Altered glycosylation in Madin-Darby canine kidney (MDCK) cells after transformation by murine sarcoma virus

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(Received 25 May 1989; accepted 20 November 1989)

The changes in glycosylation of an immortalized epithelial cell line (MDCK) before and after progression towards a more malignant phenotype have been studied. The parental MDCK-3 cells were immortalized after long-term passage in vitro and have shown no tendency for spontaneous acquisition of malignancy-related phenotypes such as tumorigenicity, invasion and metastasis. They conserved morphological and functional characteristics of the epithelial tissue of origin. The ras-MDCK cells acquired the fully malignant phenotype after transformation with a Harvey murine sarcoma virus; they were immortalized, invasive in vitro and produced invasive and also metastatic tumors after subcutaneous injection into nude mice. Using immobilized lectins and gel chromatography, before and after liberation of O-linked glycans from the peptide moieties and also after removal of terminal sialic acid, we have found differences in the glycosylpeptides of both whole cells and cell surface trypsinates from ras-MDCK cultures as compared to the parental MDCK-3 cultures: (i) more sialic acid in the N-linked tri- and tetra-antennary structures; (ii) more fucosylation in the N-glycosylpeptides; (iii) more bi-antennary N-glycosylpeptides and less O-linked glycans; and (iv) a lower molecular weight of the O-linked glycans probably due to a decreased sialylation. It is concluded that alterations in sialylation and fucosylation of the cell surface exposed glycans accompanied progression of MDCK-3 cells towards a more malignant phenotype.

Introduction

Alterations of glycans in cell surface exposed N-glycosylpeptides have been correlated with the expression of malignancy-related phenotypes in a range of experimental systems [29]. The strategy of such experiments has been to compare cell populations with differences in malignancy-related phenotypes: non-tumorigenic versus tumorigenic [10, 13, 32]; non-invasive versus invasive [4, 5]; and non-metastatic versus metastatic [9, 15, 28].

Alterations of the glycans have been mostly found in the terminal sugar residues: sialic acid and fucose. NIH 3T3 cells transfected with DNA from human bladder carcinoma, colon carcinoma or HL60 promyelocytic leukemia cells were tumorigenic in nude mice and showed higher-branched, sialic acid containing glycosylpeptides [10]. Human uroepithelial cells that were tumorigenic in nude mice possessed more highly branched, tri- and tetra-antennary N-acetyl-lactosaminic type glycans than their non-tumorigenic counterparts [13].
Glycosylpeptides, isolated from the surface of non-invasive cells, eluted behind those from invasive cells on a gel chromatography column, and this was ascribed to an increased sialylation of the glycosylpeptides of the latter cells. The invasive phenotype was acquired after mutagenesis, oncongene transfection or epigenetic induction with 1-O-octadecyl-2-O-methylglycerol-3-phosphocholine [4, 5, 20]. Decreased fucose incorporation in cell surface carbohydrates was associated with inhibition of invasion of MO₄ cells at reduced temperature and in gap junction defective L929 cells [3, 6]. Sialylation of particular cell surface carbohydrates was also correlated with the metastatic phenotype of a range of murine tumor cells as reviewed by Schirrmacher et al. [29], of T-cell hybridomas [9] and of mutants from the high-metastatic mouse tumor, MDA-MD₂ [15]. Marked inhibition of [³H]fucose incorporation was found in murine tumor cells showing a decrease in metastatic capacity [14, 31]. By contrast, differences in the metastatic potential of B16 melanoma cell variants did not correlate with differences in total cell surface sialic acid [28].

We have compared the glycosylpeptides of whole cells and cell surface trypsinates of an epithelial cell line (MDCK) before and after progression towards a more malignant phenotype. The parental MDCK cells conserved morphological and functional characteristics of the renal epithelial tissue of origin; they were immortalized after long-term passage in vitro and have shown little or no tendency for spontaneous acquisition of more malignant phenotypes. After transformation with a Harvey murine sarcoma virus, MDCK cells acquired a more malignant phenotype, since they were immortalized, tumorigenic, invasive and metastatic in nude mice.

This pair of cell lines provided us with the opportunity to examine whether alternations of glycans were related to immortalization or to further steps of malignant progression.

**Materials and methods**

MDCK cells (obtained from J. Leighton, Department of Pathology, Medical College of Pennsylvania, PA) are Madin-Darby canine kidney cells, coined MDCK-3 to distinguish them from other MDCK variants [2]. MDCK-3 cells have repeatedly been shown to be non-invasive in vitro [2, 30]; they were not tumorigenic after s.c. injection in nude mice (our unpublished results in collaboration with F. Van Roy, Laboratory of Molecular Biology, State University of Ghent, Belgium).

The ras-MDCK cell line (obtained from M. C. Lin, Bethesda, MD through W. Birchmeier, Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, Tübingen, F.R.G.) are MDCK cells transformed with a Harvey murine sarcoma virus [11]; they are invasive in vitro [2] and produced invasive and metastatic tumors after s.c. injection into nude mice (unpublished results in collaboration with F. Van Roy).

Samples from both cell lines were expanded to obtain three sets of subconfluent monolayers (4 x 150 cm²/set) on tissue culture plastic substrate (Flow, Irvine, Scotland, cat. no. 61-450 B5) at 37°C with Dulbecco’s modification of Eagle’s medium (DMEM, Flow, cat. no. 12-332-54) supplemented with 10 per cent (v/v) fetal bovine serum (Flow, cat. no. 29-101-54), 0.05 per cent (w/v) L-glutamine, 250 IU/ml penicillin and 100 μg/ml streptomycin.