Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 3542–3546, May 1990
Biochemistry

Molecular cloning of cDNA for the carcinoma-associated antigen GA733-2

(monoclonal antibody/COS cell expression system/antigen GA733-1)

STANISLAW SZALA, MONIKA FROEHLICH, MAUREEN SCOLLON, YASUSHI KASAI, ZENON STEPLEWSKI, HILARY KOPROWSKI, AND ALBAN J. LINNENBACH*

The Wistar Institute of Anatomy and Biology, 3601 Spruce Street, Philadelphia, PA 19104

Contributed by Hilary Koprowski, February 23, 1990

ABSTRACT **Defined by monoclonal antibody GA733, the GA733-2 antigen is a cell surface 40-kDa glycoprotein associated with human carcinomas of various origins. Molecular clones for the GA733-2 antigen were isolated from a colorectal carcinoma cell line cDNA library using the high-efficiency COS cell expression system. A 1.4-kilobase cDNA species was enriched by immunoselection with monoclonal antibody. The authenticity of individual clones was established by immunologic and sequence criteria. At the amino acid sequence level, GA733-2 was found to be >99% identical to the previously described KSA antigen defined by monoclonal antibody KS1/4. The amino acid sequence derived from the previously described GA733-related gene, GA733-1, was found to be 49% identical to GA733-2. The positions of 12 cysteine residues in the extracellular domains of the two GA733 antigens are conserved, as is the overall distribution of hydrophobic and hydrophilic residues. A 1.45-kilobase transcript of the GA733-2/KSA gene was found to be expressed in cell lines derived from colorectal and pancreatic carcinoma.**

Monoclonal antibodies (mAbs) defining tumor-associated cell surface antigens are currently being evaluated for use in the diagnosis and therapy of human cancer. Initial studies of mAbs CO17-1A and GA733 (1), demonstrating cytotoxic activity *in vitro* and tumoricidal activity in experimental animals (for review, see ref. 2), led to an evaluation of their immunotherapeutic potential in cancer patients. Idiotypic cascades have been demonstrated in cancer patients treated with mAb CO17-1A, providing support to the hypothesis that antigen-specific anti-anti-idiotypic antibody (Ab3) responses may explain some of the observed delayed clinical responses (3).

The independently derived mAbs GA733 and CO17-1A define the same 40-kDa cell surface glycoprotein (4), hereafter referred to as the GA733-2 antigen. It is expressed by carcinomas of several histologic types and to some extent by normal tissues (5). The molecular cloning of cDNA for the tumor-associated GA733-2 antigen will provide a more detailed structural characterization of the antigen and will facilitate the production of antigen needed for immunization strategies.

DNA and protein sequence analyses have identified a family of GA733 antigens (6). The GA733-2 antigen has been purified from a colorectal carcinoma cell line and its partial amino acid sequence has been determined. A genomic DNA clone isolated with an oligonucleotide probe was found to be a heretofore unknown gene, termed GA733-1, encoding a protein similar but not identical to the protein sequence of the GA733-2 antigen. The GA733-1 gene is intronless. It is transcribed into a 1.8-kilobase (kb) mRNA in pancreatic carcinoma cell lines at high levels, relative to that observed in a colorectal carcinoma cell line. Both GA733-2 and GA733-1 sequences have significant homology to an exon-encoded repeat unit in thyroglobulin and the *HLA-DR*-associated invariant chain.

Here we report the isolation of cDNA clones for the GA733-2 antigen using the high-efficiency COS cell expression system (7–9) and the sequence of the GA733-2 cDNA.† Molecular clones for the GA733-2 antigen were enriched from a colorectal carcinoma cell line cDNA library by iterating cycles of expression of the plasmid library in COS cells, immunoselection of antigen-positive cells by "panning" with the mAb GA733, and transfer of plasmid DNA from COS cells into *Escherichia coli*. The sequence of GA733-2 was compared to that of the carcinoma-associated antigen KSA (10, 11) and to the related GA733-1 antigen (6). A group of related mAbs (1, 12, 13) were assayed for reactivity to the antigen expressed by the GA733-2 clone. The size and expression of the GA733-2 mRNA in tumor cell lines were also studied.

