Effect of Cancer-related and Drug-induced Alterations in Surface Carbohydrates on the Invasive Capacity of Mouse and Rat Cells

Jan G. M. Bolscher,2 Denis C. C. Schallier,3 Lou A. Smets, Henny van Rooy, John G. Collard, Eric A. Bruyneel, and Marc M. K. Mareel

Division of Cell Biology, The Netherlands Cancer Institute, Antoni van Leeuwenhoek Huis, 121 Plesmanlaan, 1066 CX Amsterdam, the Netherlands [J. G. M. B., D. C. C. S., L. A. S., H. v. R., J. G. C. J., and Laboratory of Experimental Cancerology, Department of Radiotherapy and Nuclear Medicine, University Hospital, 185 De Pintelaan, B-9000 Ghent, Belgium [E. A. B., M. M. K. M.]

ABSTRACT

The effect of alterations in cell surface carbohydrates on invasion of mouse and rat cells into embryonic chick heart fragments in organ culture was studied. Matching pairs of malignant and nonmalignant cells, including all categories of carcinogenic induction (i.e., viral, chemical, or oncogenic), were compared for their alterations in cell surface carbohydrates and invasive behavior. Glycopeptides derived from the surface of malignant cells expressed cancer-related changes in carbohydrate composition, demonstrated by gel filtration chromatography as a shift in size distribution in comparison with those from nonmalignant counterparts. This phenotypic property strictly correlated with the acquisition of the invasive capacity. Morphological transformation of cells without simultaneous alteration in surface carbohydrates was, however, insufficient for invasion.

To test the possible mechanistic role of altered surface carbohydrates in the invasive capacity of cells, the surface molecules of noninvasive cells were modified by incubation with an alkyl-lyso phospholipid (racemic 1-O-octadecyl-2-O-methyl glycerol-3-phosphocholine). Alkyl-lyso phospholipid induced an increase in surface sialylation resembling the changes found in malignant and invasive cells. After pretreatment with alkyl-lyso phospholipid, morphologically transformed but nonmalignant and noninvasive cells became able to invade chick heart tissue. These findings indicate that alterations in cell surface carbohydrates, induced by entirely different mechanisms, endowed cells with invasive capacity.

INTRODUCTION

The fucose containing carbohydrate moieties of membrane glycoproteins from malignant cells are characteristically different in their gel filtration size distribution in comparison with those from nonmalignant cells. The carbohydrates from malignant cells contain more highly branched or elongated chains with increased amounts of sialic acid residues (for reviews see Refs. 1–5). The most striking feature is their general occurrence in all malignant tissues and cell lines, independent of transforming principle and cellular origin.

Since carbohydrates are known to be involved in establishing and maintaining functional contacts between cells, alterations in surface carbohydrates may enable tumor cells to override normal control functions, e.g., endowment of the capacity to invade normal tissue. Invasion includes infiltration and subsequent destruction of normal tissue, both being progressive in time and space (6), and is an important step in the sequence of events leading to metastasis. In several cell systems metastatic capacity is influenced and regulated by the degree of sialylation of specific cellular binding sites (7–13). In addition, differences in invasive capacity between T-cell lymphoma variants (7) and hybrids of noninvasive T-lymphoma (BW 5417) and activated T-cells (14) have been ascribed to differences in sialylation of cell surface carbohydrates. Also in normal cells a correlation between cell surface carbohydrate expression and functional aspects related to invasion has been described. Mature granulocytes transiently express leukemic-like carbohydrates during regression from the bone marrow (15). Temporal changes in cell surface carbohydrate composition and lectin binding of mouse trophoblasts have been connected with infiltration in the uterine wall (16–17).

To further investigate the apparent participation of cell surface carbohydrates in tissue invasion, an organ culture model was used which meets the above-mentioned criteria of invasion (18, 19). Matched pairs of malignant and nonmalignant cells were studied for the existence of a possible correlation between the occurrence of cancer-related carbohydrate changes and the invasive capacity. Moreover, to go beyond correlative observations and to obtain a direct indication of a functional relationship it was attempted to transiently modify the surface carbohydrates of noninvasive, nontumorigenic cells. In view of its reported activity on invasion (20) the ether analogue ET-18-OCH₃ of the naturally occurring 2-lyosphatidylcholine was tested for possible effects on cell surface carbohydrates. This ALP transiently modified the carbohydrates to an extent that they resembled those found on cancer cell surfaces.

