J. Appl. Genet. 36(3), 1995, pp. 279-284

GA733 genes expression in human gastrointestinal tumours

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Abstract. Expression of GA733-1 and GA733-2 genes in gastrointestinal tumours was investigated. Twelve cases of colorectal and stomach cancers were analysed. The Northern blot and RT-PCR methods were used. The second method appeared to be much more sensitive and useful in these investigations. No expression of GA733-1 gene was found in the investigated cases. GA733-2 expression was observed in 83% of colon cancers (five to six analysed cases) and in 33% of stomach cancers (two to six analysed cases). No correlation was found between the expression and clinical properties of tumours. Moreover, in two cases the presence of GA733-2 mRNA was observed in the surrounding morphologically normal tissue. These results indicate that GA733-2 gene can be a good marker of colon but not stomach cancers.

Key words: GA733 neoantigen, colon adenocarcinoma, stomach adenocarcinoma.

GA733-1 and GA733-2 genes are members of a family which determines at least two type I membrane proteins. These 40-kDa glycoproteins are expressed on the surface of various human tumours including colon, pancreas, gastric, lung, and bladder carcinomas (SZALA et al. 1990). Moreover, GA733 antigens have been found at a high concentration in most murine plasmocytomas and at a much lower level in normal plasma cells (BERGSAGEL et al. 1992). Monoclonal antibodies recognizing these glycoproteins (GA733, 17-1A) have been evaluated for the experimental diagnosis and immunotherapy of gastroin-

Received: May 1995.
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testinal tumours (DOUILLARD et al. 1986, WETTENDORFF et al. 1989, BUCHSBAUM et al. 1990, STRASSBURG et al. 1992).

Until now two genes of the GA733 family have been cloned and sequenced – GA733-1 (LINNENBACH et al. 1989) and GA733-2 (SZALA et al. 1990). Both appear to encode integral membrane proteins. GA733-1 is an intronless gene while GA733-2 consists of nine exons (LINNENBACH et al. 1989, SZALA et al. 1990). These genes are identical to 49% but their putative promoter regions are unrelated. These findings suggest that GA733-1 gene was formed by retroposition of GA733-2 gene via an mRNA intermediate. However, GA733-1 gene appears not to be a defective retropseudogene but rather an intronless gene encoding protein with structural features in common with GA733-2 protein (LINNENBACH et al. 1993). Both sequences have been discovered to be homologous to the human thyroglobulin type I repeat unit, subunit of the IL-2 growth factor receptor and to some matrix adhesion molecules like nidogen (SIMON et al. 1990). Recently GA733-2 protein has been found to mediate a homophilic cell-cell adhesion of murine cells transfected with its complete cDNA (LITVINOV et al. 1994). These findings suggest that the epithelial glycoprotein can be a cell-cell or cell-matrix adhesion molecule.

Here we report the investigation of GA733-1 and GA733-2 genes expression in colon and stomach adenocarcinomas at mRNA level. We have also tried to find some correlation between GA733 expression and histological type and stage of cancer.

Material and methods

In our studies we have used fresh or frozen surgical preparations of human colon and stomach cancers and normal mucosa tissue surrounding them (5 cm from a tumour).

mRNA isolation: 0.5g of tissue was homogenised with 3 ml of lysis buffer (2M ammonium sulphosalicylate, 50 mM EDTA, 0.3% SDS, 20 µl/ml β-mercaptoethatnol, pH 4.8) at 10°C. The homogenate was shaken with 1 ml of phenol (saturated with lysis buffer) and 2 ml of chloroform and then centrifuged (9000 rpm, 15 min., 4°C). This step was repeated 2 times. The obtained aqueous phase was precipitated with 1 vol. of 96% ethanol (1 hour at –20°C) and dissolved in TE buffer.

Northern blot analysis: RNA electrophoresis was performed under denaturing conditions on 2% agarose, 6% formaldehyde gel. Separated RNA was blotted to nitrocellulose membrane with 10×SSC buffer (1.5 M NaCl, 0.15M

sodium citrate) (MANIATIS et al. 1992). The filter was hybridized to a nick-translated GA733-2 cDNA and GA733-1 DNA probes in 50% deionized formamide, 5×SSPE, 0.1% SDS , 5×Denhardt's solution, 1% dextran sulfate at 42°C and then washed twice in 1×SSPE, 0.5% SDS and once in 0.2×SSPE, 0.5% SDS (HAMES, HIGGINS 1985).