MATERIALS AND METHODS

cDNA Library Preparation. Cytoplasmic RNA was isolated from the colorectal carcinoma cell line SW948 by the vanadium method (14) and then twice fractionated by oligo(dT)-cellulose column chromatography (15). cDNA was synthesized using 4 μg of poly(A)$^+$ mRNA; the double-stranded cDNA was ligated to *Bst*XI adaptors and then size-selected on a 5–20% (wt/vol) potassium acetate gradient (8). cDNA ≥0.8 kb long was ligated to *Bst*XI-digested gradient-purified CDM8 expression vector (9), and the recombinants were transfected into *E. coli* MC1061/P3 cells. A cDNA library of 1×10^7 recombinants was obtained.

Immunoselection of cDNA Clones. Three sequential transfers of SW948 cDNA between COS cells and *E. coli* cells were performed.

In the first transfer, recombinant bacteria were amplified overnight in the presence of chloramphenicol, and library DNA was isolated by alkaline lysis and centrifugation on cesium chloride/ethidium bromide gradients. The DEAE-dextran transfection method was used to introduce library DNA into COS cells. Twenty-four 60-mm dishes containing COS cells at ≈50% confluency were incubated with DNA at 0.2 μg per dish (7). At 24 hr after transfection, the cells were treated with trypsin and distributed to 48 dishes. The cells were detached from the dishes after an additional 48 hr with a buffer containing EDTA (7) and then incubated with affinity-purified mAb GA733-2 at 1 μg/ml. After centrifugation through a cushion of 2% (vol/vol) Ficoll-400 and passage

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

Abbreviations: mAb, monoclonal antibody; MHA, mixed hemadsorption assay.

*To whom reprint requests should be addressed.

†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M33011).

through a 100-μm nylon mesh, the cells were distributed onto bacteriological Petri dishes that had been coated with sheep anti-mouse IgG (Cooper Biomedical). The dishes were rinsed, and the adherent cells were lysed by addition of a buffer containing 0.6% SDS (16). Episomal DNA was recovered (16) and reintroduced into *E. coli* MC1061/P3 cells. Ampicillin- and tetracycline-resistant bacterial colonies were harvested from plates and expanded by liquid culture in the presence of chloramphenicol.

For the second and third transfers, the recombinant bacteria were converted to spheroplasts and fused to COS cells by addition, and then immediate removal, of PEG 1450. After an expression period of 72 hr, GA733-2 cDNA was further selected by "panning" (7). To monitor the enrichment of a specific size-class of cDNA insert, 10 bacterial colonies after each transfer were randomly selected for isolation of plasmid DNA by the alkaline minipreparation method (17). The cDNA inserts were evaluated by digestion at flanking *Xho* I sites.

Analysis of Immunoselected Clones. Twenty percent of the DNA derived from a 1.5-ml minipreparation was transfected into COS cells by the DEAE-dextran method. The cells were cultured as described above and evaluated 3 days after transfection for expression of the GA733-2 epitope. Mixed hemadsorption assay (MHA) was carried out as described (18). For Western blot analysis, cells were lysed in a buffer containing Nonidet P-40 (4) and 40-μg aliquots of total protein were treated with sample buffer without 2-mercaptoethanol (19) at 100°C for 1.5 min. Samples were electrophoresed on a 12% polyacrylamide gel containing SDS (19) and electroblotted onto a nitrocellulose filter, which was sequentially incubated with affinity-purified mAb GA733 at 1 μg/ml, affinity-purified goat anti-mouse IgG-alkaline phosphatase conjugate, and substrates for color development (Promega Biotec).

DNA Sequence Determination and Analysis. The GA733-2 cDNA clone was sequenced on both strands by the dideoxynucleotide method (20). To resolve compressions, *Thermus aquaticus* DNA polymerase reactions were performed substituting 7-deaza-deoxyguanosine 5'-triphosphate for dGTP (United States Biochemical). Initially, DNA sequence data were obtained using an M13 universal primer; the internal DNA sequence was then established by the specific-primer-directed method (21).

To examine the relationship of the GA733-2 sequence to known sequences, release 60 of GenBank and release 18 of EMBL databases were searched with the program WORDSEARCH (22); release 22 of the NBRF protein database was searched with the program FASTP (23). The programs ALIGN (24) and GAP (22) were used to analyze the relationship between GA733-2 and the GA733-1 gene.

