Overexpression of the CD155 gene in human colorectal carcinoma

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Abstract

Background and aims—The Tage4 gene (tumour associated glycoprotein E4) is overexpressed in rat colon tumours and Min mouse intestinal adenomas. The rat Tage4 protein has approximately 40% identity with human CD155, a member of the immunoglobulin superfamily coding for a transmembrane protein capable of serving as an entry receptor for poliovirus, porcine pseudorabies virus, and bovine herpesvirus 1. Analysis of the rat Tage4 gene has revealed structural and functional similarities with the human CD155 gene. We therefore investigated expression of the CD155 gene in human colorectal carcinomas.

Methods—Overall CD155 expression was assessed by semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemical analysis using tissue specimens from patients with colorectal adenomas and adenocarcinomas. We also used a qualitative RT-PCR assay to determine relative expression of different splicing variants in each sample.

Results—mRNA levels of CD155 were increased in six of six colorectal cancer tissues compared with the tumour free colon mucosa. Immunohistochemical analysis revealed an increased level of CD155 protein in 12 of 12 samples. The qualitative RT-PCR assay revealed that relative expression of the different CD155 variant transcripts was similar in the different normal and cancer samples tested, indicating that this overexpression is not associated with a particular mRNA variant generated by alternative splicing of the CD155 gene.

Conclusion—We have shown for the first time that the CD155 gene is overexpressed in colorectal carcinoma and that this overexpression begins at an early stage in tumorigenesis and continues to late stages. (Gut 2001;49:236–240)

Keywords: CD155; colorectal cancer; immunoglobulin superfamily; poliovirus receptor

As our understanding of the biology of colorectal cancer progresses, new knowledge about tumorigenesis and tumour biology can be used to diagnose, treat, and prevent this type of cancer. In an initial study of an animal model for immunotherapy of colon tumours, monoclonal antibodies directed against rat colon carcinoma cells were raised.1 One of these monoclonal antibodies (E4) reacts strongly with the primary tumours and lung metastases, as determined by immunohistochemistry. The epithelia of the gastrointestinal tract and of the bronchi are only slightly stained and no staining of other normal rat tissues is observed. Initial experiments have indicated that the antigen recognised by E4 is a transmembrane glycoprotein.2 The corresponding Tage4 gene (tumour associated glycoprotein E4) has since been found to be overexpressed in chemically induced rat colon tumours3 and in Min mouse intestinal adenomas.4 In contrast, a barely detectable level was found in the normal adult rat and mouse tissues tested. The Tage4 cDNA has been isolated.5 Sequence analysis using ALIGNp software revealed 40.9% identity between the Tage4 protein and the human CD1556 a member of the immunoglobulin (Ig) superfamily coding for a transmembrane protein capable of serving as an entry receptor for poliovirus, porcine pseudorabies virus, and bovine herpesvirus 1. The Tage4 gene has been mapped to rat chromosome 1q127 and mouse 7A2-B1,8 regions that are homologous to the long arm of human chromosome 19 where the CD155 gene is located.10 To gain insight into the molecular mechanisms involved in its overexpression in cancer cells, we isolated and characterised the rat Tage4 gene.11 This gene covers ~15 kb and is composed of eight exons and seven introns. The human CD155 gene also contains seven introns in identical positions and phases. In addition, the protein encoded by the Tage4 gene was tested for ability to mediate entry of several viruses. The results indicated that the Tage4 gene is probably orthologous to the gene for CD155. We therefore decided to analyse expression of the CD155 gene in human colorectal carcinoma (CRC). This was performed by reverse transcription-polymerase chain reaction (RT-PCR) analysis and by immunohistochemistry. We also analysed relative expression of the different variants of the CD155 gene by a qualitative RT-PCR assay.

