Biology of the Cell (1997) **89**, 467-473

Original article

Lack of modulation of the galectin-3 and asialoglycoprotein receptor transcription in hepatocarcinoma of transgenic mice

Eric Hébert and Michel Monsigny

Glycobiology, Centre de Biophysique Moléculaire et Université d'Orléans, rue Charles-Sadron, 45071 Orléans cedex 2, France

Galectin-3 and asialoglycoprotein receptor are lectins belonging to the classes of soluble lectins and of membrane C type lectins respectively. Conflicting results have been reported concerning their transcription level in the time course development of tumours. In the present study we investigated the abnormalities and the transcription levels of galectin-3 and asialoglycoprotein receptor genes in liver-targeted SV40 large T transgenic mice related to normal mice. In the strain expressing the highest level of large T, 100% of the male mice reproducibly developed an hepatocarcinoma. We provide evidence that the galectin-3 and asialoglycoprotein receptor genes are stable in such mice. The galectin-3 gene is weakly transcribed and its level is identical and constant in normal and transgenic mice, suggesting a lack of involvement in the development of large T-induced hepatocarcinoma. The asialoglycoprotein receptor gene is actively transcribed and its level remains high all along the development of the tumour; therefore, in such an hepatocarcinoma the asialoglycoprotein receptor could be used to take up drugs, genes or oligonucleotides associated with glycosylated carriers bearing galactose residues in a terminal non-reducing position. (© Elsevier, Paris)

galectin-3 / asialoglycoprotein receptor / SV40 large T antigen / transgenic mice

INTRODUCTION

Animal lectins are recognised as molecules playing important roles in a variety of biological processes through binding to glycoconjugates (Drickamer, 1988; Sharon and Lis, 1989). Within the past few years, lectins became a well-established means for understanding varied aspects of cancer and metastasis and evidence is now emerging that lectins are dynamic contributors to tumour cell recognition (Hébert and Monsigny, 1993; Mody *et al*, 1995). To increase the understanding of these lectin-dependent processes, attempts are made to measure lectin gene expression in the time course development of various tumours. Among lectins, galectins are members of a growing family of β-galactoside-binding lectins and galectin-3 is one of the most extensively studied members (Barondes *et al*, 1994a, b). *In vitro*, it has been found that virally or oncogene-transformed mouse or human cells express a higher level of galectin-3 as compared with untransformed cells (Moutsatsos *et al*, 1987; Hébert and Monsigny, 1994; Hébert *et al*, 1996).

The relationship between galectin-3 expression and neoplastic transformation is particularly noteworthy. Increased galectin-3 expression has been related to the metastatic potential of several tumourigenic cells (Raz *et al*, 1990). However, in human colorectal carcinomas, galectin-3 has been reported to increase (Irimura *et al*, 1991) or to decrease (Castronovo *et al*, 1992; Lotz *et al*, 1993) with progression towards a metastatic state. Several highly metastatic melanoma cell lines express galectin-3 at a level similar to that of normal human melanocytes (Mey *et al*, 1994). Conversely, its expression was implicated in

Abbreviations: ASGR, asialoglycoprotein receptor; BSA, bovine serum albumin; EDTA, ethylene diamine tetraacetic acid; SDS, sodium dodecylsulfate; SSC, sodium salt citrate buffer; Tris, tris-[hydroxymethyl]aminomethane.

the metastatic potential of murine melanomas (Inohara and Raz, 1994). Moreover, it is reported that galectin-3 is down-regulated in human breast cancer in association with the acquisition of the invasive and metastatic phenotype (Castronovo *et al*, 1996) whereas other data (Le Marer and Hughes, 1996) suggest that galectin-3 expression in human breast cancer is not correlated directly with their invasive potential *in vitro*.

In the light of these controversial findings for which one explanation could be related to heterogeneity of the tumours during their development, we were prompted to study galectin-3 gene modification and/or transcription in an highly reproducible *in vivo* model system with regard to tumour development.

