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Exon skipping in the E-cadherin gene transcript in metastatic human gastric carcinomas

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Altered expression of the homophilic cell-adhesion molecule E-cadherin is implicated in tumor metastasis. We have analysed E-cadherin mRNA in 26 surgically resected human gastric carcinomas. In four diffuse type carcinomas according to Laurén's classification we observed alterations of the processed E-cadherin gene transcript. Direct sequencing of PCR-amplified cDNA revealed that in all four tumors single exons containing putative calcium binding sites were lacking in the spliced E-cadherin mRNA. The same type of alteration was detected in metastatic tumor cells infiltrating lymph nodes. The altered forms of E-cadherin mRNA were not present in normal gastric epithelial tissue. This is the first report describing structural alterations of E-cadherin that may influence calcium-dependent cell adhesion and thus promote one of the critical steps in cancer metastasis: the detachment of carcinoma cells from the primary tumor mass.

Tumor metastasis is a complex multistep phenomenon and remains the most life-threatening aspect of the oncogenic process (1). Recent studies have shown that downregulation of E-cadherin expression is associated with dedifferentiation and metastasis of human carcinomas *in vivo* (2, 3). E-cadherin mediates a calcium-dependent homophilic interaction between epithelial cells essential for regulating cell polarity and maintaining tissue integrity (4). A reduction in the E-cadherin-mediated cell adhesion would impair cohesion and facilitate escape of cells from a tumor mass (4).

Gastric carcinomas are highly metastatic tumors; many of them show loose cell–cell adhesion although the tumor cells express considerable amounts of E-cadherin protein (5). We have analysed E-cadherin by PCR and cDNA sequencing to see if, in these tumors, structural alterations have occurred that may affect function but not expression. Although the cDNA sequence of human E-cadherin is only partially available at present (6), we have used the high homology of the cadherin gene family members to design PCR primers able to amplify the human sequence.

Fresh cancer tissues, normal gastric epithelium and lymph nodes were obtained at surgery from 26 patients with primary gastric carcinomas, snap frozen and stored in liquid nitrogen. The tumors were composed of 14 diffuse type, 8 intestinal type, and 4 mixed according to the classification of Laurén. Total cellular RNA was isolated by guanidinium thiocyanate extraction and caesium chloride centrifugation method (7). For PCR amplification the mRNA was first reverse transcribed and then subjected to 40 cycles of amplification using various primers. One primer pair (Ex7, (5'-ACCTCTGTGATGGAGGTC) and rEx10, (5'-GGGGGC(T,C)TC(G,A)TTCAC(G,A)TC) detected altered PCR products in the region between exon 7 and exon 10 (see Figure 1A). Conditions were 1 min denaturation at 94°C, 1 min annealing at 55°C and 1 min elongation at 72°C. Products were visualized by agarose gel electrophoresis and specific products excised and purified using glass milk (GeneCleanII, Bio101 Inc., La Jolla, CA, USA). Purified DNA was sequenced using Sequenase Version 2.0 (USB, Cleveland, OH) and either PCR primers or internal primers.

Amplification of E-cadherin cDNA from the exon 7–10 region in all normal gastric epithelial tissues analysed and in the majority of tumors (22/26) revealed a single PCR product having the anticipated length of approximately 630 base pairs. In four tumors, however, the amplification product in each case was shorter: in two diffuse type carcinomas the PCR product was only 450 bp in length, whereas in additional two diffuse type carcinomas the amplification product was only 500 bp long (see Figure 1).

Direct sequencing of the 450 bp PCR products revealed that they both diverged from the normal sequence at the same point (see Figure 1 for example). Alignment of mouse E-cadherin (9) and chicken L-CAM (10) sequences with those we obtained from the normal and truncated human E-cadherin mRNA indicates that 183 nucleotides corresponding to the entire exon 9 are absent in these two gastric carcinomas. Comparison between the sequences of the 500 bp amplification products with the normal human E-Cadherin sequence showed that 129 nucleotides corresponding exactly to exon 8 were missing. The deleted regions are located in the extracellular portion of the molecule and include putative calcium binding sites (8). The open reading frame is maintained because both exons start and end with complete codons. Tumor cell-infiltrated lymph nodes from three of the four patients expressing altered E-cadherin molecules were available for analysis. The same exon deletions found in the primary tumors were identified in tumor cells infiltrating lymph nodes in all three patients (see Figure 1), indicating that the alteration arose prior to metastatic spread. So far, no exon skipping has been identified in the remaining 22 gastric carcinomas analysed.