RNA Blot Analysis. Aliquots (2 μg) of poly(A)$^+$ cytoplasmic mRNAs were electrophoresed on a 2.2 M formaldehyde/1% agarose gel (25) and transferred to nitrocellulose. The filter was hybridized to a nick-translated GA733-2 cDNA probe in 0.05 M Na_2PO_4, pH 6.5/1× Denhardt's solution (50× Denhardt's solution is 1% Ficoll 400/1% polyvinylpyrrolidone/1% bovine serum albumin)/50% (vol/vol) deionized formamide/0.1% SDS/5× SSC (20× SSC is 3.0 M NaCl/0.3 M sodium citrate, pH 7.0) at 42°C and then washed at high stringency in 0.1× SSC/0.1% SDS at 65°C. The filter was then rehybridized to the control α-enolase cDNA probe (26).

RESULTS

The authenticity of cDNA clones enriched by the high-efficiency COS cell expression system was established by several criteria. Five of the first six cDNA clones studied were found to encode the GA733-2 epitope. From this group of five, a detailed characterization of clone GA733-2-2 was carried out; clone GA733-2-10 was partially sequenced to determine the basis for the heterogeneity observed in the length of the clones.

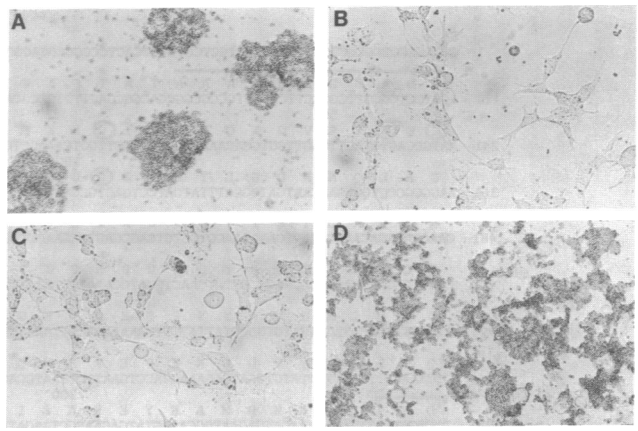

FIG. 1. MHA analysis of COS cells after transient expression of a cDNA clone representative of the enriched species. (*A*) Positive control SW948 colorectal carcinoma cells reacted with mAb GA733. (*B*) Nontransfected COS cells with mAb GA733. (*C*) COS cells 3 days after transfection with control P3 antibody. (*D*) COS cells 3 days after transfection with mAb GA733.

Identification of GA733-2 cDNA Clones. The shuttling of library cDNA between COS and *E. coli* cells was observed quantitatively and qualitatively. Episomal DNA isolated from COS cells transfected by the DEAE-dextran technique and selected by panning yielded 36,500 drug-resistant bacterial colonies; COS cells transfected by the spheroplast fusion technique and panned in the second and third cycles yielded 2300 and 2900 colonies, respectively. The *Xho* I restriction pattern of 10 colonies randomly selected from each cycle indicated an enrichment for a size-class insert of 1.4 ± 0.1 kb after the third cycle (data not shown).

Six members of this group were individually transfected into COS cells and assayed by MHA and Western blotting for expression of the GA733 epitope. In the MHA assay, in five of the six cultures transfected with the cDNA clones ≈30% of the cells formed rosettes. A representative positive clone is shown in Fig. 1*D*. Nontransfected COS cells were not reactive to the mAb GA733 (Fig. 1*B*). This percentage of positive cells is consistent with the efficiency of gene transfer using the DEAE-dextran technique. The MHA results were confirmed by immunoblot analysis of total cell protein, which showed that only COS cells transfected with one of the five cDNA clones (Fig. 2, lanes 3, 4, 5, 6, and 8) expressed a

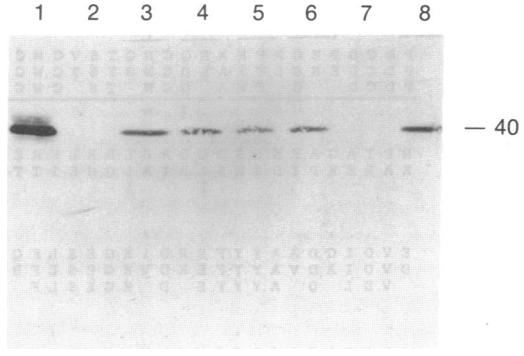

FIG. 2. Western blot analysis of transfected COS cells transiently expressing cDNA clones. Extracts were derived from SW948 colorectal carcinoma cells (lane 1), COS cells (lane 2), and COS cells transfected with cDNA clones GA733-11, -10, -6, -5, -3, and -2 (lanes 3–8, respectively). The nitrocellulose filter was incubated with mAb GA733 and the 40-kDa band is indicated.

