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Abnormalities of the cadherin–catenin complex in chemically-induced colo-rectal carcinogenesis

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β-Catenin is a multifunctional protein originally identified as a component of the cadherin cell–cell adhesion complex. It also binds the adenomatous polyposis coli (APC) tumour suppressor which controls β-catenin cellular levels through its degradation. β-Catenin and/or APC mutations result in increased cytoplasmic β-catenin and nuclear translocation. The aim of the present study was to examine the expression and cellular localisation of α- and β-catenin, p120 and E-cadherin in a chemically-induced mouse model of colo-rectal cancer using 1,2-dimethylhydrazine (DMH). Female Balb/C mice were injected subcutaneously with a solution providing 25 mg DMH base/kg body weight for 17 weeks. Animals were killed and tumours identified in the intestine with a dissecting microscope. Formalin-fixed paraffin-embedded sections of normal and dysplastic colonic mucosa were stained by an indirect avidin–biotin immunohistochemical technique using mouse monoclonal antibodies, and membranous, cytoplasmic and nuclear cellular localisation was assessed by light microscopy. Staining distribution scored as follows: 3, >90 % positive epithelial cells; 2, >50 % positive epithelial cells; 1, <50 % positive epithelial cells. Non-dysplastic colonic epithelial cells revealed β-catenin expression at the membrane (33/41 scored 3), areas of cytoplasmic expression (24/41 scored 1) and no nuclear staining. Dysplastic colonic epithelium revealed increased membranous and cytoplasmic β-catenin immunoreactivity (39/41 and 38/41 both scored 3) with focal nuclear staining (14/41). Expression patterns for α-catenin, p120, and E-cadherin were similar to β-catenin with increased membranous and cytoplasmic immunoreactivity in dysplastic mucosa, although no nuclear staining was observed. Increased cytoplasmic expression and nuclear localisation of β-catenin are consistent with a possible mutation in its gene, and this finding was in keeping with the mutational analysis of exon 3 by single-strand conformational polymorphism. Increased immunoreactivity of the other catenins also suggests further disruption in catenin regulation. In summary, alterations in the β-catenin expression and cellular localisation in the DMH-induced tumours are similar to those seen in human sporadic colo-rectal tumours. The DMH is therefore a useful model for studying the abnormalities of the E-cadherin–catenin pathway in colo-rectal carcinogenesis.

E-cadherin: Catenins: Colo-rectal carcinogenesis: Dimethylhydrazine model

Colo-rectal cancer is a multifactorial disease involving the interaction of a large number of genes and their environment. Genetic changes in colo-rectal cancer (for a review, see Fearon & Vogelstein, 1990) include an adenomatous polyposis coli (tumour-suppressor gene) mutation in 85 % of all colo-rectal cancers. E-cadherin, β-catenin and adenomatous polyposis coli all play a fundamental role in the regulation of the normal colon, with disruptions potentiating colo-rectal cancer development. The catenins are a multigene family of cytoplasmic proteins comprising α- (102 kDa), β- (92 kDa), γ- (Plakoglobin; 83 kDa) catenin, and p120$^{(CAS)}$ (Peifer *et al*. 1992; Liu *et al*. 1997; for a review, see Smith & Pignatelli, 1997). The catenins are multifunctional, associating with a large number of proteins (Brady-Kalnay *et al*. 1995; Pignatelli, 1998; for a review, see Nakamura, 1997) that direct their function within the

Abbreviations: DMH, 1,2-dimethylhydrazine; PCR, polymerase chain reaction; SSCP, single-strand conformational polymorphism; TBE, Tris-borate–EDTA.

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cell, playing a role in cell motility, growth, adhesion and signalling. Cell adhesion to neighbouring cells is via E-cadherin interacting with the catenins and the actin cytoskeleton, playing important roles in cell growth, differentiation and survival (Watabe *et al*. 1994; for reviews, see Ben-Ze'ev & Geiger, 1998; Efstathiou & Pignatelli, 1998). E-cadherin, a transmembrane glycoprotein, forms Ca-dependent homophilic interactions between cells creating 'zipper-like' adherens junctions (Gagliardi *et al*. 1995; Shiozaki *et al*. 1996; Reviewed by Smith & Pignatelli, 1997). Perturbation of any of these interactions results in changes in intercellular adhesion and cell transformation (Valizadeh *et al*. 1997; Guilford *et al*. 1998). As β-catenin is highly homologous to the armadillo protein, found in *Drosophila melanogaster*, it was shown that β-catenin plays an important role in the vertebrate wingless-wnt pathway (for reviews, see Ilyas & Tomlinson, 1997; Nusse, 1997), a pathway important in colon carcinogenesis (for a review, see Barth *et al*. 1997). Wnt-1 acts through the wingless pathway to inhibit glycogen synthase kinase (GSK)-3β (Cook *et al*. 1996), a serine–threonine kinase that negatively regulates β-catenin when in excess (Rubinfeld *et al*. 1996; Morin *et al*. 1997; for a review, see Jankowski *et al*. 1997). Inhibition of GSK-3β results in increased stability of β-catenin, resulting in cytoplasmic accumulation and nuclear translocation where it associates with the transcription factors LEF and TCF (Behrens *et al*. 1996; Korinek *et al*. 1997; for a review, see Barth *et al*. 1997) altering gene transcription.