Hepatic lectins may also be involved in malignant transformation; they are plasma membrane receptors which bind asialoglycoproteins and actively mediate their endocytosis. The major rat hepatic lectin, the asialoglycoprotein receptor (ASGR) mediates the endocytosis of desialylated glycoproteins in mammalian hepatic parenchymal cells and rare hepatoma cells such as the hepatoblastoma cell line HepG2 (Ashwell and Harford, 1982). ASGR expression usually declines in parallel with a malignant transformation (Stockert and Becker, 1980). Furthermore, it has been shown the ASGR expression was repressed in response to enhanced rates of HepG2 proliferation *in vitro* (Theilmann *et al*, 1983).

In the present study we investigated a putative modification and the level of expression of the galectin-3 and ASGR genes at the mRNA level in SV40 T harbouring transgenic mice in relation with those of normal mice. A precise targeting of the SV40 T early expression region in the liver of transgenic mice was obtained by using 700 bp of the antithrombin III regulatory sequences to control oncogene expression. In the strain expressing a high level of large T antigen, the incidence of hepatocarcinoma was 100% in males. The transgene was indeed integrated in the Y chromosome, in order that the hepatocarcinomas are restricted to the male mice. The evolution was reproducible and characterised by a marked cytolysis starting at about 4 weeks of age, a preneoplastic state marked by a progression from hyperplasia to proliferative nodules which are able to elicit a tumour formation in nude mice and to proliferate *in vitro*; this hepatocellular carcinoma is associated, in 10% of the cases, with lung metastases. The mice usually die at 7 months corresponding to the latest stage of tumour progression (Dubois *et al*, 1991). These transgenic mice are therefore a good model to study the modulation of expression of protein genes putatively involved in the development of a spontaneous tumour.

We provide evidence that the galectin-3 and ASGR genes are neither qualitatively nor quantitatively altered in transgenic mice developing hepatocarcinoma. Galectin-3 gene is weakly transcribed without variation of the transcript level between 1 and 7 months suggesting a poor involvement, if any, in the development of such hepatocarcinoma. The ASGR gene is also transcribed without any modification along the development of the tumour. This constant level of expression could provide a useful way of ASGR-mediated gene delivery in advanced tumours.

MATERIALS AND METHODS

Animals

Transgenic mice were obtained from Dr P Briand. In the line used, the transgene was integrated on the Y chromosome, which facilitates the obtention of a large number of offspring and the screening procedure (Dubois *et al*, 1991). Both normal and transgenic animals were B6/D2 F1 hybrids. Animals were killed and hepatectomized at various ages ranging from 1 to 7 months. Tissue samples were obtained from non-nodular pieces of the liver as well as from nodules with a size corresponding to 5 to 100 mg.

DNA and RNA extraction

Genomic DNA was prepared from Ultra-Turrax®-homogenized tissues by phenol extraction (Sambrook *et al*, 1989). After ethanol precipitation, the DNA samples were dissolved in 10 mM Tris-1 mM EDTA (pH 7.4) and stored at –20°C. Total RNA was extracted according to Chomczynski and Sacchi (1987), redissolved in 10 mM Tris buffer (pH 7.4), and stored at –70°C.

DNA analysis

DNA (10 μg) was digested with *Hin*dIII or *Eco*RI and electrophoresed through a 0.8% agarose gel. The DNA was blotted onto Hybond-N membrane (Amersham, Buckinghamshire, UK) and cross-linked by alkaline treatment of the membrane in 0.4 M sodium hydroxide. Hybridisation was performed according to the method of Mahmoudi and Lin (1989): briefly, the membrane was hybridised at 68°C overnight with 2×10^6 cpm mL^{-1} radiolabelled probe in 2 mM EDTA, 0.5 M sodium phosphate buffer containing 70 mg/mL SDS and 10 mg/mL BSA. The blot was then washed to high stringency (0.2 SSC at 68°C) and autoradiographed for 1–10 days at –70°C using Fuji medical X-ray film.

RNA analysis

Total RNA (50 μg per lane) was analysed by electrophoresis in 1.4% agarose glyoxal gels (McMaster and Carmichael, 1977) transferred to nylon membranes, hybridised with radiolabelled probe and washed according to the above-described method for DNA analysis.

Probes

For Southern and Northern blot analysis, the 950 bp fragment of the galectin-3 excised from the pM5 plasmid (Raz *et al*, 1987) or a plasmid containing the mouse asialoglycoprotein receptor cDNA MHL-1 (Takezawa *et al*, 1993) were used. The p91 plasmid containing β-actin cDNA (Minty *et al*, 1981) was used as a control for DNA and RNA loading and for a quality control.

