Correlation of Glycosylation in a Membrane Protein with a Molecular Weight of 150,000 with Tumorigenic Property of Rat Fibrosarcoma Variants

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ABSTRACT

Four clonal variants of fibrosarcoma cells with differing degrees of tumorigenicity and intercellular adhesive property have been isolated from a methylcholanthrene-induced tumor produced in inbred Donryu rats. The variant clones G and Z were characterized by an extremely low tumorigenicity in contrast to the high tumorigenicity of the original clone A and the variant clone P. The variant P was highly tumorigenic in the ascites form when inoculated in the peritoneal cavity, but its tumorigenicity was minimal when inoculated s.c. With scanning electron microscopy, sharp well-developed microvilli were observed in the surface of clones Z and G in contrast to smooth blebs on the surface of clones A and P. Clone G cells showed a remarkable side by side adherence to form a worm-like shape, sharing a common cell coat. Clone Z showed a similar tendency but to a lesser degree. Clones A and P did not show such a property.

Protein and glycoprotein profiles of these variant cell lines have been studied by the following surface-labeling techniques: lactoperoxidase-catalyzed [125] iodination; galactose oxidase-NaB₃H₄; sialidase-galactose oxidase-NaB₃H₄; periodate-NaB₃H₄; and by metabolic labeling with precursor sugars. These variant cell lines show a remarkable difference in degree of sialation in one glycoprotein species with a relative molecular weight of 150,000, whereas the profiles of other proteins and glycoproteins are indistinguishable between variant cell lines. The possible role of glycosylation in the ectoprotein (M.W., 150,000) in defining the tumorigenic property of variant cell lines is discussed.

INTRODUCTION

Phenotypes of tumor cells are variable. Some mutant cell lines induced by mutagens show a low tumorigenicity and are called "revertants" (12, 13, 23, 24), whereas some variants selected by repeated passage in animals show a high tumorigenicity (4, 17). The high-tumorigenic phenotype of various continuous tumor cell lines can be suppressed by cell fusion with low-tumorigenic L-cells, whereby one chromosome seems to be gained. Therefore, the tumorigenic phenotype may result from the loss of a growth control gene in one chromosome which is supplemented by nontumorigenic cells through cell fusion (27, 28). A number of hepatocarcinoma variants (Yoshida hepatoma) with different growth characteristics have been investigated, and their degree of malignancy has been correlated with their glycolytic isozyme pattern. The process of phenotypic reversion from malignant to nonmalignant status was termed "decarcinogenesis" (13, 25). The mutant clones showing decarcinogenesis were derived from the ascites form of mammary carcinoma by mutagens (18).

Tumorigenic phenotypes of cells have been correlated with the profile of cell surface membrane molecules. The following molecular changes of membranes have been most extensively studied and have been regarded as the common phenotype for the tumorigenic phenotype on variant cells derived from decarcinogenesis. The mutant clones selected by repeated passage in animals show a high tumorigenicity of 4 variant isolates.

Four phenotypic variants with a remarkable difference in tumorigenicity were isolated from a methylcholanthrene-induced tumor of inbred Donryu rats by K. Koyama at the National Cancer Center Institute (Tokyo). Subsequently, their cell surface proteins and glycoproteins have been analyzed at the Fred Hutchinson Cancer Research Center and the University of Washington, Seattle, Wash. This paper reports a noteworthy finding, namely, that the status of glycosylation in a unique glycoprotein with a molecular weight of 150,000 may reflect the tumorigenic properties of 4 variant isolates.

MATERIALS AND METHODS

History and Establishment of 4 Clones. The original tumor was a spindle-cell sarcoma induced by s.c. injection of 0.1% of methylcholanthrene in inbred Donryu rats. Tumor cells were released by trypsinization of minced sarcoma and inoculated at about 10⁷ cells into the peritoneal cavity of Donryu rats. Finally, the original free-cell type ascites sarcoma AS 653 was established. A highly metastatic variant was isolated from the original AS 653 cells by s.c. inoculation of sarcoma cells followed by trypsinization of metastatic lymph nodes. This was repeated 10 times; thus, the highly metastatic clone (termed as AS 653-HM) was isolated. The AS 653-HM was further selected by colony formation in 0.3% soft agar in Eagle's medium supplemented with 10% calf serum. Cloning was re-
peated 3 times, thus establishing the highly malignant clone A. Clone G was isolated from clone A by culturing in Eagle’s medium containing 1% calf serum for more than 130 generations. During this term, some cells become confluent to form an unusual worm-like appearance (see Fig. 1G). It appears that the cell surface coats of these cells are fused, and cells are linked together through a common surface coat. These linked cells proliferate as a linked state. The worm-like confluent cells were isolated mechanically and continuously cultured in the medium containing 1% calf serum. The morphology and growth behavior of clone G were not altered as long as it was cultured in a low concentration of calf serum.

Clone P was isolated from clone A by culturing in a soft agar plate with Eagle’s medium containing 10% calf serum. Clone Z was isolated from clone A by its adhesive property on glass surfaces. Clone Z adhered on glass whereas clone P did not.

