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Alterations of E-cadherin and β-catenin in gastric cancer

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Abstract

Background: The E-cadherin-catenin complex plays a crucial role in epithelial cell-cell adhesion and in the maintenance of tissue architecture. Perturbation in the expression or function of this complex results in loss of intercellular adhesion, with possible consequent cell transformation and tumour progression.

Methods: We studied the alterations of E-cadherin and β-catenin in a set of 50 primary gastric tumours by using loss of heterozygosity (LOH) analysis, gene mutation screening, detection of aberrant transcripts and immunohistochemistry (IHC).

Results: A high frequency (75%) of LOH was detected at 16q22.1 containing E-cadherin locus. Three cases (6%) showed the identical missense mutation, A592T. This mutation is not likely to contribute strongly to the carcinogenesis of gastric cancer, because a low frequency (1.6%) of this mutation was also found in 187 normal individuals. We also detected a low frequency (0.36%, 0%) of this mutation in 280 breast tumours and 444 other tumours, including colon and rectum, lung, endometrium, ovary, testis, kidney, thyroid carcinomas and sarcomas, respectively. We also analyzed the aberrant E-cadherin mRNAs in the gastric tumours and found that 7 tumours (18%) had aberrant mRNAs in addition to the normal mRNA. These aberrant mRNAs may produce abnormal E-cadherin molecules, resulting in weak cell-cell adhesion and invasive behaviour of carcinoma cells. Reduced expression of E-cadherin and β-catenin was identified at the frequency of 42% and 28%, respectively. Specially, 11 tumours (22%) exhibited positive cytoplasmic staining for β-catenin IHC. An association was found between reduced expression of E-cadherin and β-catenin. Moreover, an association was detected between reduced expression of E-cadherin and diffuse histotype.

Conclusion: Our results support the hypothesis that alterations of E-cadherin and β-catenin play a role in the initiation and progression of gastric cancer.

Background

E-cadherin (120 kDa; chromosome 16q) is a classical cadherin and forms the key functional component of adherence junctions between epithelial cells [1]. It is bound via a series of undercoat proteins, the catenins (α, β and γ) to the actin cytoskeleton [1]. This linkage between transmembranous cadherins and actin filaments of the cytoskeleton is necessary to form strong cell-cell adhe-

sion. An intact E-cadherin – catenin complex is required for maintenance of normal intercellular adhesion. In light of this, several groups have proposed that in carcinomas, E-cadherin functions as an invasion suppressor molecule such that its loss permits or enhances the invasion of adjacent normal tissues. Immunohistochemical studies in human cancers, including gastric cancer, have frequently shown that a proportion of invasive carcinomas and carcinomas *in situ* show aberrant levels of E-cadherin and/or catenin expression in comparison to their related normal tissue [2–4]. In general, E-cadherin and catenin staining is strong in well-differentiated cancers that maintain their cell adhesiveness and are less invasive, but is reduced in poorly-differentiated tumours which have lost their cell-cell adhesion and show strong invasive behaviour [2,3].

E-cadherin is involved in contact inhibition of cell growth by inducing cell cycle arrest [5]. It has the ability to inhibit cell proliferation by the upregulation of p27 involved in the cell cycle regulation [5], although the mechanism by which E-cadherin regulates p27 is still unclear. Therefore, E-cadherin, generally described as an invasion suppressor [6], can act as a major growth/proliferation suppressor.

An important function of β-catenin in cell signalling, has been elucidated [7]. In the absence of a mitotic signal from outside the cell, β-catenin is sequestered in a complex with the adenomatous polyposis coli (APC) gene product, a serine threonine glycogen synthetase kinase (GSK-3β) and an adapter protein axin (or a homologue conductin), enabling phosphorylation and degradation of free β-catenin by the ubiquitin-proteasome system [8]. When a mitotic signal is delivered by the Wnt pathway, by association of the Wg/Wnt family of secreted glycoproteins and their membrane receptor frizzled, it leads to activation of the dishevelled (Dsh) protein, which is recruited to the cell membrane. The activated Dsh downregulates the protein complex, so that it can no longer phosphorylate β-catenin, which is then not degraded. The release of β-catenin from the phosphorylation and degradation complex promotes β-catenin stabilization and signalling. This results in an increase of free cystolic β-catenin which translocates to the nucleus and directly binds the transcription factors Lef and Tcf, leading to the activation of gene expression. Therefore β-catenin performs distinct functions in E-cadherin-mediated cell-cell adhesion and in Wnt signalling [8].