MATERIALS AND METHODS

Cells. The cell lines R1C and HSU, established from baby rat kidney cells by transfection with genomic fragments of the oncogenic Ad12, were identical to those described by De Leijn et al. (21). The left-hand fragment (0–7.2%) of the Ad12 genome caused stable morphological transformation but no tumorigenic potential (HSU). Transfection with a larger fragment (0–16%) resulted in fully transformed, tumorigenic R1C cells (22).

Malignant rat brain cells (RB14-T) and their nonmalignant precursors (RB14-N and RB22) were obtained and cultured in the same way as described by Laerum and Rajewski (23). Briefly, neonatal rat brain cells exposed in vivo to the carcinogen ENU underwent continuous morphological transformation during serial culture in vitro, followed by malignant transformation (tested in syngeneic animals) after about 12 passages (RB14-T). The RB14-N cells were from cultures prior to tumorigenic transformation, while the RB22 cells were normal controls from animals not exposed to the carcinogen.

The mouse MO cell family has been previously described by Mareel et al. (24). The MO3, cells, transformed with KiSV, are invasive in vitro (18) and produce invasive and metastasizing tumors in syngeneic mice (19), in contrast to the nontransformed counterpart (MOH).

The NIH/3T3 cells and its transformant induced by human tumor DNA have been described by Collard et al. (25). The malignant T13

Received 12/26/85; revised 3/24/86; accepted 4/17/86.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by grants from the Netherlands Cancer Foundation, Koninklijke Wilhelmina Fonds (NKI-KWF 84-16), Amsterdam, the Netherlands; from the Nationaal Fonds voor Wetenschappelijk Onderzoek (20.093 and 39.000.983); and from NAVO and Belgisch werk legen Ranker, Brussels, Belgium.

2 To whom requests for reprints should be addressed.

3 Present address: Laboratoria Birstol Benelux, 185-187 Terhulpensesteenweg, 1170 Brussels, Belgium.

4 The abbreviations used are: ET-18-OCH₃, racemic 1-O-octadecyl-2-O-methyl glycerol-3-phosphocholine; ALP, alkyl-lyso phospholipid; ENU, ethylnitrosourea; Ad12, adenovirus type 12; KiSV, Kirsten sarcoma virus.
transformant was established by transfection with T24 bladder carcinoma DNA.

All cells were cultured in Dulbecco's modified Eagle's medium (Flow Laboratories, Ltd., Irvine, Scotland) supplemented with 10% fetal calf serum (Gibco Europe, England) and antibiotics (penicillin, 100 IU/ml; streptomycin, 50 µg/ml) in a humidified incubator at 37°C in 5% CO2.

Invasion Assay. The capacity of cells to invade normal tissue was assayed in confrontations between test cell aggregates and fragments of 9-day-old embryonic chick heart in organ culture as described earlier (18). Briefly, the aggregates (diameter, 0.2 mm) were brought into contact with precultured heart fragments (diameter, 0.4 mm) on top of a semisolid agar medium. After attachment of the aggregates to the heart fragment the confronting pairs were further incubated individually in fluid culture medium on a gyratory shaker at 120 rpm. The confronting pairs were fixed in Bouin-Holland's solution after 1–28 days, followed by embedding in paraffin or Histowax and complete serial sectioning (8 µm thick), and stained with hematoxylin-cosin or with an antiserum against chick heart tissue (26). For semiquantitative evaluation the interaction of test cells with chick heart was classified as described before (27): Grades I and II (confronting cells found at the periphery of, or intermixed with, the outer fibroblastic layer of the heart fragment, respectively) were combined to indicate the absence of invasion (denoted as −); Grade III, when confronting cells replaced the cardiac muscle to less than 50% (denoted as +); and Grade IV, when confronting cells replaced the cardiac muscle to more than 50% (denoted as ++). According to Mareel et al. (19) Grades III and IV meet the criteria of invasion in vivo. In assays with ALP-treated cells, suspensions of cells were confronted with heart fragments on top of a semisolid agar medium to provide a more rapid and maximal interaction. After 4 h the excess of unattached cells was removed, and the heart fragments with attached cells were incubated individually in fluid culture medium as usual.