The growth rate in vitro of the various clones was examined in Eagle’s medium containing 10% fetal calf serum. Transplantability was tested by inoculation of 10³ cells s.c. and i.p., and the percentage of survivors was determined by days.

Cell Culture. Cells were grown as a suspension in Dulbecco’s modified Eagle’s medium supplemented with various concentrations of calf serum or fetal calf serum as specified in the presence of kanamycin (100 µg/ml).

Metabolic Labeling of Cells. Cells were cultured in the same medium described above and supplemented with 10% fetal calf serum, but containing [3H]glucosamine (2 µCi/ml medium) for 48 hr. After incubation, cells were collected by centrifugation, washed with phosphate-buffered saline (20 mM sodium phosphate buffer, pH 7.0, containing 0.15 M NaCl), and analyzed.

Cell Surface Profile of Proteins and Glycoproteins. Proteins and glycoproteins of these cells were analyzed by polyacrylamide slab gel electrophoresis in sodium dodecyl sulfate according to the method of Laemmli (19) and the total protein profile was examined using Coomassie blue staining (5). The growth curves of 4 variants showed essentially the same growth rate in Eagle’s minimum essential medium supplemented with 10% fetal calf serum (data not shown). However, malignancy, as measured by the ability of these cells to grow progressively in vivo, showed a striking difference as seen in Chart 1. In this experiment, 10³ cells were inoculated i.p. in the inbred Donryu rats. Clone P did not grow following s.c. inoculation, although its malignancy in ascites form was greater than the original clone A. The cell surface structure of these clones as revealed by scanning electron microscopy is shown in Fig. 1. It is noteworthy that clone G which showed a remarkably low tumorigenicity (Chart 1) was characterized by an unusual confluent figure with a worm-like shape; i.e., several cells are confluent to form a chain. Microvilli appear sharp and needle-like. Clone Z cells which also showed low tumorigenicity were characterized by their sharp microvilli and tendency to attach to each other. Transplantability, chromosome mode, and histocompatible antigenicity of these clonal variants are summarized in Table 1.

Cell Surface Glycoproteins of 4 Variant Clones. The protein profiles of the 4 variant cell lines stained by Coomassie blue showed very similar patterns (see Fig. 2), and the surface-labeling patterns of proteins by lactoperoxidase-catalyzed [125I]iodination were indistinguishable among the 4 variant cell lines (data not shown). Although we have not examined the protein pattern separated by 2-dimensional gel electrophoresis, the protein components of these 4 variant cell lines may not be significantly different.

In contrast to the protein label, the labeling of galactosyl or N-acetylgalactosaminyl residues by galactose oxidase and tritated sodium borohydride showed a remarkable difference. Both clone Z and clone G hardly gave any label in contrast to a striking label at the molecular weight 150,000 region of clones P and A (see Fig. 3 left). However, intensive labeling occurred at a molecular weight of 150,000 and at other bands in clones G and Z after sialidase and galactose oxidase treatment. After sialidase treatment, however, the molecular weight 150,000 band of clone P showed only moderately intensified label, and that of clone A did not show greatly intensified label (see Fig. 3 right). Thus, sialylgalactosyl or sialyl-N-acetylgalactosaminyl residues in molecular weight 150,000 glycoproteins are much greater in number in G and Z clones than in variants P and A. The results of direct sialosyl labeling with periodate-NaB₃H₄ indicated that the sialosyl residue in the molecular weight 150,000 glycoprotein is much greater in variants G and Z than in variants P and A (Fig. 4). The pattern of glycoprotein metabolically labeled with [3H]glucosamine did not show a clear distinction between clones G, Z, and P, A, since the label is incorporated heavily in various protein species including cytoplasmic proteins (Fig. 5).

results

In Vitro and in Vivo Growth Characteristics of 4 Variant Clones. The growth curves of 4 variants showed essentially the same growth rate in Eagle’s medium containing 1% calf serum (data not shown). However, malignancy, as measured by the ability of these cells to grow progressively in vivo, showed a striking difference as shown in Chart 1. Survival rate of Donryu rat after inoculation of ascites forms of methylcholanthrene-induced fibrosarcoma and their variants. Ascites, original AS 653 cells as in the text: A, clone AS 653-HM: P, Z, and G, variants as explained in the text. Cells (10³) were inoculated on Day 0 and observed until Day 55. Each group consists of 10 rats. Mean values of 3 experiments are shown.
DISCUSSION

The results of this study clearly indicate that low-tumorigenic, low-invasive variants G and Z were characterized by a higher degree of labeling for sialyl galactose or sialosyl-N-acetyl-galactosamine in a specific glycoprotein with a relative molecular weight of 150,000. In striking contrast, the same glycoprotein of the highly tumorigenic original clone A showed a much lower degree of labeling for the same structures. The clone P, which showed preferential growth as an ascites form, showed a much lower degree of labeling for the same structures. It is noteworthy that the profiles of a number of other proteins and glycoproteins of these variant cell lines were indistinguishable. It was noticed that the less tumorigenic variants A or P showed preferential growth as an ascites form but did not grow as a solid tumor, was also characterized by a lesser degree of labeling. A positive correlation between the surface-labeling profile and the cell surface morphology is interesting but requires further extensive study.