The 1,2-dimethylhydrazine (DMH) mouse model is widely used to study chemically-induced colon cancer (Rogers & Nauss, 1985; Jackson *et al*. 1999), providing a useful model for studying early carcinogenesis and sporadic cancer development. DMH is a specific colon carcinogen that induces large bowel tumours in rodents (Klurfeld, 1995) and results in morphological changes similar to those observed in human adenomatous polyposis (Chang, 1978; Moorghen *et al*. 1998). DMH is activated in the liver, with these active metabolites reaching the dividing cells at the bottom of the crypt via the bloodstream (Weisburger *et al*. 1977; Klurfeld, 1995) or possibly via biliary secretion (Sunter *et al*. 1980). The precise nature of the mutations caused by the carcinogen DMH are currently unknown. Pro-mutagenic lesion O^6-methylguanine has been detected in DNA from various rat and mouse tissues following exposure to DMH (Jackson *et al*. 1999). Some genes found mutated in human colo-rectal cancer have been studied and a mutation in the GSK-3β consensus motif of β-catenin has been found in rats and mice (Takahashi *et al*. 1998, 2000). The aim of the present study was to examine the expression and cellular localisation of β-catenin, α-catenin, p120, and E-cadherin in non-dysplastic and dysplastic tissues of DMH-treated mice.

Methods

1,2-Dimethylhydrazine mouse model

Female Balb/C mice (2 weeks old; B & K, Hull, UK) were caged in groups of ten and housed under standard conditions. The mice were fed *ad libitum* on a basal diet (Special Diets Services, Witham, Essex, UK) supplemented with various sources of fibre, and weight and water intake were continually monitored. Female Balb/C mice were injected subcutaneously with DMH hydrochloride (Sigma-Aldrich Co. Ltd, Gillingham, Dorset, UK) dissolved in EDTA (0·4 %, w/v; Sigma-Aldrich) and normal saline (9 g NaCl/l), pH 6·5 (25 mg DMH base/kg body weight) once weekly for 17 weeks. The mice were killed by cervical dislocation, the colons removed and opened longitudinally, pinned on to corkboard serosal side down, fixed in carnoys reagent for 3 h followed by fixation in 10 % (v/v) formalin. Nodules were then counted, measured and position noted (Fig. 1) using a dissecting microscope. Representative histological samples were taken from the macroscopic nodules and adjacent normal mucosa for further analysis and histological examination.

Immunohistochemistry

The identification of microadenomas and their histology was determined using standard haematoxylin and eosin staining. Tissues were examined by two independent observers (E.L.T. and M.P.) using a light microscope.

The expression and cellular localisation of catenins were determined using a standard avidin–biotin complex immunohistochemical technique on 4 µm sections of carnoys-fixed paraffin-embedded tissue sections mounted on to polylysene (BDH, Poole, Dorset, UK) slides. Endogenous peroxidase activity was blocked by incubating the slides in 0·6 % (v/v) H_2O_2 and antigen retrieval was achieved by microwaving (Amana, 800 W, high power; Bradshaw Microwave Ltd, Bristol, UK) the slides in 0·1 M-citrate buffer (pH 6) for 20 min. Primary antibody (Table 1) was incubated overnight at 4 C. Tissues were examined under a light microscope by two independent observers (E.L.T. and M.P.) without knowledge of origin. The cellular localisation and immunoreactivity was assessed relative to adjacent non-dysplastic epithelium within the same tissue. Staining was assessed in terms of the percentage epithelial cells with membranous, cytoplasmic or nuclear staining. Tumours were scored as follows: 3, >90 % positive epithelial cells; 2, 90–50 % positive epithelial cells; 1, <50 % positive epithelial cells.