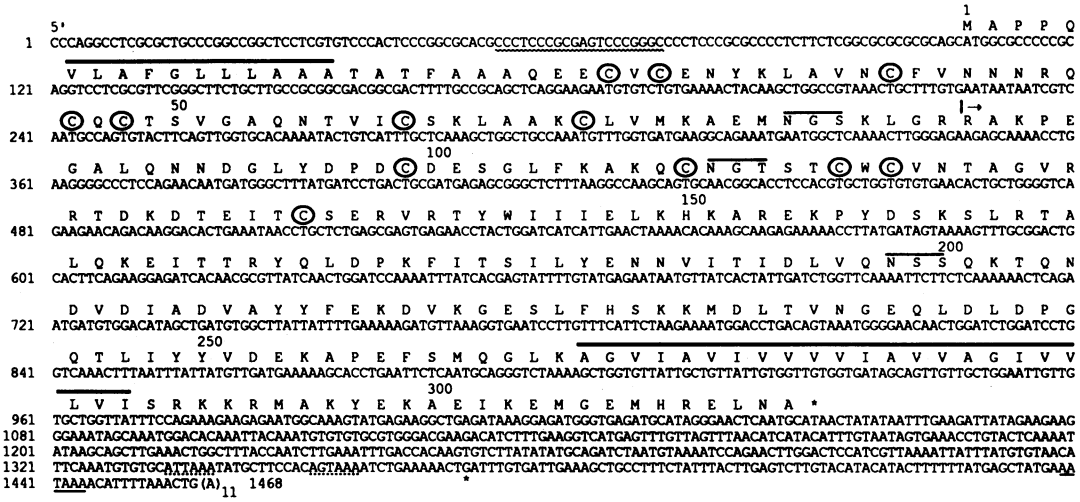

FIG. 3. DNA sequence of full-length GA733-2 cDNA. An open reading frame predicts a protein of 314 amino acids. A 30-amino acid segment with identity to the partial amino acid sequence of native GA733-2 antigen (6) begins at amino acid position 81 (arrow). A putative signal sequence (first bold overline), 12 cysteine residues (circles), three potential N-linked glycosylation sites (overline), and one transmembrane domain (second bold overline) are indicated. Other features of the DNA sequence include: an extra 20-base segment (wavy underline) in the 5′ untranslated region (see text), one typical poly(A) signal (underline), and two variant poly(A) addition signals (dotted underlines) associated with a second poly(A)-addition site at residue 1369 (asterisk). The single-letter amino acid code is used.

40-kDa protein indistinguishable from that present in positive control SW948 cells (Fig. 2, lane 1).

To determine if the GA733-2 antigen would bind mAbs related to the mAb GA733, an MHA experiment was performed on COS cells transiently expressing the GA733-2-2 cDNA clone. COS cells transfected by the clone did form rosettes when incubated with mAbs CO17-1A (1), M74 (12), and 323/A3 (13), whereas no rosettes were observed in parallel cultures treated with control P3 antibody; nontransfected COS cells were negative upon incubation with the mAbs above (data not shown).

The DNA sequence of a clone encoding the GA733-2 epitope predicted an open reading frame for 314 amino acids (Fig. 3). Beginning at amino acid 81 of the predicted sequence, 30 contiguous residues are identical to the established partial amino acid sequence of native GA733-2 antigen (6). There is further agreement over the next 15 residues of the two sequences, including the rare Cys-Trp-Cys sequence. Disagreements between the two sequences occur only at positions that had been provisionally assigned. These results prove that the cDNA clone is GA733-2. The position of the sequence correlation provides an explanation for the relationship between the 30-kDa and 40-kDa forms of the antigen (4). The 40-kDa form of the antigen, which is blocked, contains the amino terminus, and the 30-kDa breakdown product of the antigen begins at residue 81.