Loss of the E-cadherin locus on the long arm of chromosome 16 (16q22) occurs in gastric (24%), hepatocellular (50%), lobular breast (50–100%) and oesophageal (66%) carcinomas [4,9–12]. There have been several reports on E-cadherin gene mutations in human cancers [13]. In poorly-differentiated tumours, such as lobular breast cancer and diffuse-type gastric cancer, E-cadherin mutations play an important role in tumour development [14,15]. Several studies have reported germline mutations in the E-cadherin gene in families with an inherited diffuse type of gastric cancer [16,17]. Only a minority of gastric cancers can be accounted for by E-cadherin mutations. Frequent somatic mutations of β-catenin gene have been found in small colorectal adenomas and intestinal type gastric cancer [18,19]. Most of the mutations involved the loss of serines or threonines from the GSK-3β phosphorylation region. Genetic alterations in β-catenin abolishing cell-cell adhesiveness have been observed in two gastric cancer cell lines, HSC39 and 40A; both derive from the same signet ring cell carcinoma of the stomach and show a diffuse growth pattern [20,21]. This mutation results in a truncated β-catenin that lacks the region for interaction with β-catenin. Transfection of these cell lines with wild-type β-catenin restores cellular adhesiveness [21].

Here, we performed E-cadherin and beta-catenin gene mutation and expression analysis in a series of 50 primary gastric tumours in order to understand better the involvement of the alterations of E-cadherin and β-catenin in the carcinogenesis of gastric cancer.

Materials and methods

Samples

Included in the study were 50 tumours and corresponding normal samples, of which two tumours (17 and 23) were from the same family, the rest sporadic (Table 1). These cases were diagnosed by the Department of Pathology, University Hospital of Iceland. Tissue was obtained freshly on the day of surgery or from paraffin-embedded material. Information concerning the tumour stage, histotype and grade was also acquired from the same department. DNA for PCR was isolated by the proteinase K treatment [22]. RNA for RT-PCR was extracted using Tri Reagent (Molecular Research Center, INC. USA). For the A592T mutation, we screened 187 normal individuals, 280 breast and 444 other cancer patients with colon and rectum, lung, endometrium, ovary, testis, kidney, thyroid carcinomas and sarcomas. All individual identifiers were removed from the control samples prior to analysis, and investigators were thus blinded to the identification of samples which can no longer be traced to specific individuals. Consent was presumed for the patient samples. For cases 294 and 728 with A592T mutation, we analyzed their pedigrees and found that there were no other cancer cases in the pedigree of case 294, but there were other 5 cancer cases in the pedigree of case 728, including 2 prostate cancers, 1 skin cancer, 1 lung cancer and 1 cancer of unclear origin.

Table 1: Summary of alterations of E-cadherin and β-catenin in a series of 50 gastric tumours.