Materials and methods

Tissue samples

Tissue samples were obtained from 18 patients (10 men and eight women; mean age 66 years, range 45–90) undergoing surgical resections for primary CRC diagnosed at the Department of Surgery, Nantes University Hospital, France. Adenomas were collected from four patients: two villous adenoma (both high grade...
CD155 and colorectal cancer

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anti-CD155. specific staining an irrelevant monoclonal stained with Papanicolaou. For control of
phatase substrate (Vector, Burlingame, Califor-
developed using the Vector Red alkaline phos-
10 mM Tris Cl, pH 7.5), staining was
one with Tris bu-
secondary antibody (dilution 1:50). Following

goat antimouse Ig (Sigma) was used as a
(4) thermal reactor (Appligene, Illkirch,
amplications were performed in a Crocodile
III thermal reactor (Appligene, Illkirch,

NORMAL COLONIC EPITHELIAL CELLS AND
CANCER CELL LINES
Normal epithelial cells were isolated from sur-
gical specimens using a non-enzymatic disso-
ciation technique based on the use of the
calcium chelating agent EDTA, as previously
described.12
HCT8R, SW620, and SW1116 colon carci-
noma cell lines were grown in RPMI-1640
containing l-glutamine and sodium bicarbo-
nate (Sigma, Saint Quentin Fallavier, France)
supplemented with 10% fetal calf serum, peni-
cillin (100 units/ml), and streptomycin (0.1
mg/ml). Cells were maintained at 37°C in 5%
carbon dioxide and passed twice a week.

IMMUNOHISTOCHEMISTRY
Cryostat sections (5 μm) were fixed in acetone
and stored at −20°C. Before use, slides were
rehydrated with phosphate buffered saline (150
mM NaCl, 10 mM phosphate buffer, pH 7.4).
The slides were then incubated with normal

goat serum for 10 minutes. Mouse monoclonal
antibody D171 (Neomarkers, Union City,
California, USA) directed against human
CD155, a 362 bp insert amplified by PCR
from HCT8R cell cDNA with oligonucleotides
CD1 and CD2 was used as a probe.

QUALITATIVE ANALYSIS OF CD155 EXPRESSION
For analysis of CD155 variants, 35 cycles of
PCR were performed as described above. The
primers used were located on exons 5 and 7,
respectively, on regions corresponding to each
side of the transmembrane domain, where the
different splice sites are located (fig 1A). The
sequences of these primers were: CD5–3' primer: 5'-TATCTTGCTCCGAGTGCTT
GCC-3'; CD2–3' primer: 5'-ATCATAGCCA
GAGATGGGATACCC-3'.

For qualitative amplification, each
cycle was carried out at 92°C for 30 seconds,
62°C for 30 seconds, and 72°C for 60 seconds.
The reaction was stopped after 18 cycles, in the
linear range of the amplification (not shown).
Following electrophoresis, amplified DNA was
transferred to Hybond N+ membranes (Amer-
sham Pharmacia Biotech, Orsay, France). The

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quantification, the bands of interest were stored as TIFF files using the BioCapt software (Vilber Lourmat, Marne la Vallée, France) and densities were determined using the EasinWin 32 software (Herolab, Fischer Scientific, Illkirch, France). The relative ratio of the different amplicons was determined for each reaction and expressed as a percentage of the overall expression. All samples were analysed at least three times.

Results

CD155 mRNA EXPRESSION IN PRIMARY CRC BY RT-PCR ANALYSIS

Firstly, we analysed expression of the human CD155 gene in human colorectal tumours by RT-PCR and DNA hybridisation. RNA was extracted from six different tumours and the corresponding adjacent normal mucosa. Semi-quantitative RT-PCR analysis was performed. Expression of the CD155 gene was higher in all tumour samples compared with adjacent normal tissues, as illustrated in fig 2. Quantification of the signals obtained indicated that there was a 2–10-fold increase in amounts of CD155 mRNA in colorectal tumours compared with adjacent normal mucosa.

CD155 EXPRESSION IN PRIMARY CRC BY IMMUNOHISTOCHEMISTRY

To determine which cells overexpress the CD155 gene, immunostaining experiments were performed on frozen 5 µm thick sections with monoclonal antibody D171. As illustrated in fig 3A, anti-CD155 reactivity was clearly detected in tumour samples. In each case the sections used for the negative control, which were stained with an irrelevant antibody, were negative (fig 3B). CD155 expression was localised to tumour cells and not observed in the stromal reaction under these experimental conditions. Similar staining of CD155 was identified in all adenomas as well as in all adenocarcinomas. Staining was independent of tumour grade, degree of differentiation, and localisation of the tumour. All normal colon tissues presented only weak staining of epithelial cells, and no staining was detected in the lamina propria (fig 3C).