A protein molecular weight of 34,902 is predicted for the GA733-2 antigen. This is consistent with data showing GA733 to be a 40-kDa glycoprotein (4, 12), as the sequence contains three potential N-linked glycosylation sites. A sequence of 11 hydrophobic amino acids resembling a signal sequence core was observed. According to the "(−3, −1)-rule" (27), signal

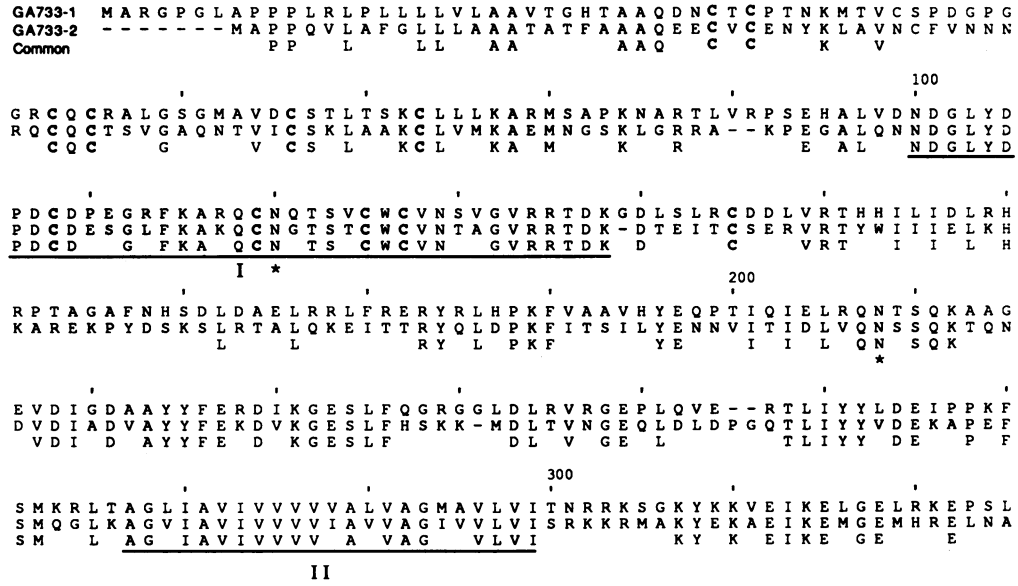

FIG. 4. Homology of GA733-2 and GA733-1 amino acid sequences. Aligned cysteine residues (boldface type), conserved potential N-linked glycosylation sites (*), and gaps to maximize the alignment (–) are shown. Two of the more prominent regions of homology are underlined. The single-letter amino acid code is used.

peptidase cleavage probably occurs 7 residues after the core sequence, between alanine and glutamine. A 242-residue cysteine-rich extracellular domain is followed by one 23-residue hydrophobic transmembrane domain and a 26-residue highly charged cytoplasmic domain. In addition to the typical poly(A)-addition signal AATAAA present in clone GA733-2-2, the DNA sequence of the shorter cDNA clone GA733-2-10 revealed the sequences ATTAAA and AGTAAA 30 and 13 bases upstream, respectively, from poly(A) addition after residue 1369 (Fig. 3). These relatively uncommon variant poly(A) addition signals have been observed elsewhere in nature (28).

Homologies of GA733-2. A comparison of the GA733-2 DNA and protein sequences with the data bases of known sequences revealed no homologies other than the relationships with thyroglobulin and the *HLA-DR*-associated invariant chain (6). Comparison with tumor-associated antigen sequences (10, 11) indicated that GA733-2 was identical to the KSA antigen sequence, with the exception of one residue in the transmembrane domain (isoleucine instead of a methionine at position 278). This is a conserved substitution and may represent a polymorphism.

The sequence of the 5′ untranslated region of GA733-2 contained the internal 20-base sequence 5′-CCCTCCCGCGAGTCCCGGGC-3′ (Fig. 3) that was not observed in the KSA sequence. Since the addition of this sequence is internal, it is probably the result of a differential splicing event, although this has not yet been directly demonstrated. Whereas the significance of this 20-base sequence is unknown, its presence is likely to have an effect on the secondary structure of the 5′ untranslated region of the mRNA. When analyzed with the program FOLD (29), a bifurcation loop in this region of the mRNA was predicted only when the 20-base sequence was present (data not shown).

The GA733 Antigen Family. The amino acid sequences of the antigens GA733-2 and GA733-1 (6) were compared. The two proteins are undoubtedly related (Fig. 4). An alignment score 76 standard deviations above the mean score of 100 random runs was obtained (24), thus establishing the high statistical significance of the relationship between the two genes. The two antigens are 49% identical. Taking into account conserved substitutions, the two antigens have a similarity of 67%. Both antigens have 12 cysteines; 11 of the cysteines align and 1 of these cysteines is shifted by one position. Two potential N-linked glycosylation sites are conserved. There are two regions of maximum homology. Region I spans 39 residues of the extracellular domains and is characterized by a 79% identity and a 97% similarity; region II represents the two transmembrane domains.