Tumour Sample	Stage	Type	Grade	LOH at 16q22.1	E-cad gene mutations and polymorphisms	Aberrant mRNA of E-cad	E-cad IHC	β-cat IHC
1	T3N3	diff		+	IVS1+6T→C	-	+/-	++/-$^{\nabla}$
3	T3N1	squ	G1	+	IVS4+10C→G	ND	-	+/-
17	T3N1	diff		ND	GTG(Val)→GTC(Val) at cd832	ND	-	-
23		met		+	GCC(Ala)→ACC(Thr) at cd592*	ND	+++/-	+++/-
43	T3N2	diff		+	-	-	-	+++/-
50	T3N1	diff		-	IVS1+6T→C	-	++/-	++/-
165	T3N1	int	G3	ND	-	-	+++/-	+++/-$^{\nabla}$
174	T3N2	int	G3	-	-	ND	+++/-	++/-
193	T3N2	int	G3	+	-	ND	-/+++	-/++
200	T3N1	int	G2	-	IVS4+10C→G	-	++/-	+++/-
231	T3N0	mi	G3	+	-	-	++/-	++/-$^{\nabla}$
283	T3N0	int	G3	ND	-	ND	++/-	++/-
287	T3N1	int	G2	+	-	-	-/+	++/-
294	T3N1M1	mi	G4	+	GCC(Ala)→ACC(Thr) at cd592♦	-	++/-	++/-$^{\nabla}$
304	T3N3	int	G2	-	-	-	-/+++	+/-
308	T3N1	int	G2	+	-	-	+++/-	++/-
314	T3N1	diff		-	CAC(His)→CAT(His) at cd632 GGC(Gly)→GGT(Gly) at cd865	-	+++/-	++/-$^{\nabla}$
360	T2N1	int	G3	+	IVS4+10C→G	+♣	++/-	+/-
369		met		+	-	+♣	+++/-	++/-
433	T3N1	mi	G3	-	IVS4+10C→G	-	-/++	-/+
435	T2N0	int	G3	+	-	-	-/+++	-/++
443	T2N0	int	G4	-	-	-	+++/-	+++/-
451	T4N0	int	G2	ND	-	ND	+++/-	++/-
474	T3N1	int	G3	ND	-	ND	-	+/-
493	T3N1	int	G3	+	-	-	-/+++	+/-
503	T3N1	int	G3	ND	-	-	-	+++/-
556	T3N2	int	G2	-	IVS1+6T→C 5'UTR-71C→G	ND	++/-	+/-
568	T3N2	mi	G3	+	-	+♣	++/-	++/-
612	T3N0	int	G2	ND	IVS4+10C→G AAC(Asn)→AAT(Asn) at cd751	-	+++/-	+/-
636	T3N1	diff		+	IVS4+10C→G	ND	+++/-	+++/-$^{\nabla}$
650	T2N0M1	int	G2	+	-	ND	-/+	++/-
675	T2N0	mi	G3	+	IVS4+10C→G	-	+/-	++/-$^{\nabla}$
676	T3N1	int	G2	+	-	-	+++/-	-$^{\nabla}$
680	T3N1	mi	G3	+	IVS1+6T→C AAC(Asn)→AAT(Asn) at cd751	-	++/-	++/-
694	T3N1	int	G1	+	-	-	+++/-	+++/-
717	T3N1	int	G3	+	-	+♣	-/+++	+++/-
726	T2N1	int	G2	+	IVS4+10C→G	-	-/+++	++/-
728	T3N0	int	G2	+	GCC(Ala)→ACC(Thr) at cd592♦	-	-/+++	++/-
729	T3N1	int	G1	-	IVS1+6T→C	-	-/+++	+++/-
732	T2N1	int	G3	+	-	-	-/+++	+++/-
735	T4N3	diff		ND	AAC(Asn)→AAT(Asn) at cd751	-	+++/-	+++/-$^{\nabla}$
738	T3N2	diff		+	-	-	-	-$^{\nabla}$
750		met		ND	AAC(Asn)→AAT(Asn) at cd751	+♣	++/-	+++/-
755	T2N1	int	G2	ND	-	+♥	+++/-	++/-
808	T3N0	int	G1	+	ACG(Thr)→ACA(Thr) at cd251	+♠	+++/-	++/-
811	T2N1	int	G2	+	-	-	+++/-	-/+
832	T3N2	int	G2	+	-	-	+++/-	+++/-$^{\nabla}$
855	T3N2	int	G2	+	-	-	+++/-	+++/-
875	T3N2	int	G2	-	IVS4+10C→G	ND	+++/-	+++/-
904	T2N0	int	G3	+	IVS4+10C→G	-	-/+++	++/-
Useful				30	3	7	21	14
Total				40	50	38	50	50
%				75	6	18	42	28