CD155 EXPRESSION IN HUMAN CANCER CELL LINES

In addition to the immunohistochemical staining experiments, D171 reactivity was observed by flow cytometry and indirect immunofluorescence with all human colon carcinoma cell lines tested so far (HT29, Colo205, SW620, HCT8R, SW1116; not shown).

CD155 EXPRESSION IN CRC BY IMMUNOBLOT ANALYSIS

To characterise further the protein detected by anti-CD155 monoclonal antibodies in human tissues, we performed western blot analyses. Proteins were extracted from four colon tumours and three colon cancer cell lines. Monoclonal antibody D171 is not suitable for western blotting. Therefore, following electrophoresis and transfer, nitrocellulose membranes were probed with monoclonal antibody 5H5 (kindly provided by Dr A Nomoto) which recognises CD155 under denaturing conditions (unpublished). This revealed an ~80 kDa protein (data not shown) which is in agreement with the reported molecular weight of the transmembrane CD155 protein.

EXPRESSION OF CD155 SPlicing VARIANTS

The CD155 gene has been shown to be expressed as several isoforms corresponding to splicing variants. To analyse the relative expression of these different transcripts in CRC, a qualitative RT-PCR analysis was performed with oligonucleotides CD5 and CD15. These primers are located on exons 5 and exon 7, respectively. Agarose gel electrophoresis revealed three amplified DNA fragments (fig 1B). These different fragments were purified, cloned, and sequenced. This analysis revealed that the major band (273 bp) corresponds to the isoform containing the transmembrane domain (isoform α). The lower 137 bp and 113 bp fragments correspond to transcripts lacking part of or the entire exon 6, respectively. They have been described as β and γ isoforms by Koike and colleagues. They lack the transmembrane domain and code for soluble forms of CD155.

Figure 2 Increased levels of CD155 in human colorectal carcinoma. Reverse transcription-polymerase chain reaction analysis of CD155 expression was performed on paired tumorous (lanes T) and non-tumorous (lanes N) colorectal tissues from four patients, as described in materials and methods.
cDNAs prepared from RNA extracted from tumours and normal tissues were analysed using this assay. The relative abundance of these three RNA transcripts was determined for six colon tumours and the corresponding normal mucosa. This revealed no qualitative difference between the samples studied (not shown).

All of these samples contain different cell types. Therefore, we applied this assay to preparations of normal intestinal epithelial cells isolated from surgical specimens obtained from patients undergoing colonic resection and compared the results with those obtained with human colon carcinoma cells lines. In normal epithelial cells, the transmembrane $\alpha$ isoform represents the main CD155 transcript (65–70%) (table 1). Variants $\beta$ and $\gamma$ correspond to 25–30% and 5–8% of the CD155 mRNAs, respectively. No significant difference was observed between the three preparations tested. Similar ratios between the different splicing variants were obtained with the colon cancer cell lines analysed (table 1). These data indicate that overexpression of the CD155 gene does not involve a particular isoform but most likely results from increased transcription of the gene.

### Discussion

The CD155 gene codes for integral membrane or soluble proteins that are members of the Ig superfamily. They share a V-C2-C2 domain structure. The members of this family have a diversity of functions but in most cases the common denominator is a recognition role at the cell surface. Most of these proteins are involved in cell-cell interaction during normal or pathological processes such as embryogenesis (for example, neural cellular adhesion molecule (N-CAM), and neurone-glia CAM (Ng-CAM)), immune response (Igs, T cell receptors, MHC antigens), inflammation and wound healing (intercellular CAM-1 (ICAM-1), CD54; vascular CAM-1 (VCAM-1), CD106), tumorigenesis and cancer metastasis (N-CAM; Ng-CAM; VCAM-1; ICAM-1; platelet endothelial CAM-1 (PECAM-1), CD31; carcinoembryonic antigen (CEA)). Some growth factor receptors also belong to the Ig family: the platelet derived growth factor receptor, colony stimulating factor 1 receptor, and fibroblast growth factor receptors. Finally, several viral infections are mediated through binding to a membrane receptor belonging to the Ig family.