Cell Surface Glycopeptide Isolation and Analysis. Cells or cell aggregates were metabolically labeled with either L-[5,6-3H]fucose (1 µCi/ml, 60 Ci/mmol) or L-[1-14C]fucose (0.5 µCi/ml, 60 mCi/mmol) for 20 h at 37°C. Radiochemicals were obtained from New England Nuclear, Boston, MA, or The Radiochemical Center, Amersham, United Kingdom. Glycopeptides were isolated from the cell surface by proteolytic enzymes as described (28) and desalted by gel filtration-centrifugation on Bio-Gel P-2 columns (29) or dialysis against bidistilled water. Glycopeptides were analyzed as such or following treatment with mild acid or neuraminidase (Vibrio cholerae, EC. 3.2.1.18; Behringwerke AG, Marburg, West Germany) to remove terminal sialic acids as described earlier (28). Gel filtration was performed on Bio-Gel P-10 Sephadex G-50 (2.1, w/v) columns, eluted with 0.1 M Tris-HCl buffer (pH 8.0) containing sodium dodecyl sulfate (0.1%, w/v), EDTA (0.01%, w/v), and 2-mercaptoethanol (0.1%, v/v) with a flow rate of 4 ml/h and 0.7-ml fraction size.

Modification of Cell Surface Carbohydrates by ET-18-OCH3. Cells were incubated with ALP (kindly provided by Dr. W. E. Berdel, Technical University, Munich, Federal Republic of Germany) 48 h prior to the invasion assay or glycopeptide analysis. The drug was used at concentrations which permitted growth to at least 75% of that of controls. These concentrations varied between 5 and 30 µg/ml, depending on the cell type.

RESULTS

Comparison of Malignant and Nonmalignant Cells. The possible involvement of cancer-related changes in cell surface carbohydrates in malignant invasion was studied in four matched pairs of malignant and nonmalignant cells. The panel included viral and chemical transfectants as well as human- and viral-DNA transfectants.

The first cell system consisted of R1C and HSU rat kidney cells transformed by specific Ad12 genome fragments (22). To test whether the differences in carbohydrate composition between these transfectants observed in monolayer cultures (21) were also present when grown in aggregates, HSU and R1C cell aggregates were metabolically labeled with [3H]- or [14C]-fucose. The size distribution pattern (Fig. 1) showed that the glycopeptides derived from tumorigenic R1C cell aggregates were of a higher apparent molecular weight relative to those from HSU cell aggregates, comparable to the differences in R1C and HSU cells grown in monolayers (cf. Ref. 21). The invasive capacity of HSU and R1C cells was studied in parallel. Multiple confronting pairs of cell aggregates and precultured heart fragments were incubated and fixed after 4 and 7 days. Characteristic stages of the invasive process are illustrated in Fig. 2, and the results are included in Table 1. The tumorigenic R1C cells were highly invasive (++, Grade IV). The histology of all cultures met the criteria of malignant invasion since R1C cells progressively replaced the cardiac muscle (Fig. 2). Counterstaining with an antiserum against chick heart tissue revealed that only remnants of the cardiac muscle were left (Fig. 2d).

However, morphological transformation alone appeared insufficient for invasion since the interaction between HSU cells and heart tissue was at most limited to the occupation of the outer fibroblastic layer of the heart fragment (Fig. 3). Even after 28 days when HSU cells had totally surrounded the heart fragment, these cells were still incapable of infiltrating and degrading the chick heart fragment (Fig. 3, e and f). Apparently, the additional information in the larger Ad12 genome fragment, responsible for alterations in cell surface carbohydrates (21), was causal in the acquisition of a tumorigenic and invasive phenotype.

Neonatal rat brain cells expressed in vivo to the carcinogenic ENU undergo continuous transformation during serial culture in vitro (cf. 23). The acquisition of the tumorigenic potential of these cells coincides with the capacity to invade into chick heart tissue (30). In a similar series of rat brain cells the surface carbohydrates were analyzed before and after transformation to tumorigenic cells. Glycopeptides derived from the rat brain cells after tumorigenic transformation (cell line RB14-T) expressed cancer-related changes in cell surface carbohydrates when compared with those from cells before malignant transformation (RB14-N) (Fig. 4). The surface carbohydrate composition of the latter cells was comparable with that of RB22 cells derived from untreated animals (data not shown).

In the third system glycopeptides derived from the tumori-
Fig. 2. Light micrographs of 8-μm-thick sections from aggregates of malignant R1C confronted with fragments of chick cardiac muscle. Fixation after 7 (a, b) and 14 (c, d) days; a and c were stained with H & E; b and d with an antiserum against chick heart. R, R1C; CH, chick heart tissue. Bars, 50 μm.