T, tumour (size and invasiveness); N, node (degree of metastasis); M, metastasis; G1, well differentiated; G2, moderately differentiated; G3, poorly differentiated; G4, not differentiated; diff, diffuse (degree of differentiation=G4); mi, mixed; int, intestine; met, metastatic tumour probably from stomach tumour; squ, squamous epithelial; LOH, loss of heterozygosity; E-cad, E-cadherin; β-cat, β-catenin; IHC, immunohistochemistry; cd, codon; UTR, untranslated region; +, positive LOH, aberrant E-cad mRNA, E-cad and β-cat IHC;-, negative LOH, E-cadherin gene mutation, aberrant E-cad mRNA, E-cad and β-cat IHC; ND, not determined or not done; +/-, ++/- and +++/-, more than 50% cells positive; -/+, -/++ and -/+++, more than 50% cells negative; *, somatic mutation; ♦, germline mutation; ♣, insertion of intron 7 between exons 7 and 8, stop codon 374; ♥, deletion of last 72 bases of exon 7, exon 8 and first 124 bases of exon 9, stop codon 322; ♠, deletion of exons 8 and 9; deletion of last 84 bases of exon 8, stop codon 358; ∇, these samples also showed cytoplasmic staining for β-catenin IHC.

LOH determination

Microsatellite markers used for LOH analysis of chromosome 16q were: D16S503, D16S496, D16S421, D16S545 and D16S512 for the region 16q22.1 containing E-cadherin locus (Genome Database). The polymerase chain reaction (PCR) products were separated in an acrylamide sequencing gel and transferred to a positively-charged nylon membrane, Hybond-N+ (Amersham, Aylesbury, UK) and baked for at least 2 h at 80°C. The non-radioactive detection method used to visualize the PCR products has been described previously [23]. Autoradiograms were inspected visually by at least two reviewers, comparing the intensity of alleles from normal and tumour DNA. The absence or a significant decrease of one allele in the tumour compared to the normal reference sample was considered as LOH.

Mutation screening

All 16 exons of E-cadherin gene and exon 3 of β-catenin gene were screened for inactivation mutations with a PCR-SSCP (single strand conformation polymorphism) analysis on genomic DNA templates. The primers for E-cadherin and β-catenin used in the SSCP analysis were described in our previous article [4] and Park et al. [1999], respectively, and ordered from Pharmacia Biotech or TAG Copenhagen A/S. Genomic DNA was used at 30 ng per 25 μl reaction mixture containing 5 pmol of the forward and reverse primers, 2.5 nmol of each dNTP, 0.5 units of DynaZyme polymerase. The samples were amplified in 35 cycles composed of 30 s of denaturation at 94°C, 30 s of annealing at 55–70°C, and finally 60 s of extension at 72°C. A hot start was used by adding the enzyme in first cycle at around 70°C, after a preincubation time of 5 min at 94°C. A 4 μl aliquot of PCR products was mixed with 7 μl of formamide dye (95% formamide, 0.05% bromophenol blue and 0.05% xylene cyanol), denatured at 94°C for 10 min and snapcooled on ice. Aliquots of 2 μl were analysed simultaneously on two non-denaturing polyacrylamide gels (5% acrylamide with 2% cross linking), either containing 5% glycerol or lacking glycerol. Electrophoresis was performed in 1× TBE on vertical gels at 6w overnight or for 6 h at room temperature. The PCR products were visualized as the microsatellite markers. Samples with abnormal mobility bands were amplified again for 35 cycles as described above. A 5 μl aliquot of PCR products was then incubated with 10 U exonulease I and 2 U shrimp alkaline phosphatase to remove excessive primers and dNTPs (US70995, Amersham). Sequences of both strands were determined by thermo sequenase DNA polymerase (Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit, Amersham) using the two original PCR primers. We performed the A592T mutation analysis on those cancers, except the cancer of unclear origin, in the family of case 728 using direct sequencing.