Probes were radiolabelled with [α-^{32}P]dCTP (3000 Ci mmol^{-1}) by the random primer method of Feinberg and Vogelstein (1984).

Densitometric scanning of the gels

The quantification of the autoradiographs intensities was carried out using a Bioprint® densitometric scanning apparatus (Vilbert-Lourmat, France) equipped with Image Quant® software.

RESULTS AND DISCUSSION

Our purpose was to investigate the galectin-3 and ASGR gene structure and transcription during the development of large T-induced hepatocarcinoma in transgenic mice.

Southern blot of transgenic mice liver DNA

It is widely accepted that multiple genetic alterations are essential for the development of malignant tumours, including hepatocarcinoma. The gene alteration frequently found in cancer is a gene amplification (Brison, 1993) and, for instance, c-*myc* oncogene amplification has been reported in rat hepatocarcinoma (Pascale *et al*, 1996). In order to rule out any increase of lectin RNA content in tumour which could be due to gene amplification we performed a study of the galectin-3 and ASGR gene organisation.

DNA extracted from various liver biopsies, including tumour nodules of various sizes from large T transgenic male mice and from female mice of the same strain was digested with *Hin*dIII restriction enzymes and analysed with a galectin-3 probe or digested with *Eco*RI restriction enzyme and analysed with a ASGR probe.

The digestion pattern obtained from *Hin*dIII digestion of DNA of two different 7-month-old transgenic males and of two different reference 7-month-old females is shown in figure 1a. This pattern showed in each case three bands corresponding to DNA fragments of 4.6, 3.3 and 1.3 kb in line with literature data (Raz *et al*, 1987; Voss *et al*, 1994); these bands correspond to the *Hin*dIII-generated fragments of the mouse galectin-3 gene which can hybridise with the mouse galectin-3 cDNA probe we have used (fig 1c) (Raz *et al*, 1987).

The digestion pattern obtained from *Eco*RI digestion of DNA of two different 7-month-old transgenic males and of two different reference 7-month-old females screened with a mouse ASGR cDNA probe (Takezawa *et al*, 1993) is shown in figure 2. In all cases the pattern revealed only one band corresponding to about 3.5 kb DNA fragment which can not be precisely assigned as the mouse ASGR gene sequence is not known.

The patterns obtained with any of the samples examined, 50 transgenic tissues and 20 non-transgenic female tissues, were alike, independent of the

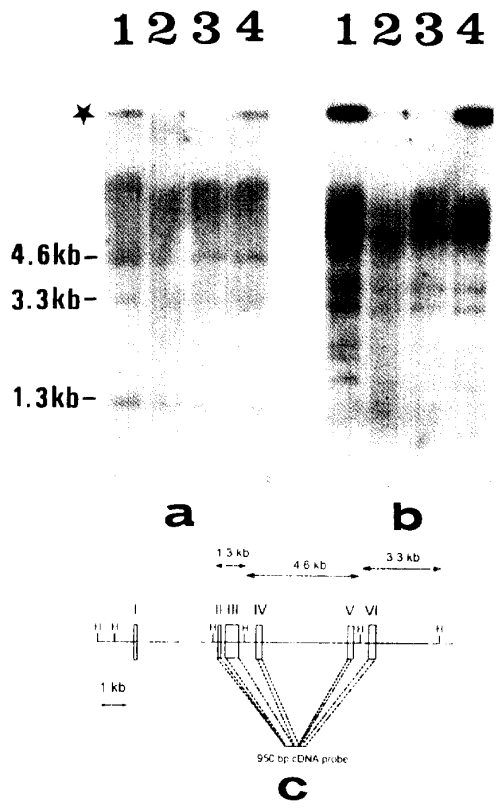

Fig 1. a, b. Southern blot analyses of 7-month-old female mice (lanes 1 and 3) and of 7-month-old transgenic mice (lanes 2 and 4). DNA was digested with *Hin*dIII restriction enzyme, transferred on nylon membrane and hybridised with a ^{32}P-labelled galectin-3 probe **(a)** or a ^{32}P-labelled β-actin probe **(b)**. The star indicates the position of the well; signals at this position in some lanes are due to some insoluble labelled material left in the well but not to solubilised undigested DNA as can be shown on ethidium bromide stained gel (data not shown). **c.** Schematic representation of the murine galectin-3 gene (after Rosenberg *et al* (1993)). The non-coding first exon (I) and coding exons (II to VI) are depicted as open boxes related to the 950 bp cDNA probe (Raz *et al*, 1987) used in Southern blot experiments. Location of *Hin*dIII (H) restriction endonuclease sites are indicated as well as the size of the DNA fragments generated.