Table 1  Semiquantitative analysis of invasion into chick heart tissue

<table>
<thead>
<tr>
<th>Cells</th>
<th>Grading* after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 4</td>
</tr>
<tr>
<td>HSU Nonmalignant rat kidney cells transfected with (0–7.2%) Ad12 DNA</td>
<td>– (5)a</td>
</tr>
<tr>
<td>RIC Malignant rat kidney cells transfected with (0–16%) Ad12 DNA</td>
<td>+ (3)</td>
</tr>
<tr>
<td>NIH/3T3 Nonmalignant mouse cells</td>
<td>– (3)</td>
</tr>
<tr>
<td>T13 Malignant transformant of NIH/3T3 induced by T24 bladder carcinoma DNA</td>
<td>+ (6)</td>
</tr>
</tbody>
</table>

Grading of invasion was according to Bracke et al. (27). –, no invasion (Grades I and II); +, less than 50% invasion (Grade III); ++, over 50% invasion (Grade IV). For details see “Materials and Methods.”

Numbers in parentheses, number of confronted pairs analyzed.

genic and invasive MO4 (18, 19) cells and the nontransformed counterparts (MOH) were analyzed as usual. The size distribution pattern (data not shown) revealed that the tumorigenic MO4 cells expressed cancer-related glycopeptide changes. The invasive capacity of MO4 cells and the absence of this property in MOH cells were confirmed in parallel experiments (data not shown).

The fourth cell system consisted of NIH/3T3 cells and its transformant established by transfection with T24 bladder carcinoma DNA (T13). Similarly, as published previously (25, 31), T13 cells expressed cancer-related carbohydrate changes relative to the parent NIH/3T3 cells (data not shown). The T13 and NIH/3T3 cells were analyzed in parallel for their ability to invade chick heart tissue. As included in Table 1, the T13 cells were invasive (++, Grade IV) while parent NIH/3T3 cells were not.

The combined observations in the various cell systems (summarized in Table 2) demonstrate a strict correlation between cancer-related carbohydrate changes and the invasive capacity of transformed cells.

Modulation of Cell Surface Carbohydrates and Invasion. It has been recently reported that ALP interferes with the invasive process when present during the assay (20). We therefore investigated whether ALP may mediate invasion by affecting the carbohydrate moieties of surface glycoproteins. The tumorigenic R1C cells and the morphologically transformed but non-tumorigenic HSU cells were incubated with ALP during 48 h in 5 and 20 μg of ALP per ml, respectively (the highest concentr-
Fig. 3. Light micrographs of 8-μm-thick sections from aggregates of morphologically transformed but nonmalignant HSU rat kidney cells confronted with fragments of chick cardiac muscle. Fixation after 7 (a, b), 14 (c, d), and 28 (e, f) days; a, c, and e were stained with H & E; b, d, and f with an antiserum against chick heart. H, HSU; CH, chick heart tissue. Bars, 50 μm.

Fig. 4. Gel filtration profiles of surface glycopeptides derived from ENU-treated, malignant RB14-T (•) and ENU-treated, premalignant RB14-N rat brain cells (O). For conditions, see legend to Fig. 1.

Table 2 Relation between cancer-related alterations in cell surface carbohydrates and invasiveness in vitro

<table>
<thead>
<tr>
<th>Cells</th>
<th>Invasiveness*</th>
<th>Cell surface carbohydrates*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSU Nonmalignant rat kidney cells transfected with (0-7.2%) Ad12 DNA</td>
<td>Noninvasive</td>
<td>Normal</td>
</tr>
<tr>
<td>RIC Malignant rat kidney cells transfected with (0-16%) Ad12 DNA</td>
<td>Invasive</td>
<td>Altered</td>
</tr>
<tr>
<td>RB22 Untreated rat brain cells</td>
<td>Noninvasive</td>
<td>Normal</td>
</tr>
<tr>
<td>RB14-N Premalignant, ENU-treated rat brain cells</td>
<td>Noninvasive</td>
<td>Normal</td>
</tr>
<tr>
<td>RB14-T Malignant, ENU-treated rat brain cells</td>
<td>Invasive</td>
<td>Altered</td>
</tr>
<tr>
<td>MOH Nonmalignant C3H mouse cells</td>
<td>Noninvasive</td>
<td>Normal</td>
</tr>
<tr>
<td>MO4 Malignant C3H mouse cells transformed by KISV</td>
<td>Invasive</td>
<td>Altered</td>
</tr>
<tr>
<td>NIH/3T3 Nonmalignant mouse cells</td>
<td>Noninvasive</td>
<td>Normal</td>
</tr>
<tr>
<td>T13 Malignant transformant of NIH/3T3 induced by T24 bladder carcinoma DNA</td>
<td>Invasive</td>
<td>Altered</td>
</tr>
</tbody>
</table>

* Invasion was measured in an organ culture assay into precultured chick heart fragments as described in "Materials and Methods."