RT-PCR analysis: cDNA was produced using oligo dT cellulose as a primer and reverse transcriptase system according to (LEVESQUE et al. 1993). PCR amplification was carried out on cDNA-oligo dT cellulose using oligonucleotide primers for GA733-1 (5'-GCCCCACCGCCGGCGCCTTC-3' and 5'-TAGATAATGGACCTGCTGTAA-3') and GA733-2 (5'-AAGCAAGA-GAAAAACCTTAT-3' and 5'-TTCAAAATAATAAGCCACAT-3') genes according to (LEVESQUE et al. 1993). Amplification conditions: denaturation – 2 minutes at 95°C, annealing – 1 minute at 52°C and synthesis – 3 minutes at 72°C in the presence of 200 µM dATP, dGTP, dTTP each, 20 µM dCTP and 1 Ci [α^{32} P] dCTP. Amplification products were autoradiographically detected after electrophoresis on denaturing 5% acrylamide, 8M urea gels.

Results

Six cases of colon (samples 1-6) and six of stomach (samples 7-12) carcinomas were investigated. RNA was extracted from pairs of normal mucosa and adenocarcinoma tissue obtained from the same patient. GA733 expression was examined by Northern blotting. Two samples of colon cancer RNAs (2 and 3) were hybridized with GA733-1 and GA733-2 probes. A single 1,4-kb transcript corresponding to GA733-2 mRNA size was observed in each sample (Fig. 1). No hybridization signal was obtained using GA733-1 probe. These results indicated that only GA733-2 gene was expressed in colon cancer tissue. To support this possibility a more sensitive RT-PCR method was used. All samples from colon and stomach adenocarcinomas and surrounding normal mucosa were examined using two pairs of primers for GA733-1 and GA733-2 genes. The expected product sizes were 300bp for GA733-1 and 200bp for GA733-2. After amplification and electrophoresis only one kind of DNA fragments corresponding to GA733-2 product size was observed (200bp) (Fig. 2). Our data indicate that A733-2 gene is expressed with a high frequency (83%) in colon cancers and with a lower frequency (33%) in stomach adenocarcinomas (Tab. 1). No correlation was observed between the expression and

Table 1. Expression of GA733-2 gene in colon and stomach carcinomas

No.	Age	Sex	Location	Stage	Expression of GA733-2		Histological type
					in tumour	in normal mucosa	
1	58	M	colon	$T_4N_2M_1$	–	–	*Adenoma muciparum partim gelatinosum II°/III°*
2	65	K	colon	$T_3N_1M_0$	+	+	*Adenoma tubulare muciparum II°*
3	71	K	colon	$T_4N_2M_1$	+	+	*Carcinoma solidum partim mucocellulare*
4	65	M	colon	$T_2N_0M_0$	+	–	*Adenocarcinoma tubulare partim gelatinosum*
5	54	K	colon	$T_2N_2M_0$	+	–	*Adenocarcinoma cylindrocellulare II°*
6	75	M	rectum	$T_2N_0M_0$	+	–	*Adenoma tubulo-villosum in adenocarcinoma papillare muciparum versum*
7	57	M	stomach	$T_3N_0M_0$	–	–	*Adenocarcinoma tubulare*
8	39	M	stomach	$T_3N_1M_0$	–	–	*Adenocarcinoma tubulare partim solidum II°/III°*
9	58	M	stomach	$T_3N_xM_0$	+	–	*Carcinoma mucocellulare lauren*
10	45	M	stomach	$T_3N_1M_0$	–	–	*Adenocarcinoma tubulare*
11	51	M	stomach	$T_3N_1M_0$	+	–	*Adenocarcinoma mucocellulare II°/III°*
12	57	M	stomach	$T_4N_1M_1$	–	–	*Adenocarcinoma tubulare partim solidum*

clinical properties of tumours. In two cases the presence of GA733-2 mRNA was observed in normal colonic mucosa (Fig. 3). No expression of the GA733-1 gene was found.