Aberrant mRNA screening

1–5 μg of the total RNA was reversely transcribed into cDNA using first strand cDNA synthesis kit (Amersham Pharmacia Biotech). All samples were examined for E-cadherin cDNA deletions and insertions. E-cadherin cDNA was amplified using primer pairs Ex7-rEx10/2 and Ex9/2a-rEx11 for the region of exons 7–10 encoding calcium binding sites [24]. PCR products were visualized by agarose gel electrophoresis. Abnormal fragments were excised and sequenced using forward and reverse primers to determine the boundaries of the deletions and insertions. Here, we used BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Foster City, CA) and automated sequencer ABI PRISM™ 3100 (Perkin-Elmer) for sequencing

Immunohistochemical staining

Immunohistochemistry for E-cadherin and β-catenin was performed on 5-μm sections from paraffin-embedded tumour tissue blocks with monoclonal antibodies E-cadherin 5H9 and Goat Anti-Catenin Beta (Research Diagnostic, Inc. NJ, USA), respectively, using the antigen retrieval protocol described by Hazelbag et al. [1995]. Tumours were graded by intensity of staining as negative (-), weakly positive (+), moderately positive (++) and strongly positive (+++).

Statistical analysis

A X^2 test or Fisher's exact test was used to assess the relationship between the above parameters.

Results

The frequency of LOH at 16q22.1 region was 75% (Table 1).

Three tumours (6%) showed the same missense mutation A592T of exon 12, of which 2 cases had germline mutation, and one case had somatic mutation. Information for detected polymorphisms was included in Table 1. Three of 187 (1.6%) normal individuals and 1 of 280 (0.36%) breast tumours showed this germline mutation. The mutation was not found in 444 other tumours (Table 2).

Additionally, 3 of 280 breast tumours showed another missense mutation GCC(Ala)→TCC(Ser) at the identical codon 592, of which 2 cases were germline mutation; the third was unclear because the normal tissue was unavailable. The histological type of the 4 breast tumours was ductal.

In the family of case 728, we found that the patient with skin cancer and one of the patients with prostate cancer showed the same germline mutation as case 728. Interestingly, no mutations were detected in the tumour sam-

Table 2: Frequency of A592T missense mutation in gastric cancer, other cancer and normal individuals

Variables	A592T/total	%
Gastric cancer	3/50	6
Breast cancer	1/280	0.36
Other cancer*	0/444	0
Normal population	3/187	1.6

* including colon and rectum, lung, endometrium, ovary, testis, kidney, thyroid carcinomas and sarcomas.

ples of these two cases. Probably, the mutated alleles were lost during tumour development. The onset ages for the cases with germline mutations in the pedigree were 78 years for case 728, 73 years for the skin cancer and 76 years for the prostate cancer.

We did not detect mutation by SSCP and DNA sequencing in exon 3 of the β-catenin gene in the 50 gastric tumours.

Seven gastric tumours showed aberrant transcripts of the E-cadherin. Tumours 360, 369, 568, 717 and 750 displayed insertion of intron 7 between exons 7 and 8. Tumour 755 showed deletion of last 72 bases of exon 7, exon 8 and first 124 bases of exon 9. Tumour 808 displayed two aberrant mRNAs, of which one had deletion of exons 8 and 9, and one case with deletion of last 84 bases of exon 8 (Table 1).

Finally, we performed immunohistochemical staining of E-cadherin and β-catenin. A regional variation of the staining was detected across the tumours. The scorings +/-, ++/- and +++/- refer to more than 50% cells positive, and scorings -/+, -/++ and -/+++ indicating more than 50% cells negative. Negative (-) or reduced (-/+, -/++, -/+++ and +/-) expression of E-cadherin and β-catenin was detected in 21/50 (42%) and 14/50 (28%) cases, respectively. Furthermore, for β-catenin IHC, 11 tumours also showed positive cytoplasmic staining (Table 1). A significant association was found between negative or reduced expression of E-cadherin and β-catenin ($p = 0.048$, X^2 test). Also we found an association between reduced expression of E-cadherin and diffuse histotype ($p = 0.04$, Fisher's exact test).

