viral gene function(s) or simply by selection of cells during in vivo passage. In either case, the present results on this experimental system provide evidence that a change in the cell surface is an important event leading to neoplasia.

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