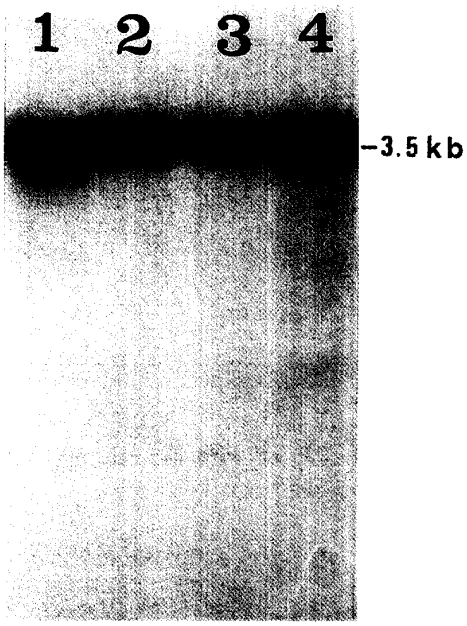

Fig 2. Southern blot analysis of 7-month-old female mice (lanes 1 and 3) and of 7-month-old transgenic mice (lanes 2 and 4). DNA was digested with *Eco*RI restriction enzyme, transferred on nylon membrane and hybridised with a ^{32}P-labelled asialoglycoprotein receptor probe.

Fig 3. Northern blot analysis of liver tissues from two 7-month-old transgenic mice (lanes 1 and 3) and from a reference 7-month-old female mouse (lane 2). Total RNA was electrophoresed in a glyoxal agarose gel and transferred to a nylon membrane as described under *Materials and methods*. A galectin-3 transcript of 1.6 kb was detected when the membrane was hybridised with a ^{32}P-labelled galectin-3 probe **(a)**. A β-actin transcript of 2.1 kb was detected upon a subsequent hybridisation with a ^{32}P-labelled p91 plasmid (Minty *et al*, 1981) **(b)**.

age of the transgenic mice or of the reference female and irrespective of the localisation, nodular or not, and of the size of the biopsy in the liver. The differences in intensity between the fragments in different lanes reflected the differences in loading of tissues DNA as controlled by the densitometric scanning of the bands obtained, in line with literature data (Minty *et al*, 1983), after rehybridisation with the β-actin probe (fig 1b for the galectin-3 experiment).

The data reported suggest that neither an amplification nor a drastic rearrangement of the galectin-3 and ASGR genes took place in the tumour tissue of transgenic mice developing a large T-induced hepatocarcinoma. However, tumours are known to be heterogeneous: first, within the same tumour sample, tumour cells can be found at different stages of tumour progression and could therefore display different abnormalities; second, tumour cells are associated with other cells which are not expected to present genomic abnormalities.

The fact that the DNA signal intensity from a tumour is very similar to that of non-tumorigenic tissues whatever the liver localisation or the size of the nodule, ranging from 1 mm in diameter for 4-month-old animals to more than 1 cm in diameter for 7-month-old animals, supports the lack of amplification of the lectin genes investigated in large T-induced hepatocarcinoma.

Northern blot

We first analysed total RNA extracted from nodular and non-nodular tissues of several transgenic males for the latest stage of tumour progression, *ie* for 7-month-old animals and of several reference non-transgenic females. An apparently normal size transcript (1.6 kb) (Raz *et al*, 1987) was detected after several days of autoradiography exposure when total tissue RNA was subjected to Northern blot analysis with galectin-3 probe (fig 3a). The differences in intensity between the bands of different lanes again reflected the differences in loading of tissue RNA as controlled by the densitometric scanning after rehybridisation with the β-actin probe (fig 3b) indicating that the galectin-3 gene is transcribed weakly but at the same level in normal tissue and in tissue from the most advanced stage of the hepatocarcinoma, *ie* for 7-month-old transgenic mice.