Discussion

The high frequency of LOH at 16q22.1 region strongly suggests that there are one or more tumour suppressor genes in this region, whose loss could trigger carcinogenesis of gastric cancer. The E-cadherin gene has been mapped to chromosome 16q22.1 [26]. The reduced expression and gene mutations of E-cadherin in several types of cancers including gastric and lobular breast cancer have been identified, indicating that the E-cadherin gene is a tumour suppressor gene [2–4], [13–17,27]. In this study 3 cases (6%) showed the identical missense mutation A592T. The calcium-binding motifs located in the extracellular domains 1–5 are regarded as the key element for the function of E-cadherin, since a synthetic molecule with a single amino-acid substitution in a calcium-binding motif showed no adhesiveness [28]. Also, in human cell line MKN45 from gastric cancer, which lacked tight cell-cell adhesion, a 4-amino-acid deletion was found at the boundary between exons 6 and 7, which was considered to alter the conformation surrounding the key calcium-binding motifs and to abolish the adhesive property of the E-cadherin molecules [29]. The single amino-acid substitution in the present study is located within the fifth extracellular domain of E-cadherin, where a calcium-binding motif could exist. It is conceivable, therefore, that the mutations in the three cases also destroyed function of the E-cadherin. Interestingly, the three cases also showed LOH at 16q22.1 containing the E-cadherin locus. So it can be considered that two genetic events resulting in inactivation of the gene occurred in the two alleles of E-cadherin gene, respectively. The findings above indicated that the E-cadherin gene is a tumour-suppressor gene because it is in accordance with the classical two-hit theory for tumour suppressor genes [30]. Previous studies in lobular breast cancer also support this opinion [4,14]. In cell line MKN45 mentioned above (poorly differentiated adenocarcinoma) with weak cell-cell adhesion, a 12-bp in frame deletion of E-cadherin gene and loss of the wild type allele were detected [29]. But this cell line still showed strong expression of mRNAs and proteins, suggesting that not only reduced expression but also structural abnormalities themselves may result in inactivation of the E-cadherin-mediated cell adhesion system [29]. Therefore, a single amino-acid substitution found in this study may cause structural changes of E-cadherin and result in limited cell-cell adhesion, although two of the cases (23 and 294) showed sufficient protein expression.

Interestingly, the same sequence variant of somatic and germline mutation was found simultaneously in different gastric patients. Case 23 with somatic mutation had an onset age of 56 years, but cases 294 and 728 with germline mutation had onset ages of 71 and 78 years, respectively. One explanation for this phenomenon could be that loss of second allele of E-cadherin in case 23 occurred very early, thereby triggerring carcinogenesis relatively early in case 23. But there is another possibility that this mutation in case 23 played a role only in pro-

gression of the tumour, but not in the initiation, where other genetic events could probably be responsible for the initiation of the gastric cancer. But cases 294 and 728 with germline mutations had late onset age. This may be because the inactivation of another allele occurred very late. An article reported that the obligatory carriers with truncated mutations in E-cadherin gene in their 80s and 90s remained unaffected [31]. Therefore, further identification of genetic and/or environmental modifiers that might account for the variable age of onset should be carried out. Specially, case 294 had three tumours in the stomach, which is in line with genetic tumours usually being multiple [30].

The frequency (6%) of mutation A592T in 50 gastric tumours is almost four-fold that in normal population, suggesting again that this mutation indeed contributed to the tumourigenesis in a subset of gastric tumours. Futhermore, 0.36% of breast tumours, and no other tumours, showed identical germline mutation, indicating that there could be a histological difference for this mutation in gastric cancer and other cancer.