Discussion

The presented studies include 12 cases of malignant solid tumours of human colon and stomach. No expression of GA733-1 gene was observed in the examined samples. This, however, is in agreement with previous data suggesting that GA733-1 is actively transcribed only in pancreatic carcinoma cell

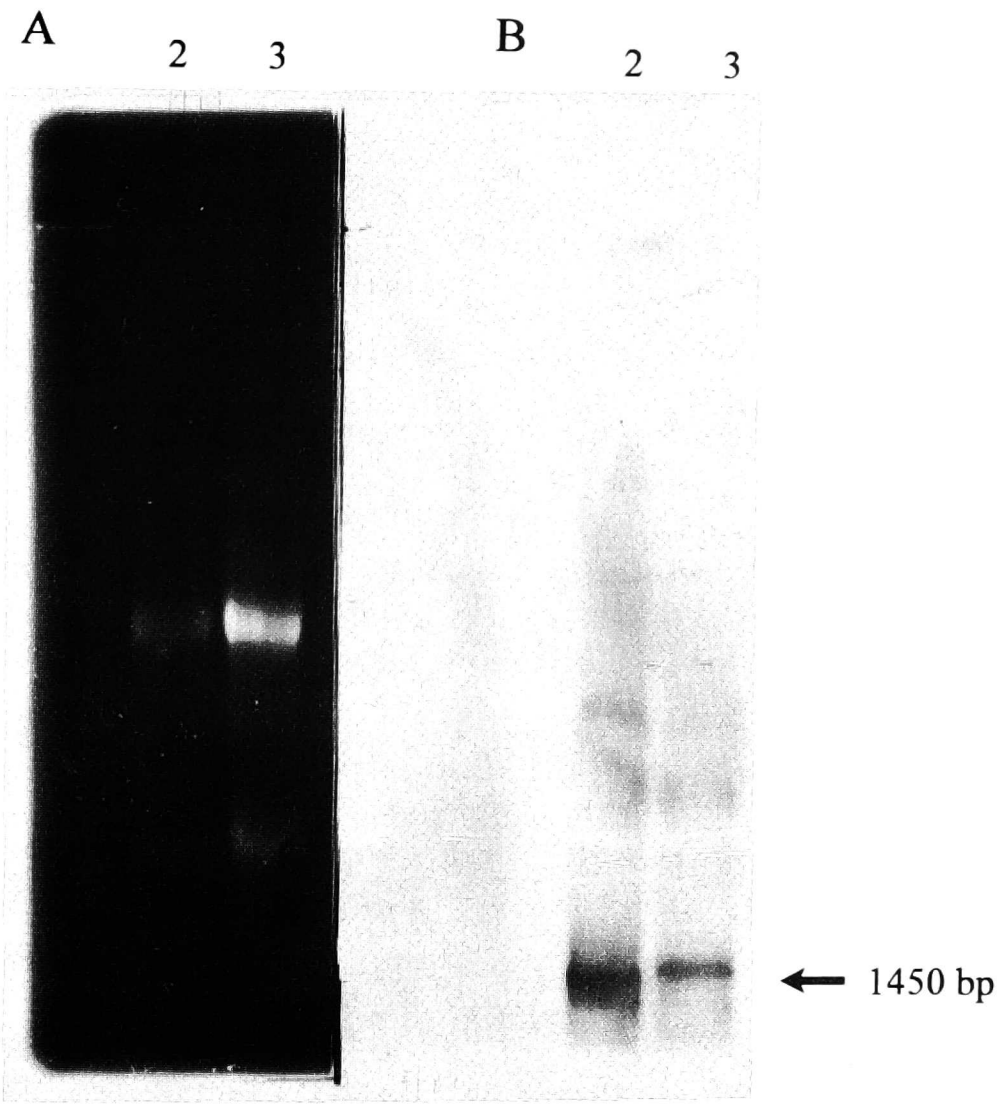

Fig. 1. A – Electrophoretic separation of RNA preparations from two cases of colon adenocarcinomas (samples 2 and 3) on 2% agarose, 6% formaldehyde gel stained with 1μg/ml ethidium bromide; B – Autoradiography of Northern blot showing hybridization of separated RNAs to ^{32}P dCTP labelled GA733-2 cDNA probe. Numbers of lines correspond to those of samples in Table 1.