Table 1. Antibodies used for immunohistochemical staining

	Supplier	Concentration
Primary antibodies		
β-catenin	BD Transduction Labs*	1:500
α-catenin	BD Transduction Labs	1:100
p120	BD Transduction Labs	1:250
E-cadherin	BD Transduction Labs	1:500
Secondary and tertiary antibodies		
Biotin goat anti-mouse	DAKO Ltd†	1:250
Peroxidase-labelled streptavidin	DAKO Ltd	1:100

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†Ely, Cambs., UK.

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β-Catenin mutational analysis

Primer design. β-Catenin was amplified using primers designed by Takahashi *et al*. (2000) that were obtained from MWG-Biotech, NC, USA.

Polymerase chain reaction. DNA extracted from frozen tissues (– 80 C) using a Qiagen® kit (Qiagen Ltd, Crawley, West Sussex, UK). Polymerase chain reaction (PCR) reaction mixture (0·5 µl (50 pMol) primer, 5 µl 2 mM-dNTP (Gibco , Invitrogen Corp., Carlsbad, CA, USA), 5 µl PCR buffer (〈 10; Gibco), 3 µl 50 mM-$MgCl_2$ (Gibco), 0·3 µl platinum Taq (5 U/µl, Gibco), 6 µl DNA (tissue lysate) and sterile injection water) with total volume of 50 µl, amplified by forty cycles (94 C for 1 min, 58 C for 1 min, 72 C for 1 min, except the last cycle which lasted 7 min) using a DNA engine (MJ block, Peltier Thermal cycler; Dyad , MJ Research Inc., Waltham, MA, USA). A negative and, where possible, a positive control were run in parallel. Samples (15 µl) of PCR product in 5 µl Ficoll buffer (% (v/v); 0·25 bromophenol blue, 0·25 xylene cyanol FF, 15 Ficoll (type 400; Pharmacia Biotech AB, Uppsala, Sweden), water (molecular cloning; Maniatis *et al.* 1989), were fractionated by gel electrophoresis on a 1 % (w/v) Tris-borate–EDTA (TBE)–agarose gel containing 0·14 µg ethidium bromide/ml, running in 1 〈 TBE buffer (0·045 M-Tris-borate, 0·001 M-EDTA, pH 7·5) at 100 V for 1 h. The separated DNA and 100 bp marker was visualised on a u.v. transilluminator and recorded using a digital camera (Kodak, Hemel Hempstead, Herts., UK).

Single-strand conformational polymorphism. DNA (2–8 µl) and 2 µl single-strand conformational polymorphism (SSCP) loading buffer (5 M-NaOH, 0·05 M-EDTA, 50 % glycerol, 5 % bromophenol blue–xylene cyanol and water; Orita *et al.* 1989) were heated to 37 C for 2 min, cooled with ice for 5 min, loaded on to SSCP gel (6·3 ml Mutational detection enhancement, 3 ml 5 〈 TBE, 16·1 ml double-distilled water, 60 µl Tetramethyl-1-2-diaminomethane, 400 µl 10 % Ammonium persulphate) and run at 4 C with 0·6 〈 TBE for 7 h at 200 V. Band visualisation was achieved by washing with 10 % (v/v) ethanol inacetic acid, incubating for 15 min with 0·1 % (w/v) $AgNO_3$, washing with double-distilled water, and then developed using 1·5 % (w/v) NaOH and 0·1 % (v/v) formaldehyde (37 %), fixed in 0·75 % (w/v) Na_2CO_3 and visualised using a light box and recorded using a digital camera. Nucleotide sequencing of shifted band in SSCP analysis was also performed in the Department of Biochemistry, University of Bristol, Bristol, UK.

Results

Increase in cytoplasmic expression and nuclear localisation of β-catenin in dysplastic mucosa

Within the non-dysplastic tissues (Fig. 1) of the DMH-exposed tissues > 90 % of the colonic epithelial cells were positive for β-catenin expression at the cell membrane in thirty-three of forty-one tissues examined. The areas of cytoplasmic expression within these tissues were found to be much less, with twenty-four of forty-one tissues having areas of < 50 % positivity. There was no focal nuclear expression of β-catenin within the non-dysplastic tissues. The dysplastic tissues revealed increased membranous and cytoplasmic β-catenin immunoreactivity with > 90 % of the epithelial cells being positive for β-catenin in the majority of tissues (thirty-nine of forty-one and thirty-eight of forty-one tissues respectively scored 3). Within these dysplastic tissues focal nuclear expression was also observed in fourteen of forty-one tissues. Thus, when comparing the non-dysplastic tissues with the dysplastic tissues there was an increase in cytoplasmic and focal nuclear expression for β-catenin within the dysplastic areas, an increase in immunoreactivity was also noted (Fig. 1 (A)) but not quantified.