† Glycopeptides derived from matching pairs of cells were analyzed by cochromatography as described in "Materials and Methods." "Altered" means that the cells expressed cancer-related alterations in carbohydrate moieties of membrane glycoproteins relative to nonmalignant counterparts (normal).

‡ From De Ridder and Laerum (30); measured in similar series of rat brain cells using the same assay.
treatment completely abolished the ALP effect (data not shown), suggesting that increased surface sialylation was the underlying cause of this shift in size distribution. A full account of the biochemical effects of ALP will be reported separately.\(^5\) To study the effect of ALP pretreatment on invasion, HSU cells were confronted with the heart tissue as a single cell suspension on semisolid agarose medium to provide a rapid and maximal interaction. After 2, 4, and 7 days histological examination indicated a clear occupation (+, Grade III) of the heart tissue by ALP-treated HSU cells, whereas in control cultures both cardiac muscle and HSU cells remained sharply delineated (Fig. 6; Table 3). Occupation and penetration of the host tissue were to some extent progressive in time, meeting the criteria of at least Grade III of invasion. However, after 7 days the chick heart fragment was not totally abolished by the ALP-treated HSU cells in contrast to the very progressive invasion of R1C (+++, Grade IV). Pretreatment of R1C cells with ALP had no effects on the invasive capacity.

The same ALP-mediated induction of invasion was achieved with NIH/3T3 cells (Table 3) tested as suspended cells. Unlike the results with NIH/3T3 cells in aggregates (Table 1), occasionally some untreated NIH/3T3 cells appeared to be able to penetrate the chick heart muscle. The background invasion in this assay is as yet unexplained but possibly due to selection of variant cells facilitated by the increased interaction area.

DISCUSSION

The biological consequences of cancer-related alterations in cell surface carbohydrates have been discussed in several reviews (1-5). A prevailing conclusion was that such changes may not affect a single cellular function but rather provoke a general infidelity in various cell surface-mediated processes controlling normal cell behavior. In the present study it was attempted to associate specific surface carbohydrate expressions with the capacity to invade normal tissue. It was observed in four different cell systems (Table 2) that only cell lines with altered carbohydrates were capable of invading normal tissue. Immortalization and morphological transformation without simultaneous alteration in surface carbohydrates, as in the HSU and RB14-N cells, were in themselves insufficient for invasion. Thus, a previously established correlation between invasive capacity in vitro and tumorigenicity in vivo (18, 30) can be extended with the concomitant occurrence of cell surface carbohydrate changes. Accordingly, cancer-related changes in cell surface carbohydrates may be conditional if not causative in the invasive potential.

Supportive evidence for the latter contention follows from the studies with ALP. The ALP-induced epigenetic change in the degree of sialylation of surface molecules transiently endowed nontumorigenic HSU cells with invasive capacity (Fig. 6) and stimulated that of NIH/3T3 cells (Table 3). Quantitatively, the invasion by ALP-treated HSU cells was less than obtained with permanently malignant cell lines. This is ascribed to the disappearance of the effect of ALP 64 h after its removal (Fig. 5d). On the other hand the limited invasion by ALP-treated cells was persistent after 7 days (Table 3; Fig. 6) and to some extent progressive in time (cf. Table 3, Days 2 and 4). It is assumed that proliferation of already invaded cells accounts for these observations. The precise mechanisms through which ALP alters the level of surface sialylation are as yet unknown, but probably different from those operational after malignant transformation as will be reported in detail separately.\(^5\)

How alteration in glycosylation could be instrumental in tissue invasion might be considered in the context of cellular adhesion and contact inhibition of movement (reviewed in Refs. 32-35). Abercrombie (32) concluded from studies with confronting colonies that pairs of malignant cells do not intermix and that a polarity in contact inhibition of movement between malignant and normal tissue is required to bring about the invasion-like growth pattern in this system. The differential adhesion hypothesis proposed by Steinberg (36), based on thermodynamic considerations of homotypic and heterotypic adhesive forces, predicts that cells will move into an environment with maximal cellular interaction. Interaction between cells is mediated by cell adhesion molecules (cf. Ref. 37), and the strength of intercellular adhesion can be strongly influenced by

Fig. 6. Light micrographs of 8-μm-thick sections of nonmalignant HSU rat kidney cells after pretreatment with (a) or without (b) ALP, confronted as cell suspensions with fragments of chick heart muscle. Fixation after 7 days and staining with H & E. HSU, CH, chick heart tissue. Bars, 50 μm.