To our knowledge this is the first report describing alterations of the human E-cadherin gene transcript in epithelial tumors. Amino acid substitutions in a single calcium-binding domain of

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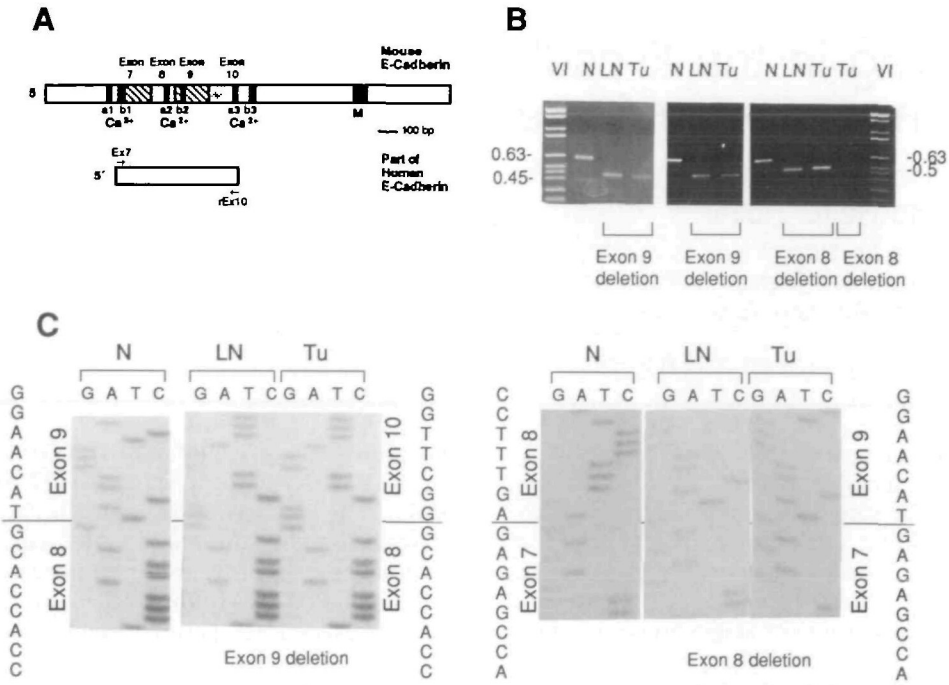

Figure 1. Detection of altered human E-cadherin cDNA in diffuse type gastric carcinomas by polymerase chain reaction and sequencing. **A.** Position of primers relative to the structure of mouse E-cadherin (8, 9) (a and b, calcium binding motifs; M, transmembrane domain). **B.** Ethidium bromide stained gel of the amplification products from four patients (only the tumor tissue from the fourth patient was available) identifying alterations in the human E-cadherin cDNA (VI, marker VI (Boehringer Mannheim); N, normal; LN, lymph node; Tu, tumor). **C.** Sequence of the normal and altered human E-cadherin fragments showing the abutting of exons 8/10 and exons 7/9 in tumors and infiltrated lymph nodes.

E-cadherin abolished the adhesive function (11). E-cadherin molecules lacking entire calcium-binding sites would, therefore, be expected to be non functional and facilitate the escape of metastasizing cells from the primary tumor.

Immunohistochemical analysis using three different commercial antibodies (6F9, DECMA-1, and HECD-1) revealed that the E-cadherin protein was strongly expressed in tumor tissue and tumor cells infiltrating lymph nodes in the patients with altered E-cadherin mRNA (data not shown). The level of expression was comparable to the one seen in normal gastric epithelium.

There is no evidence for alternative splicing in the mouse E-cadherin gene (9). Since no length alterations could be detected in the human E-cadherin cDNA in all normal gastric epithelial tissues and in most tumors analysed in this study (nor in normal kidney, placenta, and thyroid), we postulate that mutations — rather than alternative splicing mechanisms — occurred in the E-cadherin gene in four diffuse type gastric carcinomas that resulted in skipping of either exon 8 or exon 9.

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