Cytoplasmic E-cadherin cellular localisation is not associated with reduced membranous expression in dysplastic mucosa

Within the non-dysplastic tissues > 90 % of the epithelial cells were positive for E-cadherin (Fig. 2) membrane expression within the majority of the tissues (thirty-nine of forty-one scored 3). Cytoplasmic expression showed > 50 % of the epithelial cells being positive for E-cadherin in some tissues (twenty-three of forty-one scored 2), the remainder of tissues having lower expression levels. There was no nuclear E-cadherin expression within the non-dysplastic tissues (zero of forty-one). Of the epithelial cells > 90 % were positive for E-cadherin membrane expression within the dysplastic tissues, suggesting membrane expression was maintained (thirty-nine of forty-one scored 3). This positive outcome was accompanied by an increase in cytoplasmic expression with 90 % of the epithelial cells being positive in the majority of tissues (thirty-two of forty-one scored 3) but no nuclear localisation (zero of forty-one) was observed. Thus, the results show an increase in cytoplasmic expression for E-cadherin in the dysplastic tissues (Fig. 2 (A)).

Increase in α-catenin and p120 cytoplasmic expression in dysplastic mucosa is not associated with nuclear localisation

Expression of α-catenin (Fig. 3) within the non-dysplastic tissues revealed membranous expression in all tissue in > 90 % of all colonic epithelium (forty of forty scored 3). Of the epithelial cells > 50 % were positive for α-catenin within the cytoplasm in the majority of these tissues (thirty-five of forty scored 2) and no nuclear expression (zero of forty-one) was observed. Within the dysplastic areas of these tissues there was maintenance of membrane expression, with membranous expression in > 90 % of the epithelial cells in all tissues (40/40 scored 3). There was also an increase in cytoplasmic localisation of α-catenin within the majority of these tissues, with 90 % of the cells being positive in the majority of tissues (twenty-nine of forty scored 3); this increase was accompanied by no focal nuclear expression. Thus, within the dysplastic tissues α-catenin expression was shown to be maintained at the membrane and increased within the cytoplasm. Expression of p120 (Fig. 4) within the non-dysplastic areas of the DMH tissues showed membranous expression in 90 % of epithelial cells for the majority of tissues (thirty-nine of forty scored 3); expression was throughout the crypt, although greatest at the mid to top point of the crypt. Some cytoplasmic expression was

