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Errata

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

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Methods, pages 230 and 231

Please replace the *Immunohistochemistry* and *β-Catenin mutational analysis* sections with the following:

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 4ºC. 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.

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 (50pMol) 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.