There is a strong conservation in the position of hydrophobic and hydrophilic residues in the two antigens, as indicated by nearly identical Kyte–Doolittle hydropathy plots (30) (Fig. 5). Based on the above observations, a model for the GA733 family of cell surface antigens is presented in Fig. 6.

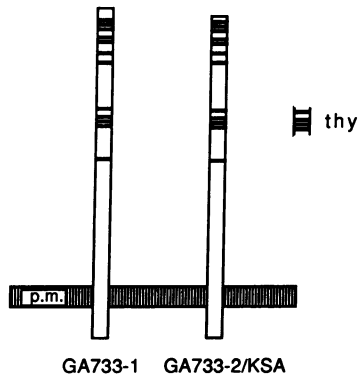

FIG. 6. Model of the GA733 family of membrane antigens. Characteristic features include an extracellular domain with 12 conserved cysteine residues (heavy lines), a single transmembrane domain, and a short cytoplasmic anchor. Region of homology with the cysteine-rich thyroglobulin (thy) repeat motif (6) is indicated. p.m., Plasma membrane.

mRNA Expression Pattern of GA733-2. A Northern blot experiment was performed on a panel of mRNAs from tumor cell lines to determine the size of the GA733-2 transcript and to begin to establish the pattern of expression of the GA733-2 gene. The GA733-2-2 CDM8 recombinant plasmid was used as a hybridization probe under conditions of high stringency (Fig. 7*A*). The nitrocellulose filter was washed and then hybridized with a cDNA probe for α-enolase, to verify that all mRNA preparations were intact and that approximately the same amount of mRNA was loaded in each lane (Fig. 7*B*).

Two colorectal carcinoma lines were positive for GA733-2 mRNA (Fig. 7*A*, lanes 1 and 2). The size of the transcript was 1.45 kb, which correlated with the size of the full-length cDNA clone. The mRNA was also present at various levels in two pancreatic carcinoma lines (Fig. 7*B*, lanes 3 and 4). As expected, two melanoma cell lines did not express the GA733-2 gene (Fig. 7*A*, lanes 5 and 6).

DISCUSSION

When mice are immunized with cells derived from human carcinomas, the 40-kDa cell surface glycoprotein GA733-2 is one of the immunodominant antigens that is recognized. Immunization with cells derived from carcinoma of the colon by three groups of investigators has resulted in the development of mAbs with binding specificities for this same antigen (1, 12, 31). Immunization with carcinoma cell lines derived from lung (32) and breast (13) have yielded mAbs defining antigens with a tissue distribution comparable to that of GA733. Molecular clones for the KSA antigen, defined by mAb KS1/4 (32), have been isolated from a lung carcinoma cDNA library by two laboratories (10, 11).

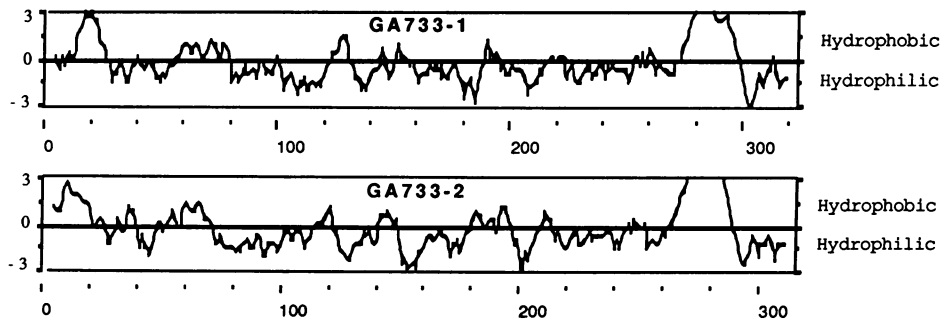

FIG. 5. Kyte–Doolittle hydropathy plots of GA733-1 (*Upper*) and GA733-2 (*Lower*). The overall similarity of the two plots is apparent. The hydrophobic peaks at the amino-terminal ends are predictive of signal peptides; the other prominent hydrophobic peak probably represents the transmembrane domains.