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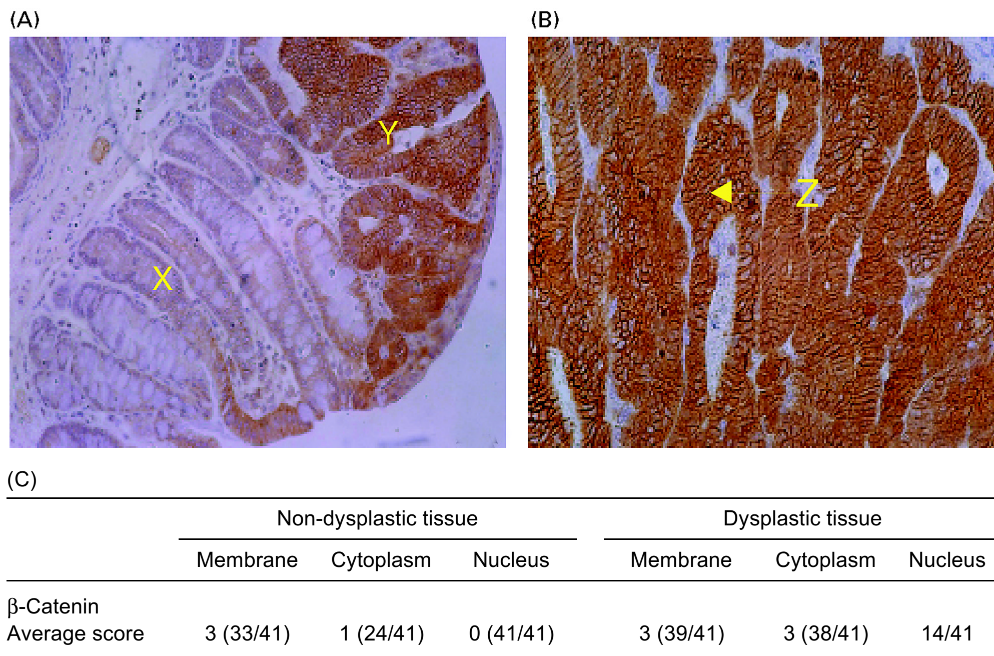
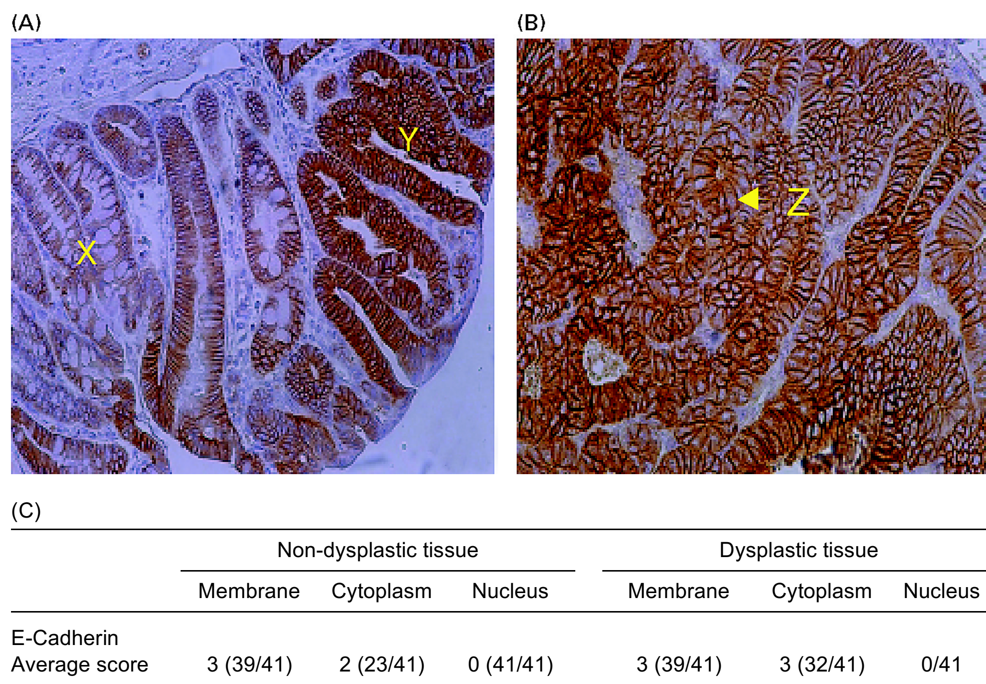


	Non-dysplastic tissue			Dysplastic tissue		
	Membrane	Cytoplasm	Nucleus	Membrane	Cytoplasm	Nucleus
β-Catenin Average score	3 (33/41)	1 (24/41)	0 (41/41)	3 (39/41)	3 (38/41)	14/41

Fig. 1. (A) Representative 1,2-dimethylhydrazine-treated mouse tissue section showing β-catenin localisation and expression in non-dysplastic (X) and dysplastic (Y) tissues. (B) Area of dysplasia with increased membranous, cytoplasmic and focal nuclear localisation (Z). (C) Non-dysplastic tissues revealed β-catenin expression at the membrane with areas of cytoplasmic expression but no focal nuclear expression. Dysplastic tissue revealed increased membranous and cytoplasmic β-catenin immunoreactivity with focal nuclear localisation. Thus, within the dysplastic tissue there was an increase in cytoplasmic and focal nuclear localisation for β-catenin when compared with non-dysplastic tissues. An increase in immunoreactivity was also noted. For details of procedures, see p. 230.



	Non-dysplastic tissue			Dysplastic tissue		
	Membrane	Cytoplasm	Nucleus	Membrane	Cytoplasm	Nucleus
E-Cadherin Average score	3 (39/41)	2 (23/41)	0 (41/41)	3 (39/41)	3 (32/41)	0/41

Fig. 2. (A) Representative 1,2-dimethylhydrazine-treated mouse tissue section showing E-cadherin localisation and expression in non-dysplastic (X) and dysplastic (Y) tissues. (B) Area of dysplasia with increased membranous and cytoplasmic localisation but no focal nuclear localisation (Z). (C) Non-dysplastic tissues showed that for E-cadherin the membranous expression was >90 % in the majority of the tissues, with evidence of cytoplasmic expression in areas, but there was no nuclear expression. Within the dysplastic tissues membrane expression was maintained, accompanied by an increase in cytoplasmic localisation but with no nuclear localisation. The results show an increase in cytoplasmic localisation of E-cadherin in the dysplastic tissues. Increased immunoreactivity within dysplastic tissue was also noted. For details of procedures, see p. 230.