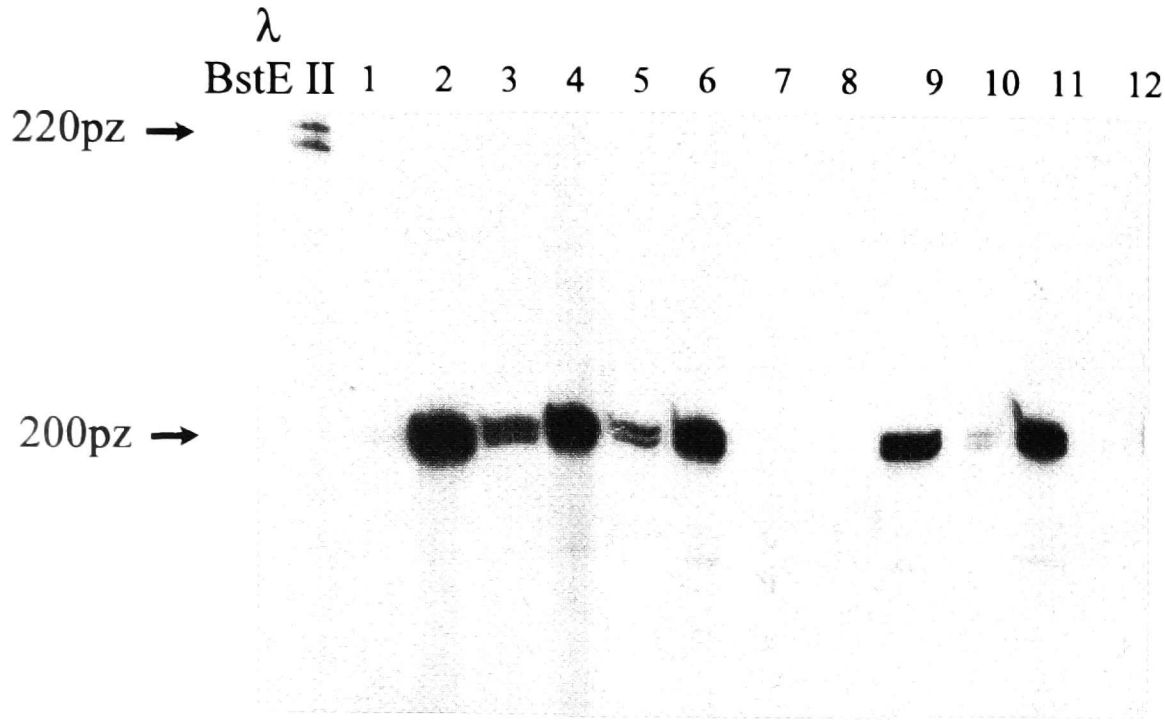

Fig. 2. Autoradiography of ^{32}P dCTP labelled RT-PCR amplification products from neoplastic tissues separated on 5% polyacrylamide gel with 8M urea. cDNAs were examined using two pairs of primers for GA733-1 and GA733-2 genes. Only one kind of products (200 bp) corresponding to the GA733-2 product size was observed. Numbers of lines correspond to those of samples in Table 1. No product was observed in the sample amplified in the absence of any template (line corresponds to the control sample not shown in the figure).

