Desmocollin switching in colorectal cancer

The desmocollins are members of the desmosomal cadherin family of cell–cell adhesion molecules. They are essential constituents of desmosomes, intercellular junctions that play a critical role in the maintenance of tissue integrity in epithelia and cardiac muscle. In humans, three desmocollins (Dsc1, Dsc2 and Dsc3) have been described. The desmocollins exhibit tissue-specific patterns of expression; only Dsc2 is expressed in normal colonic epithelium. We have found switching between desmocollins in sporadic colorectal adenocarcinoma with a reduction in Dsc2 protein (in 8/16 samples analysed by immunohistochemistry) being accompanied by de novo expression of Dsc1 (16/16) and Dsc3 (7/16). Similar results were obtained by western blotting of a further 16 samples. No change was found in Dsc2 mRNA, but de novo expression of Dscs 1 and 3 was accompanied by increased message levels. Loss of Dsc2 (8/19) and de novo expression of Dsc1 (11/19) and Dsc3 (6/19) was also found in colorectal adenocarcinomas on a background of colitis. The data raise the possibility that switching of desmocollins could play an important role in the development of colorectal cancer.

MATERIALS AND METHODS

Immunohistochemistry

Formalin-fixed, paraffin-embedded sections of sporadic colorectal adenocarcinomas and of colorectal adenocarcinomas arising on a background of colitis were obtained from the archives of University Hospital, Birmingham and the Royal Free Hospital, London respectively. Sections were examined by immunohistochemistry using the streptavidin–biotin indirect immunoperoxidase method. Antibodies JCMC against Dsc1 (North et al, 1996), 610120 against Dsc2 (Progen, Heidelberg, Germany) and U114 against Dsc3 (Progen) were used. Immunostained material was assessed by two independent observers and immunoreactivity compared to that of normal colon.

Western blotting

Sporadic tumour samples with matched specimens of histologically normal, large bowel mucosa were collected with patient consent at the time of tumour resection according to local ethical guidelines. Normal mucosa was obtained from a cancer-free patient undergoing a total colectomy. Protein lysates were prepared using TRI Reagent (Molecular Research Center, Cincinnati, OH, USA) and Western blots were probed with antibodies (as above) against Dsc1, Dsc2 and Dsc3. An antibody against keratin 8 (clone C51; Zymed, San Francisco, CA, USA) was used as a loading control for epithelial protein.
RT–PCR
Total RNA was prepared using TRI Reagent and first-strand cDNA synthesis was performed using a kit (Roche, Lewes, Sussex, UK), random primers and total RNA (1 μg). For semi-quantitative RT–PCR, an aliquot (1 μl) of the first-strand cDNA reaction was amplified with primer pairs 1, 2 and 3 (Supplementary Table 1) against Dsc1, Dsc2 and Dsc3, respectively. Normalisation was carried out using primer pair 4 against the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase. For quantitative RT–PCR, an aliquot of the first-strand cDNA reaction was amplified with primer pairs 5, 6 and 7 (Supplementary Table 1). A Sybr Green PCR master mix (Applied Biosystems, Warrington, Cheshire, UK) was used and normalisation was carried out using primer pair 8 against the gene encoding the epithelial protein keratin 8 (Supplementary Table 1).

Southern blotting
DNA was resolved by agarose gel electrophoresis, transferred onto Biodyne B membrane (Pall) and probed using a T4 kinase end-labelled Dsc1-specific oligonucleotide (CCAGTGGTGAAGGCTT AAGGT).

RESULTS

Reduced expression of Dsc2 in sporadic colorectal adenocarcinoma
In normal colonic epithelium Dsc2 showed strong staining at the cell membrane as expected (Figure 1A). A reduction in Dsc2 protein expression was found in eight of 16 sporadic cancer specimens examined (Figure 1B). There was no relationship between Dsc2 expression status and any of the clinicopathological variables analysed (Supplementary Table 2). In tumour samples, Dsc2 showed both a reduction in expression and a relocation from the membrane to the cell cytoplasm (Figure 1B). To confirm that Dsc2 expression is lost in colorectal cancer a further 16 specimens were analysed by Western blotting. Reduced levels of Dsc2 protein were found in 11 out of 16 (Figure 1C).