When samples of RNA from 7-month-old transgenic mice and non-transgenic females were subjected to Northern blot analysis with a ASGR probe, the already reported ASGR 1.4 kb transcript was detected (Stockert, 1993) at the same level in transgenic and female mice as indicated by rehybridisation with a β-actin probe and densitometric scanning (fig 4a, b, lanes 7 and F).

We then performed a study of galectin-3 and ASGR transcription during the time course development of the tumour: the densitometric analysis of the autoradiography of the Northern blots conducted as above indicates that the level of transcription remains constant for galectin-3 (data not shown) or ASGR (fig 4, lanes 1 to 7) at any step of the hepatocarcinoma development between 1 and 7 months. These observations were found in 50 transgenic mice and 20 non-transgenic female control mice again irrespective of the liver localisation, nodular or not, and of the size of the tissue sample investigated.

As far as galectin-3 is concerned, the lack of variation of RNA level we observed may be surprising in the light of the well documented literature reporting either an increased or a decreased expression of galectin-3 in tumour of various sources (Raz *et al*, 1990; Irimura *et al*, 1991; Castronovo *et al*, 1992, 1996; Lotz *et al*, 1993; Inohara and Raz, 1994; Mey *et al*, 1994; Le Marer and Hughes, 1996). Nevertheless, it should be noticed that the RNA signal of galectin-3 is very weak in the liver tissues we examined, even in the non-transgenic animal, and was only detectable after a long autoradiography exposure. This observation reflects a very low transcription level of the galectin-3 gene in any cell type of the mouse liver. In line with this result is the reported apparent lack of galectin-3 transcript in both the tumoral and the normal mouse liver tissue (Raz *et al*, 1987).

These data suggest that the galectin-3 modulation of transcription is poorly involved, if at all, in the development of SV40 large T-induced hepatocarcinoma. Because of limited availability of tumour tissues we have not measured the level of galectin-3 RNA in the lung metastases. Therefore we can not rule out the possibility that an increase in galectin-3 gene transcription occurs only in the metastatic cells emerging from the hepatocarcinoma as has been reported for pulmonary metastasis-derived cell lines (Raz *et al*, 1987, 1989) or metastatic carcinomas (Lotan *et al*, 1994; Schoeppner *et al*, 1995).

The limited availability of tumour tissue did not allow us to investigate the galectin-3 protein level in liver tissues but, in mouse cells, galectin-3 was shown to belong to the immediate early class of genes (Agrwal *et al*, 1989) for which the regulation

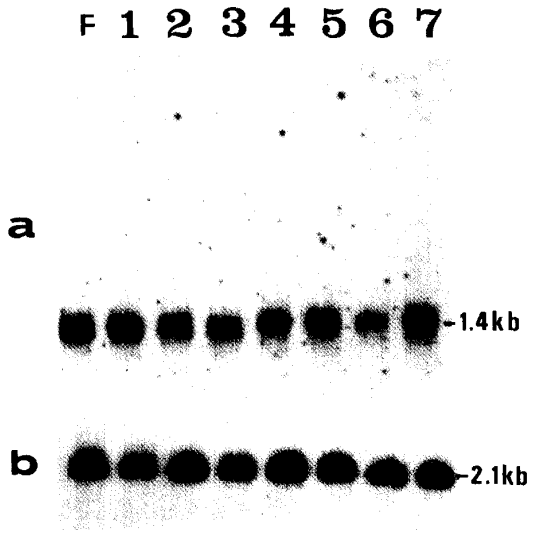

Fig 4. Northern blot analysis of liver tissues from 7-month-old non-transgenic female mouse (lane F) and from 1–7-month-old transgenic mice (lane 1 to 7): lane 1 corresponds to 1-month-old mouse; lane 2 to 2-month-old mouse and so on. An ASGR transcript of 1.4 kb was detected when the membrane was hybridised with a ^{32}P-labelled ASGR **(a)**. A β-actin transcript of 2.1 kb was detected upon a subsequent hybridisation with a ^{32}P-labelled p91 plasmid (Minty *et al*, 1981) **(b)**.

of expression occurs mainly at the RNA level (Marcu *et al*, 1992).