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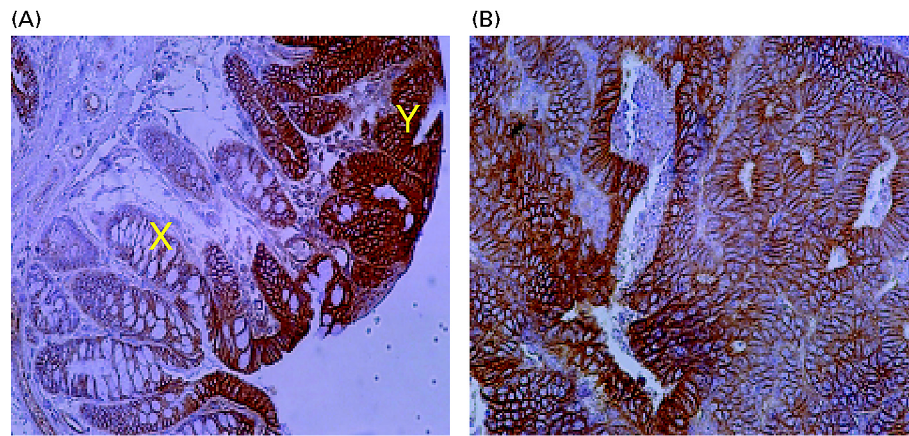
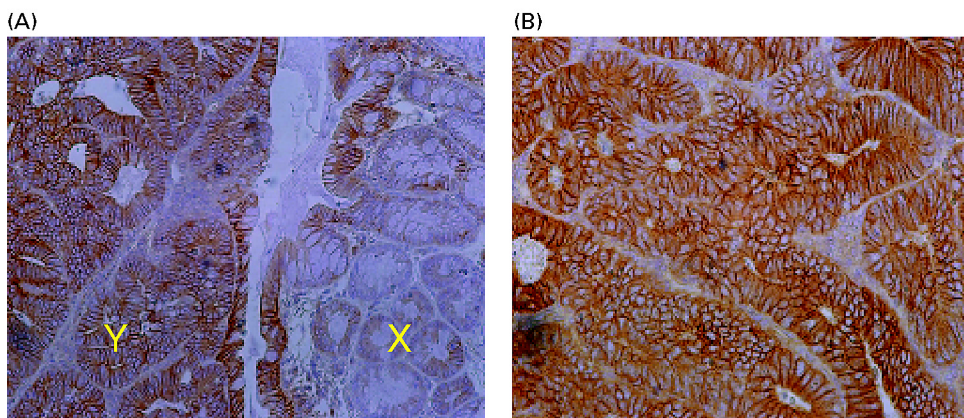


(C)

	Non-dysplastic tissue			Dysplastic tissue		
	Membrane	Cytoplasm	Nucleus	Membrane	Cytoplasm	Nucleus
α-Catenin Average score	3 (40/40)	2 (35/40)	0 (41/41)	3 (40/40)	3 (29/40)	0/40

Fig. 3. (A) Representative 1,2-dimethylhydrazine-treated mouse tissue section showing α-catenin localisation and expression in non-dysplastic (X) and dysplastic (Y) tissues. (B) Area of dysplasia with increased membranous and cytoplasmic expression but no nuclear expression. (C) α-Catenin expression within non-dysplastic tissues revealed membranous expression in all tissue, with cytoplasmic localisation found in some of these tissues with no nuclear expression. Dysplastic tissues revealed membranous expression in > 90 % of all colonocytes within the dysplastic tissues, with an increase in cytoplasmic localisation but with no nuclear localisation. Thus, within the dysplastic tissues α-catenin localisation was shown to increase within the cytoplasm. For details of procedures, see p. 230.