Recently, Bramwell and Harris (3) found that a membrane protein having a relative molecular weight of 100,000 in a wide range of tumorigenic cells showed affinity to wheat germ lectin and Con A different from that of a similar glycoprotein of nontumorigenic cells. The glycoprotein derived from tumorigenic cells showed less affinity to wheat germ lectin than to Con A, whereas the glycoprotein from nontumorigenic cells showed an opposite affinity to the 2 lectins. This indicates that the glycoprotein of less tumorigenic cells may contain a higher proportion of carbohydrate "peripheral structure," such as sialic acid and N-acetylgalcosamine which bind to wheat germ lectin, than of the core mannose structure which binds to Con A. The results of the present study also clearly indicate that glycoproteins of less tumorigenic cells contain a larger number of other proteins and glycoproteins of these variant cell lines were indistinguishable. It was noticed that the less tumorigenic clones A or P showed preferential growth as an ascites form but did not grow as a solid tumor, was also characterized by a lesser degree of labeling. A positive correlation between the surface-labeling profile and the cell surface morphology is interesting but requires further extensive study.

The present finding and those of Bramwell and Harris (3) could be interpreted as a core structural difference by Ogata et al. (21). Our present finding and those of Bramwell and Harris (3) could be an additional cell surface parameter that indicates tumorigenicity. Recently, Yogeeswaran et al. (29) described the surface-exposed glycolipid and glycoprotein of in vivo selected melanoma cell lines which showed different metastatic properties. In their study, however, highly metastatic cells showed a greater concentration of sialosylgalactosyl or sialosyl-N-acetyl-galactosaminyl label which is different from our present results. More recently, Lloyd et al. (20) demonstrated the presence of a common surface-labeled glycoprotein in various clones of human melanoma cells.

All of these studies strongly support the idea that a certain change of glycosylation pattern in a defined membrane protein may occur during transformation and that the pattern of glycosylation may define cell social behavior. As a consequence, this change may influence the degree of tumorigenic and invasive properties of tumor cells. The nature of abnormal glycosylation has been clearly defined in glycolipids (10, 11); however, that occurring in glycoproteins has not been well defined. It may be a matter of abnormal processing in glycosylation, and thus may cause an anomalous branching structure as has been postulated by Ogata et al. (21). Abnormal sialylation patterns of the different clones indicated in this study may also be related to the change in "core structure" of the glycoprotein.

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REFERENCES

Fig. 1. Scanning electron micrographs of variant clones of rat fibrosarcoma. Clones A, P, Z, and G are indicated by each letter. Magnification of the pictures of clones A, P, Z, and G, × 5,800, × 6,400, × 7,800, and × 4,600, respectively.
Fig. 2. Total protein bands of various rat fibrosarcoma clones stained by Coomassie blue. Lanes G, P, Z, and A, clones G, P, Z, and A, respectively. Note that total protein pattern of each clone is identical and that high-molecular-weight components are hardly detectable. Arrow, protein (M.W., 150,000); the molecular weight marker used for this determination is the same as in Fig. 3.

Fig. 3. Cell surface glycoprotein pattern of various clones of rat fibrosarcoma. Cells were labeled with galactose oxidase-NaB₃H₄, and shown on the left or labeled with sialidase-galactose-oxidase-NaB₃H₄, and shown on the right. Lanes G, P, Z, and A, are clones G, P, Z and A, respectively. Each gel contained 125 µg of protein (left) or 100 µg of protein (right). Note that the original clone A showed an intense band at molecular weight of 150,000 without neuraminidase treatment (A, left) (arrow). The band was not intensified after neuraminidase treatment (A, right). The same band was not stained by galactose oxidase alone for clone G and clone Z (G, left; Z, left). These bands became strongly stained after neuraminidase treatment (G, right; Z, right). The staining behavior of the same band for clone P was intermediate, namely, the band appeared with galactose oxidase alone but significantly intensified after neuraminidase treatment. The following standard proteins were used for estimation of apparent molecular weight in polyacrylamide gel electrophoresis: skeletal myosin, 200,000; Escherichia coli RNA polymerase (β' and β subunit), 165,000 and 155,000; bovine serum albumin, 68,000; ovalbumin, 43,000.

Fig. 4. The glycoprotein pattern labeled by periodate-NaB₃H₄ of various rat fibrosarcoma clones. Lanes G, P, Z, and A, clones G, P, Z, and the original clone A, respectively. Note that the band for molecular weight of 150,000 (arrow) labeled strongly for clones G and Z, but not for clones P and A. This finding supports the presence of a highly sialylated carbohydrate chain at molecular weight of 150,000 protein in clones G and Z, supporting the results as shown in Fig. 3.

Fig. 5. Metabolic labeling of various glycoproteins of rat fibrosarcoma clones. Lanes G, P, Z, and A, clones G, P, Z, and A, respectively. Note that the pattern of total carbohydrate labeled by glucosamine is entirely different from those indicated by cell surface labeling. Arrow, Protein (M.W., 150,000).
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