Table 3 Semiquantitative analysis of ALP-induced invasion into chick heart tissue

Cells were pretreated with ALP and confronted as cell suspensions with chick heart fragments in the absence of the drug as described in “Materials and Methods.”

<table>
<thead>
<tr>
<th>Cells</th>
<th>ALP (μg/ml)</th>
<th>Day 2</th>
<th>Day 4</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSU</td>
<td>0</td>
<td>-(3)</td>
<td>-(4)</td>
<td>-(2)</td>
</tr>
<tr>
<td>HSU</td>
<td>20</td>
<td>+(3); + (2)</td>
<td>+(5)</td>
<td>+(2)</td>
</tr>
<tr>
<td>NIH(3T3)</td>
<td>0</td>
<td>-(3)</td>
<td>-(3)</td>
<td>-(4); + (2)</td>
</tr>
<tr>
<td>NIH(3T3)</td>
<td>30</td>
<td>-(4)</td>
<td>+(4)</td>
<td>+(4)</td>
</tr>
</tbody>
</table>

* See Table 1, Footnote a.
* Numbers in parentheses, number of confronted pairs analyzed.

the degree of sialylation of these cell adhesion molecules (37, 38). If such mechanisms are also relevant in the invasive process, it is conceivable that the increased carbohydrate sialylation in malignant cells or in ALP-treated HSU and NIH/3T3 cells could alter in a similar fashion the adhesive forces to meet the conditions for penetration into the heart tissue.

Paradoxically, ALP, which stimulated invasiveness in HSU and NIH/3T3 cells, is antiinvasive when present during the confrontation of malignant MO4 cells and chick heart tissue (20). In the latter experiments, however, the drug was continuously present during the invasion assay, thus acting on both the malignant cells and the heart tissue. As already demonstrated for R1C and HSU cells (Fig. 5), increased sialylation by ALP is most pronounced in the nontumorigenic HSU cells. Also in normal chick heart fibroblasts and myoblasts large increases in surface carbohydrate sialylation have been observed.

As a result, preexisting differences in the degree of sialylation (and consequently in the relative intercellular adhesion) may diminish or disappear during confrontation of malignant cells and chick cardiac tissue in the continuous presence of ALP and abolish the invasive process.

In conclusion cancer-related and drug-induced alterations in cell surface carbohydrates are associated with the invasive capacity and may play a mechanistic role in this process. However, whether increased sialylation is the crucial alteration remains to be established. Moreover, transient carbohydrate changes may have physiological functions in normal cell migration as well. Van Beek et al. (15) demonstrated that metamyelocytes during bone marrow egress temporarily express surface carbohydrate properties such as those permanently expressed on myeloid leukemic cells, but absent on mature granulocytes and promyelocytic precursors. They concluded that these changes assisted in the penetration through the sinus epithelium. Corresponding observations have been made in implanting trophoblastic cells (16, 17). Accordingly, tumorigenic cells could acquire invasive capacity by a permanent expression of the gene(s) responsible for the generation of cancer-related carbohydrates. In all invasive cell lines in this study (Table 2) transformation involved introduction or activation of ras genes or functional analogous DNA sequences, namely the 0–16% fragment of the Ad12 genome in R1C cells (39), the v-Ki-ras gene in MO4 cells transformed with KiSV (24), and c-Ha-ras in the bladder carcinoma DNA-transfected T13 cells (25, 31). Finally, in the tumorigenic rat brain cells (RB14-T) N-ras was activated as proved in the NIH/3T3 focus assay.

Accordingly, it would appear that in the cell lines studied (activated) oncogenes of the ras family accompanied the acquisition of invasive capacity, associated with specific alterations in surface carbohydrates.

ACKNOWLEDGMENTS

The authors thank Dr. P. F. Lens for kindly providing the rat brain cells and A. Rietor in for isolation, characterization, and cultivating of RB cells. L. Kuyper is gratefully acknowledged for expert histological assistance.

REFERENCES


* P. F. Lens, personal communication.


Effect of Cancer-related and Drug-induced Alterations in Surface Carbohydrates on the Invasive Capacity of Mouse and Rat Cells

Jan G. M. Bolscher, Denis C. C. Schallier, Lou A. Smets, et al.


Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/46/8/4080

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.