(C)

	Non-dysplastic tissue			Dysplastic tissue		
	Membrane	Cytoplasm	Nucleus	Membrane	Cytoplasm	Nucleus
p120 Average score	3 (39/41)	2 (23/41)	0 (41/41)	3 (39/41)	3 (28/41)	0/41

Fig. 4. (A) Representative 1,2-dimethylhydrazine-treated mouse tissue section showing p120 localisation and expression in non-dysplastic (X) and dysplastic (Y) tissues. (B) Area of dysplasia with increased membranous and cytoplasmic expression but no nuclear expression. (C) p120 localisation within non-dysplastic tissues revealed membranous expression, with some cytoplasmic localisation but with no nuclear expression. Within the dysplastic tissues there was a maintenance of membrane expression, with an increase in cytoplasmic localisation but with no nuclear expression. Thus, in the dysplastic tissues p120 localisation was shown to increase within the cytoplasm. For details of procedures, see p. 230.

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observed with twenty-three of forty-one tissues having >50 % (but <90 %) epithelial cells positive for p120. As was found for the other catenins and E-cadherin, there was no nuclear expression (zero of forty-one) within the non-dysplastic tissues. Within the dysplastic tissues there was maintenance of p120 membrane expression (thirty-nine of forty-one scored 3), as for α-catenin, accompanied by an increase in cytoplasmic expression, with twenty-eight of forty-one tissues scoring 3 (>90 % of epithelial cells being positive) but with no nuclear expression (zero of forty-one), as was found for α-catenin and E-cadherin. Thus, in the dysplastic areas of the DMH-exposed mouse tissues p120 expression was shown to increase within the cytoplasm.

Mutation analysis of the β-catenin gene

Having shown cytoplasmic and focal nuclear localisation of β-catenin within the dysplastic tissues it was interesting to examine β-catenin for possible mutations. PCR primers were used to amplify β-catenin exon 3, containing the GSK-3β consensus sequence (phosphorylation sites) and a product length of 123 bp was produced. Fig. 5 shows the successful amplification of the GSK-3β consensus sequence of β-catenin. Fig. 6 gives two examples of SSCP mutational analysis of this PCR-amplified β-catenin sequence, showing that there were possible band shifts in three of four DMH-exposed tissue samples, suggesting a possible mutation within this region.

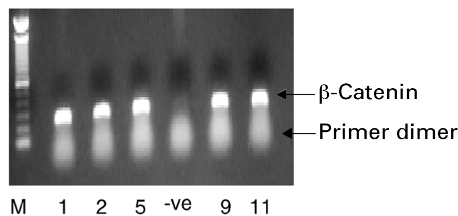

Fig. 5. Polymerase chain reaction amplification of β-catenin from frozen distal colonic tissue samples from 1,2-dimethylhydrazine-treated Balb/c mice. 1, group 1 control (non-DMH); 2, group 2 ispaghula husk–DMH; 5, group 5 pectin–DMH; 9, group 9 cellulose–DMH; 11, group 11 basal–DMH; M, 100 bp marker. For details of procedures, see pp. 230–231.

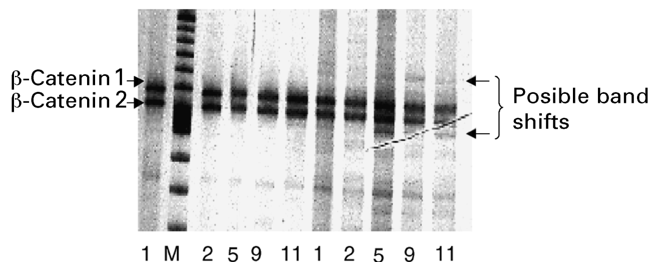

Fig. 6. Single-strand conformational polymorphism revealing possible β-catenin mutations in three of the four 1,2-dimethylhydrazine (DMH)-treated Balb/c mice. 1, group 1 control (non-DMH); 2, group 2 ispaghula husk–DMH; 5, group 5 pectin–DMH; 9, group 9 cellulose–DMH; 11, group 11 basal–DMH; M, 100 bp marker. For details of procedures, see pp. 230–231 .

Discussion

Catenins play a critical role in the regulation of cellular proliferation and carcinogenesis. The abrogation of catenin function has been shown to occur through a number of genetic and epigenetic factors, along with changes in expression and subcellular localisation of the associated proteins. In normal colonic mucosa β-catenin is mainly membranous, whereas in adenomas and carcinomas it is expressed in the cytoplasm and in the nucleus. Nuclear β-catenin increases from early adenomas to adenocarcinomas (Hao *et al*. 1997) and higher levels of nuclear expression have been found at the invasive front of colo-rectal adenocarcinomas.