Only other two cases exhibited A592T mutation in the family of case 728. Specially, this mutation was detected only in corresponding normal tissue, but not in the tumour tissue, suggesting that the mutated alleles were lost during the tumourigenesis. From these we can conclude that this mutation may not play a role in the tumourigenesis of prostate and skin cancer, but it could be gastric-cancer specific.

We conclude that the A592T mutation may increase the lifetime risk of developing gastric cancer, but is clearly a sequence variant of low penetrance. Our findings of two different sequence variants at codon 592 (A592T and A592S), as germline and somatic mutations, suggest that this codon is a hotspot of mutations in tumour pathogenesis.

The breakpoints for insertions of intron 7 and deletion of exons 8 and 9 are at splicing sites, in accordance with "GU-AG" rule for mRNA splicing. It may be speculated that these alterations were not generated by alternative splicing, since no evidences for alternative splicing were found within mouse E-cadherin gene [32] and no aberrant mRNAs were detected in noncancerous tissues. The mutations at splicing sites ought to be responsible for the alterations of E-cadherin mRNAs, although no mutations were found on DNA level in this study, presumably because the SSCP used for mutation screening has a low efficiency. An additional 2 deletions showed breakpoints at non-splicing sites, whose splicing is not in line with "GU-AG" rule, possibly indicating that a rearrangement in genomic level may cause the aberrant mRNAs. Previous studies have shown skipping of exon 8 or 9 in gastric cancer [24,33]. These aberrations may result in E-cadherins losing calcium-binding motifs due to lack of exons 8 and 9, and truncated molecules because of frameshift mutations caused by insertions and deletions, finally facilitating scattering of carcinoma cells.

Reduced expression of E-cadherin and β-catenin has been found in some cancers including gastric cancer [8]. Heterogeneous or unstable expression for both E-cadherin and β-catenin across the tumours was found. It has been demonstrated that in 40% of adenocarcinomas E-cadherin levels were raised in their intravascular tumour components in comparison to their extravascular compartments [34]. One explanation may be that entrance of a carcinoma into an intravascular compartment is associated with an upregulation of E-cadherin expression, and that subsequent exit into extravascular tissues is associated with downregulation [35]. Since E-cadherin and β-catenin are the crucial components to form cell-cell adhesion complex, loss of them may result in the disruption of the function of the complex, which may cause weak cell-cell adhesion and confer invasive properties on a tumour. Furthermore, reduced cell-cell adhesion is associated with loss of contact inhibition of proliferation, thereby allowing escape from growth control signal, finally triggering carcinogenesis of human cancer [8]. In this study, an association between abnormal expression of E-cadherin and β-catenin was found, suggesting that loss of E-cadherin binding may cause a redistribution of β-catenin from the cell membrane to the cytoplasm. The increased free β-catenin in the cytoplasm could translocate to the nucleus and lead to activation of gene expression. This is supported by the findings that two cases (676 and 738) showed negative staining in the inner surface of membrane and positive staining in cytoplasm, simultaneously. But other 9 tumours (table 1) showing moderate or strong staining in membrane also exhibited positive staining in cytoplasm, suggesting that the normal degradation of the free β-catenin in cytoplasm is inhibited. Also, we found an association between abnormal expression of E-cadherin and diffuse histotype, indicating that the alterations of the E-cadherin may play a role in poorly-differentiated gastric tumours. Interestingly, aberrant expression of E-cadherin and/or the catenins has been shown to be an independent prognostic marker for short survival in gastric cancer patients [8]. Of particular interest is the finding that E-cadherin is an independent predictor of occult lymph node and micrometastasis in nodes classified as No by routine histopathological methods [8].

Conclusions

Our results support that alterations of E-cadherin and β-catenin play a role in the initiation and progression of

gastric cancer. Expression of both genes are reduced in gastric cancer, but the mechanism of downregulation is not clear. LOH, mutations and alterations in RNA splicing could explain a part of the downregulation of the E-cadherin in gastric cancer.

Competing interests

None declared

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