It has been reported that galectin-3 gene has an internal promoter activity down-regulated by the wild type p53 but not by a mutated form of p53 (Raimond *et al*, 1995). SV40-induced damages in cellular proliferating program rely mainly on SV40 large T interactions with various cellular proteins involved in the regulation of the cellular cycle among which is the p53 protein (Lane and Crawford, 1979; Linzer and Levine, 1979). Because such an interaction could be involved in the development of the hepatocarcinoma in the transgenic mice we studied, SV40 large T may have an indirect effect on the internal promoter of galectin-3. So far, it is not known whether p53 is mutated or not in the mice hepatocarcinoma and such a role of SV40 large T on galectin-3 transcription remains putative.

In the case of ASGR, it is known that its gene expression regulation takes place mainly at the RNA level (Stockert, 1993). There is a repression of ASGR expression in response to enhanced rates of proliferation of rat hepatocytes (Conti Devirgiliis *et al*, 1994) or of the human hepatoblastoma cell line, HepG2 (Stockert, 1993). The few *in vivo* results are more controver-

sial because a down modulation of ASGR content in chemically-induced hepatocarcinoma has been reported (Stockert and Becker, 1980) whereas ASGR messenger RNA levels did not significantly differ from controls in neoplastic rat liver (Huber *et al*, 1986).

Although our analysis has been performed on tissues containing various cell types (see above) and we can not rule out that some sparse tumoural cells display a down modulation which could be masked by normal RNA content in the surrounding cells, tumoural or not, our data suggest that the ASGR gene transcription is not significantly down-regulated in SV40 large T-induced hepatocarcinoma. Even taking into account that ASGR is already expressed by normal hepatocytes, its constant level of expression in advanced tumour suggests that it could be used to perform *in vivo* hepatocarcinoma gene therapy *via* an ASGR-mediated gene delivery. Various glycosylated carriers were indeed shown to efficiently transfer DNA *via* ASGR to hepatocytes as well as to hepatocarcinoma cells in a sugar-dependent manner (Wu *et al*, 1991; Plank *et al*, 1992; Midoux *et al*, 1993; Monsigny *et al*, 1994).

ACKNOWLEDGMENTS

We are grateful to Dr P Briand for providing transgenic mice. We thank Dr Y Watanabé for the gift of MHL-1 plasmid and Dr A Raz for the gift of pM5 plasmid. This work was supported by grant 2063 from the Association pour la Recherche sur le Cancer and by La Ligue Nationale contre le Cancer.

REFERENCES

Agrwal N, Wang JL and Voss PG (1989) Carbohydrate-binding protein 35. Levels of transcription and mRNA accumulation in quiescent and proliferating cells. *J Biol Chem* 264, 17236–17242

Ashwell G and Harford J (1982) Carbohydrate-specific receptors of the liver. *Annu Rev Biochem* 51, 531–554

Barondes SH, Castronovo V, Cooper DNW, Cummings RD, Drickamer K, Feizi T, Gitt MA, Hirabayashi J, Hughes C, Kasai K, Leffler H, Liu FT, Lotan R, Mercurio AM, Monsigny M, Pillai S, Poirier F, Raz A, Rigby PWJ, Rini JM and Wang JL (1994a) Galectins: a family of animal β-galactoside-binding lectins. *Cell* 76, 597–598

Barondes SH, Cooper DNW, Gitt MA and Lefler H (1994b) Galectins: structure and function of a large family of animal lectins. *J Biol Chem* 269, 20807–20810

Brison O (1993) Gene amplification and tumor progression. *Biophys Biochim Acta* 1155, 25–41

Castronovo V, Campo E, van den Brule F, Claysmith A, Cioce V, Liu F, Fernandez P and Sobel M (1992) Inverse modulation of steady-state messenger RNA levels of two non-integrin laminin binding proteins in human colon carcinoma. *J Natl Cancer Inst* 84, 1161–1169

Castronovo V, van den Brule F, Jackers P, Claussee N, Liu FT, Gillet C and Sobel M (1996) Decreased expression of galectin-3 is associated with progression of human breast cancer. *J Pathol* 179, 43–48