Human poliovirus infections are mostly targeted to the gastrointestinal tract through binding to the transmembrane protein coded by the CD155 gene. This is the only known function of the CD155 protein at present but it probably has important biological activities.

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**Table 1** Relative expression (%) of the different CD155 variants in normal epithelial cells and colon cancer cells

<table>
<thead>
<tr>
<th>Splicing variant</th>
<th>Normal epithelial cells</th>
<th>Colon cancer cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD155$\alpha$</td>
<td>CD155$\beta$</td>
</tr>
<tr>
<td>IEC1</td>
<td>65.3 (3.6)</td>
<td>29.8 (1.5)</td>
</tr>
<tr>
<td>IEC2</td>
<td>68.5 (3.4)</td>
<td>24.8 (2.3)</td>
</tr>
<tr>
<td>IEC3</td>
<td>66.1 (4.5)</td>
<td>26.2 (3.0)</td>
</tr>
</tbody>
</table>

The relative ratio of the different amplicons, determined as described in material and methods, was evaluated for each reaction separately and expressed as a percentage of the overall expression in each sample. All reactions were performed at least three times. Mean (SD) values are presented.
Indeed, CD155 belongs to a small subfamily of the Ig superfamily that includes nectin 1, nectin 2, and nectin 3, all of which have been shown to mediate cell-cell adhesion and to localise to sites of cadherin-based cell junctions.

As it is overexpressed in cancer cells, it is tempting to speculate that CD155 expression may play a role in neoplastic transformation and/or progression. CD155 overexpression was seen in all adenomas which are established premalignant lesions in colorectal carcinogenesis. Thus CD155 expression may play a role at early stages of tumorigenesis.

Several members of the Ig family are overexpressed in cancer cells. The CD47 and NCA genes are overexpressed in human CRC. The human B-CAM gene is highly expressed in ovarian carcinomas in vivo. Muc18 has been identified as a marker of tumour progression in human malignant melanoma that may have a role in the development of metastasis. Finally, de novo expression of ICAM-1, the major human rhinovirus receptor, and correlates with increased risk of metastasis in melanoma.

The qualitative RT-PCR that we have developed revealed that the relative abundance of the three RNA transcripts was not significantly different between cancer cells and normal epithelial cells. This indicated that there is no difference between RNA splicing mechanisms in normal and colon cancer cells but rather that there is increased transcription of the CD155 gene in colon cancer cells.

The promotor of the human CD155 gene has been isolated. Solecki and colleagues recently reported mapping of three cis elements within the core promotor, and demonstrated that the AP-2 transcription factor is involved in transcription of the CD155 gene in HeLa cervical cancer cells. Further investigation into the regulation of the CD155 gene in colorectal cancer cells is necessary. We are also investigating the potential use of soluble CD155 molecules as tumour markers. In addition, demonstration that the CD155 gene is overexpressed at early stages of colorectal carcinogenesis should initiate studies on the function(s) of the different CD155 isoforms.

Finally, during completion of our manuscript, Gromeier and colleagues demonstrated that highly attenuated poliovirus recombinants can infect and propagate in cell lines derived from malignant gliomas and, most interestingly, halted tumour progression and eliminated tumours in athymic mice. They proposed that susceptibility of these malignant cells to poliovirus may be mediated by expression of the CD155 gene in gial neoplasms. Our demonstration that the CD155 gene is overexpressed in colorectal cancer cells suggests that oncolytic poliovirus recombinants may also be suitable for the treatment of colorectal cancer.

This work was supported by a grant from the Ligue Nationale Contre le Cancer. B Baury is the recipient of a research fellowship from the Ligue Nationale Contre le Cancer. We thank Dr Akio Nozito (Department of Microbiology, Tokyo University, Japan) for providing the monoclonal antibodies directed against human CD155. A Jarry and B Baury contributed equally to this work.

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Gut 2001 49: 236-240
doi: 10.1136/gut.49.2.236

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