In our DMH mouse model similar β-catenin expression was found within the non-dysplastic tissues when compared with the cellular localisation in human colonic mucosa. Within the dysplastic tissues there were also similarities, with an increase in cytoplasmic expression and focal nuclear staining for β-catenin, but this increase was accompanied by maintenance of membrane expression. This finding suggests a possible difference in β-catenin activation without disruption of the E-cadherin–catenin complex. The focal nuclear expression shown for β-catenin could possibly be the result of a number of genetic and epigenetic events. Mutation of either the adenomatous polyposis coli or the β-catenin genes results in nuclear accumulation of β-catenin and leads to activation of Tcf–Lef-dependent transcription and up regulation of target genes (e.g. c-myc, cyclin D1) important in carcinogenesis. Activating mutations in the β-catenin gene are thought to be responsible for the excessive β-catenin signalling involved in the majority of carcinogen-induced colonic carcinomas. The most common mutation that has been found in the human gene encoding β-catenin affects the serine and threonine residues of the protein that are targeted by GSK-3β. This mutation allows β-catenin to escape from the proteosomal degradation, with subsequent accumulation in the cytoplasm and translocation into the nucleus. In our study SSCP mutational analysis of dysplastic tissue revealed a possible mutation within the GSK-3β consensus sequence of β-catenin. However, sequence analysis failed to confirm a mutation, but this result could be due to the differences in sensitivities of the different procedures, with SSCP being highly sensitive. Indeed, our result is in keeping with that of Takahashi *et al*. (2000) who revealed a β-catenin mutation in the GSK-3β consensus motif in mice using the carcinogen azoxymethane, a metabolite of DMH.

Changes in cellular expression and distribution of E-cadherin and of other components of the catenins subfamily have been found in human colo-rectal adenomas and carcinomas, suggesting that they also could play a fundamental role in cancer development. Indeed, in the present study the expression patterns of α-catenin, p120, and E-cadherin were similar to those of β-catenin, with increased membranous and cytoplasmic immunoreactivity in dysplastic tissues, although no nuclear staining was observed.

Loss of E-cadherin-mediated adhesion appears to be a fundamental aspect of the neoplastic process, allowing cells to escape normal growth-control signals, resulting in loss of differentiation and increased cell proliferation associated with invasive behaviour (Frixen *et al*. 1991). In human normal colo-rectal epithelial cells there is typical

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membranous staining at the adherens junctions, but in adenomas and carcinomas there are changes in the immunoreactivity and cellular localisation. These changes in E-cadherin expression are correlated with tumour size, histopathology, growth patterns and the extent of dysplasia (Dorudi *et al*. 1993; Gagliardi *et al*. 1995). Loss of E-cadherin expression was not observed in our study, but this result may be explained by the fact that the model used explores colo-rectal carcinogenesis at an early stage, with the majority of lesions examined being adenomas.

In normal human epithelium α-catenin is strongly expressed and is required for stable E-cadherin-mediated cell adhesion (Shiozaki *et al*. 1994; Imamura *et al*. 1999). However, α-catenin expression is reduced or absent in a number of primary tumours of the oesophagus, stomach and colon (Shiozaki *et al*. 1994). Skoudy *et al*. (1996) also reported altered α-catenin expression in human colo-rectal cancer, suggesting that some human cancer cells may have impaired E-cadherin-mediated cell adhesiveness through the down regulation of α-catenin expression (Shiozaki *et al*. 1994). Within the DMH mouse model there were similarities with human α-catenin expression within the non-dysplastic tissues but differences within the dysplastic tissues, as membrane expression was maintained and cytoplasmic localisation was increased not reduced as for human cells. This finding could reflect the early stage reached within the adenoma to carcinoma sequence in the DMH mouse model or be related to the maintenance of E-cadherin expression.

In normal human colon p120-catenin is present in the crypt and surface epithelium, with cells showing reactivity in both the membrane and cytosol (Skoudy *et al*. 1996), and staining intensity being greatest in proliferating crypt cells (Valizadeh *et al*. 1997). Reduced expression of p120 was observed in 20 % of the adenomatous polyps, with loss of membranous p120 expression correlating with reduced E-cadherin expression. Decreased expression for p120 was found to correlate with the larger size tumour (Skoudy *et al*. 1996). Although in our study an increased cytoplasmic localisation of p120 was found in dysplastic mucosa, the overall expression and the association with E-cadherin was maintained.

In conclusion, alterations in β-catenin expression and localisation in DMH-exposed mice are similar to the changes seen in human sporadic colo-rectal tumours. Thus, this chemically-induced model of carcinogenesis appears to be useful for studying the abnormalities of the cadherin–catenins pathway at the early stages of tumour development.

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