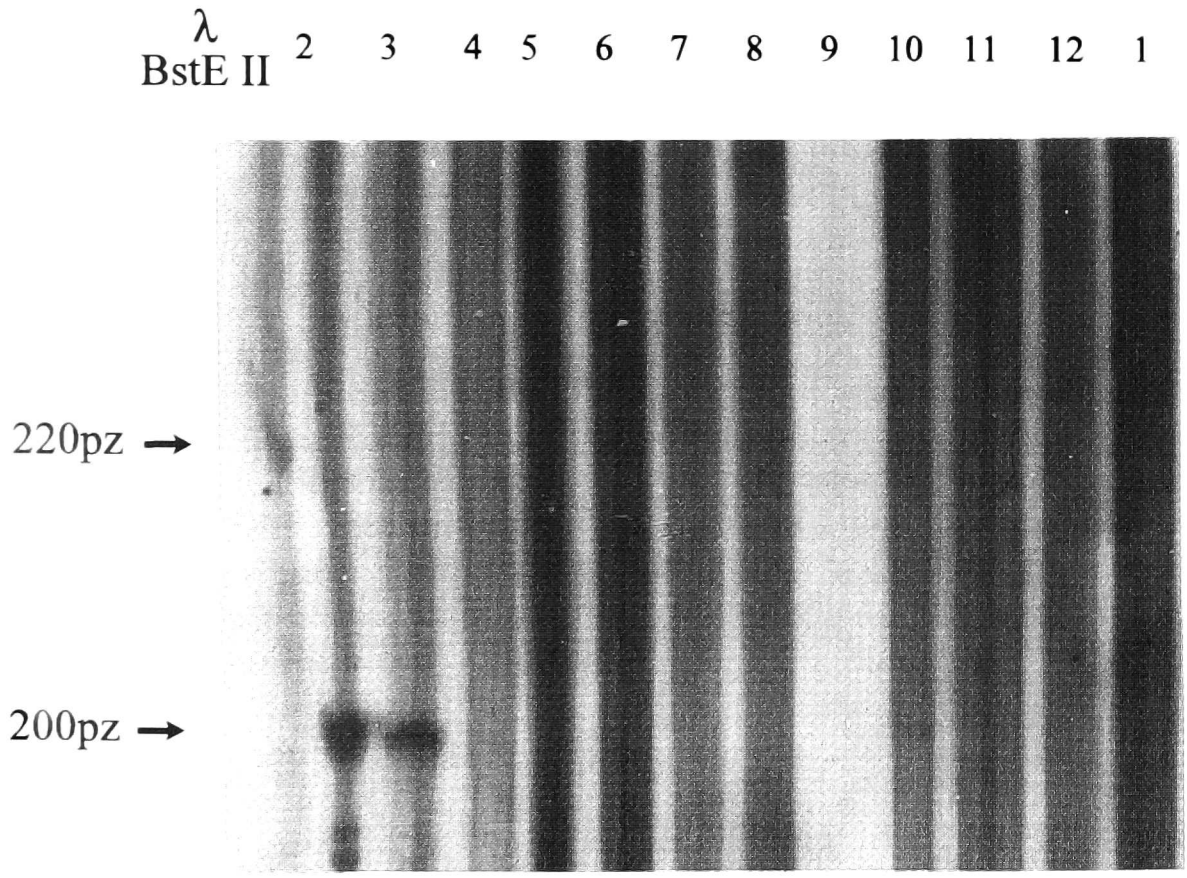

Fig. 3. Autoradiography of ^{32}P dCTP labelled RT-PCR amplification products from normal colon tissues separated on 5% polyacrylamide gel with 8M urea. cDNAs were examined using two pairs of primers for GA733-1 and GA733-2 genes. Only one kind of products (200bp) corresponding to the GA733-2 product size was observed. Numbers of lines correspond to those of samples in Table 1. No product was observed in sample amplified in the absence of any template (line corresponds to the control sample not shown in the figure).

lines (LINNENBACH et al. 1989, 1993). GA733-2 gene appears to be expressed more frequently, especially on the surface of gastrointestinal tumour cells. Our results indicated that GA733-2 gene is more often transcribed in colon cancer (83%) then in stomach adenocarcinomas (33%). However, no correlation was found between the examined expression and the age and sex of patients as well as between the stage and metastatic properties of tumours. The presence of GA733-2 mRNA in some morphologically normal tissue surrounding cancer (about 5 cm from tumour) can reflect an expansion of carcinomas. Similar data was obtained by Jacoby who found K-ras mutations in premalignant but histologically normal colonic mucosa (JACOBY et al. 1991).

These results suggest that GA733-2 gene can be a good marker of colon carcinogenesis. Investigation of its expression in colon tissue is necessary to establish its usability for early diagnosis and definition of tumour margins. Perhaps it could be interesting to examine the presence of GA733-2 mRNA in stool of patients suspected of colon cancer.

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