Chomczynski P and Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162, 156–159

Conti Devirgiliis L, Massimi M, Bruscalupi G, Felici A and Dini L (1994) Regulation of asialoglycoprotein receptor expression in rat hepatocytes cultured under proliferative conditions. *Exp Cell Res* 210, 123–129

Drickamer K (1988) Two distinct classes of carbohydrate-recognition domains in animal lectins. *J Biol Chem* 263, 9557–9560

Dubois N, Bennoun M, Allemand I, Molina T, Grimber G, Daudet-Monsac M, Abelanet R and Briand P (1991) Time-course development of differentiated hepatocarcinoma and lung metastasis in transgenic mice. *J Hepatol* 13, 227–239

Feinberg AP and Vogelstein B (1984) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 132, 6–13

Hébert E and Monsigny M (1993) Oncogenes and expression of endogenous lectins and glycoconjugates. *Biol Cell* 79, 97–109

Hébert E and Monsigny M (1994) Galectin-3 mRNA level depends on transformation phenotype in *ras*-transformed NIH 3T3 cells. *Biol Cell* 81, 73–76

Hébert E, Roche AC, Nachtigal M and Monsigny M (1996) Transformation but not *ras*-transfection increases the expression of galectin-3 in human HOS cells. *CR Acad Sci Paris* 319, 871–877

Huber BE, Glowinski IB and Thorgeirsson SS (1986) Transcriptional and post-transcriptional regulation of the asialoglycoprotein receptor in normal and neoplastic rat liver. *J Biol Chem* 261, 12400–12407

Inohara H and Raz A (1994) Effects of natural complex carbohydrate (citrus pectin) on murine melanoma cell properties related to galectin-3 functions. *Glycoconjugate J* 11,527–532

Irimura T, Matsushita Y, Sutton RC, Carralero ED, Ohannesian DW, Cleary KR, Ota DM, Nicolson GL and Lotan R (1991) Increased content of an endogenous lactose-binding lectin in human colorectal carcinoma progressed to metastatic stages. *Cancer Res* 51, 387–393

Lane DP and Crawford LV (1979) T antigen is bound to a host protein in SV 40-transformed cells. *Nature* 278, 261–263

Le Marer N and Hughes RC (1996) Effects of the carbohydrate-binding protein galectin-3 on the invasiveness of human breast carcinoma cells. *J Cell Physiol* 168, 51–58

Linzer DIH and Levine A (1979) Characterization of a 54K dalton cellular SV40 tumor antigen present in SV 40-transformed cells and uninfected embryonal carcinoma cells. *Cell* 17,43–52

Lotan R, Ito H, Yasui W, Yokozaki H, Lotan D and Tahara E (1994) Expression of a 31-kDa lactoside-binding lectin in normal human gactric mucosa and in primary and metastatic gastric carcinomas. *Int J Cancer* 56, 474–480

Lotz MN, Andrews CW, Korzelium CA, Lee EC, Steele GD, Clarke A and Mercurio AM (1993) Decreased expression of Mac-2 and loss of its nuclear localisation are associated with the neoplastic progression of colon carcinoma. *Proc Natl Acad Sci USA* 90, 3466–3470

Mahmoudi M and Lin V (1989) Comparison of two different hybridization systems in northern transfer analysis. *Biotechniques* 7, 331–332

Marcu KB, Bossone SA and Patel AJ (1992) Myc function and regulation. *Annu Rev Biochem* 61, 809–860

McMaster GK and Carmichael GG (1977) Analysis of single and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. *Proc Natl Acad Sci USA* 74, 4835–4839

Mey A, Vergnes OB, Apoil PA, Dore JF and Revillard JP (1994) Expression of galactose binding protein Mac 2 by human melanoma cell lines. *Cancer Lett* 81, 155–163

Midoux P, Mendes C, Legrand A, Raimond J, Mayer R, Monsigny M and Roche AC (1993) Specific gene transfer mediated by lactosylated poly-L-lysine into hepatoma cells. *Nucleic Acids Res* 21, 871–878