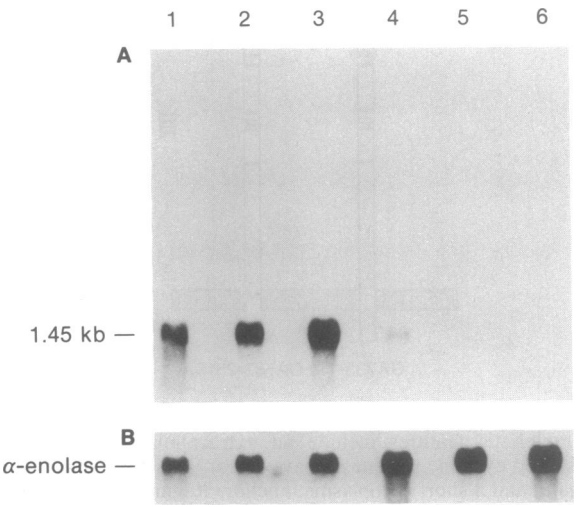

FIG. 7. (*A*) Northern blot analysis of GA733-2. mRNAs were derived from the SW948 colorectal carcinoma cell line (lane 1), the SW707 rectal carcinoma cell line (lane 2), the pancreatic carcinoma cell lines Capan-2 (lane 3) and BXPC-3 (lane 4), and the melanoma cell lines WM1158 (lane 5) and WM35 (lane 6). (*B*) Rehybridization with a control probe, α-enolase.

The high-efficiency COS cell expression system (7–9) was chosen to obtain cDNA clones for GA733-2, given the availability of mAb, the evidence that GA733-2 was not a multimeric protein encoded by more than one gene, and the expression of antigen at the cell surface. Full-length cDNA clones for GA733-2 were enriched and individually tested by MHA in about 4 weeks.

Comparison of the GA733-2 and KSA protein sequences indicated that they are identical, explaining the observed similarities between GA733 and KSA in molecular mass and tissue distribution. At the cDNA sequence level, differences between GA733-2 and KSA were observed in the sequences of the 5′ and 3′ untranslated regions. The extra 20-base segment within the 5′ untranslated region of GA733-2 mRNA may be the result of differential splicing. DNA sequence analysis of a second GA733-2 clone revealed unusual poly(A) addition signals in association with an alternate poly(A)-addition site.

MHA of COS cells transfected with the GA733-2-2 cDNA clone indicated that the CO17-1A and M74 mAbs reacted with the GA733-2 antigen, as expected. The mAb 323/A3, developed by the immunization of mice with a breast carcinoma cell line (13), also reacted with the GA733-2 antigen. Thus, the epitopes recognized by the GA733, KSA, CO17-1A, M74, and 323/A3 mAbs are all encoded by the same gene. It will be interesting to determine which of these mAbs, if any, will bind to the product of the related GA733-1 gene.

The protein sequences of the two members of the GA733 antigen family were found to be identical at 49% of 312 possible positions. The distribution of conserved positions was nonrandom. The most prominent conserved region is the 39-residue region I of the extracellular domain. Although the function of this family of glycoproteins remains unknown, it is noteworthy that region I is that part of the GA733 antigen family that is homologous to the type I repeat of thyroglobulin and the *HLA-DR*-associated invariant chain (6). The identification of an area of sequence conservation between four genes is an indication that these sequences are of functional importance.

With the availability of molecular clones of the GA733 gene family, it will be possible to study the mRNA expression pattern of these genes in different cell types. Limited Northern blot data have indicated a difference between the expression of the GA733-2 gene and the intronless GA733-1 gene. We have previously demonstrated a high level of GA733-1 mRNA in the pancreatic carcinoma cell line BXPC-3 relative to the colorectal carcinoma cell line SW948 (6). Here we report a low level of GA733-2 mRNA in BXPC-3 relative to a high level in SW948. Such a finding is not unanticipated, since a characteristic feature of a retroposon like GA733-1 is the acquisition of a heterologous promoter. It was also noted that under the conditions of these Northern blots, no cross hybridization between the two different-sized transcripts was observed; this reflects the fact that the two coding regions are only 54% identical at the DNA level.

The expression of cDNA clones encoding the epitopes recognized by the GA733 and CO17-1A mAbs will meet a critical need for tumor-associated antigen. For instance, it will now be possible to compare recombinant tumor-associated antigen with internal image anti-idiotypic antibodies as agents for the immunotherapy of carcinoma.