Minty AJ, Caravatti M, Robert B, Cohen A, Daubas P, Weydert A, Gros F and Buckingham ME (1981) Mouse actin messenger RNAs. *J Biol Chem* 256, 1008–1014

Minty AJ, Alonso S, Guenet JL and Buckingham ME (1983) Number and organization of actin-related sequences in the mouse genome. *J Mol Biol* 167, 77–101

Mody R, Joshi S and Chaney W (1995) Use of lectins as diagnostic and therapeutic tools for cancer. *J Pharmacol Toxicol Methods* 33, 1–10

Monsigny M, Roche AC, Midoux P and Mayer R (1994) Glycoconjugates as carriers for specific delivery of therapeutic drugs and genes. *Adv Drug Deliv* 14, 1–24

Moutsatsos IK, Wade M, Schindler M and Wang JL (1987) Endogenous lectins from cultured cells: nuclear localization of carbohydrate-binding protein 35 in proliferating 3T3 fibroblasts. *Proc Natl Acad Sci USA* 84, 6452–6456

Pascale RM, De Miglio MR, Muroni MR, Simile MM, Daino L, Seddaiu MA, Nufris A, Gaspa L, Deiana L and Feo F (1996) C-myc amplification in pre-malignant and malignant lesions induced in rat liver by the resistant hepatocyte model. *Int J Cancer* 68, 136–142

Plank C, Zatloukal K, Cotten M, Mechtler K and Wagner E (1992) Gene transfer into hepatocytes using asialoglycoprotein receptor mediated endocytosis of DNA complexed with an artificial tetra-antennary galactose ligand. *Bioconjug Chem* 3, 533–539

Raimond J, Rouleux F, Monsigny M and Legrand A (1995) The second intron of the human galectin-3 gene has a strong promoter activity down-regulated by p53. *FEBS Lett* 363, 165–169

Raz A, Avivi A, Pazerini G and Carmi P (1987) Cloning and expression of cDNA for two endogenous UV-2237 fibrosarcoma lectin genes. *Exp Cell Res* 173, 109–116

Raz A, Pazerini G and Carmi P (1989) Identification of the metastasis-associated, galactoside-binding lectin as a chimeric gene product with homology to an IgE-binding protein. *Cancer Res* 49, 3489–3493

Raz A, Zhu D, Hogan V, Shan N, Raz T, Karkash R, Pazerin G and Carmi P (1990) Evidence for the role of 34 kD galactoside-binding lectin in transformation and metastasis. *Int J Cancer* 46, 871–877

Rosenberg IM, Iyer R, Cherayil B, Chiodino C and Pillai S (1993) Structure of the murine *Mac-2* gene. *J Biol Chem* 268, 12393–12400

Sambrook J, Fritsch EE and Maniatis T (1989) *Molecular Cloning. A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA

Schoeppner HL, Raz A, Ho SB and Bresalier RS (1995) Expression of an endogenous galactose-binding lectin correlates with neoplastic progression in the colon. *Cancer* 75, 2818–2826

Sharon N and Lis H (1989) Lectins as cell recognition molecules. *Science* 246, 227–246

Stockert RJ (1993) Regulation of the human asialoglycoprotein receptor by cAMP. *J Biol Chem* 268, 19540–19544

Stockert RJ and Becker FF (1980) Diminished hepatic binding protein for desialylated glycoproteins during chemical hepatocarcinogenesis. *Cancer Res* 40, 3632–3634

Takezawa R, Shinzawa K, Watanabe Y and Akaike T (1993) Determination of mouse major asialoglycoprotein receptor cDNA sequence. *Biochim Biophys Acta* 1172, 220–222

Theilmann L, Teicher L, Schildkraut CS and Stockert RJ (1983) Growth-dependent expression of a cell surface glycoprotein *Biochim Biophys Acta* 762, 475–477

Voss PG, Tsay Y-G and Wang JL (1994) Galectin-3: differential accumulation of distinct mRNAs in serum-stimulated mouse 3T3 fibroblasts. *Glycoconjugate J* 11, 353–362

Wu GY, Wilson JM, Shalaby F, Grossman M, Shafritz DA and Wu CH (1991) Receptor-mediated gene delivery *in vivo*. *J Biol Chem* 266, 14338–14342

Received 25 July 1997; accepted 12 December 1997