We thank Drs. F. Melchers, B. Seed, and A. Aruffo for helpful discussions. Oligonucleotides were prepared by the Wistar Institute DNA Synthesis Facility. This work was supported by National Institutes of Health Grant CA 21124-11 and a grant from the J. Seward Johnson Central and East European Studies Fund.

1. Herlyn, M., Steplewski, Z., Herlyn, D. & Koprowski, H. (1986) *Hybridoma* Suppl. 5, S3–S8.
2. Ross, A. H., Herlyn, D., Steplewski, Z. & Koprowski, H. (1989) in *Human Immunogenetics, Basic Principles and Clinical Relevance*, ed. Litwin, S. D. (Dekker, New York), pp. 107–122.
3. Wettendorff, M., Iliopoulos, D., Tempero, M., Kay, D., DeFreitas, E., Koprowski, H. & Herlyn, D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3787–3791.
4. Ross, A. H., Herlyn, D., Iliopoulos, D. & Koprowski, H. (1986) *Biochem. Biophys. Res. Commun.* **135**, 297–303.
5. Herlyn, D., Herlyn, M., Ross, A. H., Ernst, C., Atkinson, B. & Koprowski, H. (1984) *J. Immunol. Methods* **73**, 157–167.
6. Linnenbach, A. J., Wojcierowski, J., Wu, S., Pyrc, J., Ross, A. H., Dietzschold, B., Speicher, D. & Koprowski, H. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 27–31.
7. Seed, B. & Aruffo, A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3365–3369.
8. Aruffo, A. & Seed, B. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8573–8577.
9. Seed, B. (1987) *Nature (London)* **329**, 840–842.
10. Strnad, J., Hamilton, A. E., Beavers, L. S., Gamboa, G. C., Apelgren, L. D., Taber, L. D., Sportsman, J. R., Bumol, T. F., Sharp, J. D. & Gadski, R. A. (1989) *Cancer Res.* **49**, 314–317.
11. Perez, M. S. & Walker, L. E. (1989) *J. Immunol.* **142**, 3662–3667.
12. Gottlinger, H. G., Funke, I., Johnson, J. P., Gokel, J. M. & Riethmuller, G. (1986) *Int. J. Cancer* **38**, 47–53.
13. Edwards, D. P., Grzyb, K. T., Dressler, L. G., Mansel, R. E., Zava, D. T., Sledge, G. W., Jr., & McGuire, W. L. (1986) *Cancer Res.* **46**, 1306–1317.
14. Berger, S. L. & Berkenmeier, C. S. (1979) *Biochemistry* **18**, 5143–5149.
15. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
16. Hirt, B. (1967) *J. Mol. Biol.* **26**, 365–369.
17. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York), pp. 368–369.
18. Herlyn, M., Clark, W. H., Mastrangelo, M. J., Guerry, D., Elder, D. E., LaRossa, D., Hamilton, R., Bondi, E., Tuthill, R., Steplewski, Z. & Koprowski, H. (1980) *Cancer Res.* **40**, 3602–3609.
19. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
20. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
21. Strauss, E. C., Kobori, J. A., Siu, G. & Hood, L. E. (1986) *Anal. Biochem.* **154**, 353–360.
22. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acid Res.* **12**, 387–395.
23. Lipman, D. J. & Pearson, W. R. (1985) *Science* **227**, 1435–1441.
24. Dayhoff, M. O., Barker, W. C. & Hunt, T. L. (1983) *Methods Enzymol.* **91**, 524–545.
25. Lehrach, H., Diamond, D., Wozney, J. M. & Boedtker, H. (1977) *Biochemistry* **16**, 4743–4751.
26. Giallongo, A., Feo, S., Moore, R., Croce, C. M. & Showe, L. C. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6741–6745.
27. von Heijne, G. (1986) *Nucleic Acid Res.* **14**, 4683–4690.
28. Bernstiel, M. L., Busslinger, M. & Strub, K. (1985) *Cell* **41**, 349–359.
29. Zuker, M. & Stiegler, P. (1981) *Nucleic Acids Res.* **9**, 133–148.
30. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157**, 105–132.
31. Girardet, C., Vacca, A., Schmidt-Kessen, A., Schreyer, M., Carrel, S. & Mach, J.-P. (1986) *J. Immunol.* **136**, 1497–1503.
32. Varki, N. M., Reisfeld, R. A. & Walker, L. E. (1984) *Cancer Res.* **44**, 681–687.