De novo expression of Dsc1 and Dsc3
Dsc2 is the only desmocollin produced by simple epithelial tissues such as the colon (Nuber et al., 1995). To determine whether loss of Dsc2 was accompanied by de novo expression of Dsc1 and Dsc3, we examined sporadic colorectal cancers (those previously analysed for Dsc2 expression by immunohistochemistry) using antibodies specific for Dscs 1 and 3. As expected, no Dsc1 or Dsc3 expression was detected in normal colon (Figure 2A and B). Surprisingly, Dsc1 expression was detected in all 16 tumour samples examined (Figure 2C). In these samples, staining was found both at the cell membrane and in the cell cytoplasm. Dsc3 exhibited a similar pattern of expression in seven of 16 samples (Figure 2D). Inappropriate expression of Dsc1 and Dsc3 was restricted to morphologically abnormal glands. De novo expression of Dsc1 and Dsc3 was not entirely dependent on loss of Dsc2 as Dsc1 was expressed in all tumour specimens and Dsc3 was detected in three of eight samples that showed normal levels of Dsc2 (Supplementary Table 3). Loss of E-cadherin has been

Figure 1 Reduced expression of Dsc2 in sporadic colorectal cancer: Dsc2 is localised at the cell membrane (brown staining) in normal colonic epithelium (A) but shows reduced immunoreactivity and a relocalisation from the membrane to the cell cytoplasm in colorectal adenocarcinoma (B). (C) Loss of expression of Dsc2 by Western blotting. Note that the antibody used does not detect the smaller Dsc2 ‘b’ protein. N, normal; T, tumour; K8, keratin 8 loading control for epithelial protein. Bar, 50 μm.

Figure 2 De novo expression of Dsc1 and Dsc3 in sporadic colorectal cancer. Dsc1 (A) and Dsc3 (B) are not expressed in normal colonic epithelial tissue, but exhibit de novo expression in colonic adenocarcinoma (Dsc1, C; Dsc3, D). In (C) and (D) staining is present both at the membrane and in the cell cytoplasm. Dsc1 and Dsc3 are not present in normal colonic epithelium (lane 8), but bands of the expected size (corresponding to the Dsc ‘a’ and ‘b’ proteins) were detected by Western blotting in a subset of tumour samples. Samples in lanes 3–5 were adjudged to be positive for Dsc1 (a and b proteins) and Dsc3 (a and b), while that in lane 7 was adjudged positive for Dsc1 (a and b) alone. Bands corresponding in size to Dsc1b and Dsc3b were also detected in the samples in lanes 1 and 7 respectively. The band in lane 6 (lower panel) is nonspecific. Samples in lanes 1 and 3 showed no change in Dsc2, while those in lanes 4–7 showed a reduction in Dsc2 expression (not shown). The sample in lane 2 was not tested for Dsc2. N, normal; T, tumour; Bar, 50 μm.
transcription of Dsc2 downregulated. As before, increases in Dsc1 and Dsc3 mRNA (>2-fold) were detected in a significant number of cases (three of seven and four of seven tumours respectively) (Figure 3B).

A summary of the results from immunohistochemistry, Western blotting and RT–PCR is given in Supplementary Table 4.

**Desmocollin switching occurs in colorectal adenocarcinoma on a background of colitis**

To determine whether desmocollin switching was exclusive to sporadic tumours, we examined expression of Dscs 1–3 in a series of colitis-associated colorectal adenocarcinomas. Analysis was restricted to immunohistochemistry as samples were available only as paraffin-embedded sections. The median age of patients with colitic tumours was less than those with sporadic tumours, but tumour stage, differentiation grade, site and microsatellite instability were comparable between groups (Supplementary Table 2). In tumours on a background of colitis, loss of Dsc2 was observed in eight of 19 specimens examined, and de novo expression of Dsc1 and Dsc3 was detected in 11 of 19 and six of 19 specimens respectively (Supplementary Table 4). E-cadherin expression was lost in 16 of 19 colitic tumours. Thus, in common with previous studies (Aust et al, 2001), we found that E-cadherin more commonly showed decreased expression in colitic rather than sporadic tumours (84% vs 56%). Dsc2 expression was lost in all 16 colitic tumours that showed loss of E-cadherin (data not shown).

**DISCUSSION**

In this report, we show for the first time that Dsc2 protein expression is reduced in colorectal cancer, and that this is accompanied by de novo expression of Dsc1 and Dsc3. These changes in Dsc expression pattern may result in significant alterations in desmosome function, but do not result in the complete loss of desmosomes from colorectal cancer tissue (Collins et al, 1990). However, it is possible that the size and number of desmosomes is reduced (Bosch et al, 2005). To date, only switching between classical cadherin molecules has been described (e.g. Tomita et al, 2000; Hardy et al, 2002). The functional significance of switching between desmosomal cadherins remains unknown, but will be an important area of future investigation.

In no case was loss of Dsc2 message detected, suggesting that transcriptional mechanisms are not responsible for the loss of Dsc2 protein. The most plausible explanation for the reduction in Dsc2 protein is loss of stability. One possibility is that this could be caused by a loss of cell–cell contact, perhaps as a result of loss of expression of E-cadherin, as desmocollins are rapidly degraded in the absence of intercellular contacts (Penn et al, 1987). However, it should be noted that in some cases it appears that Dsc2 expression is lost in the presence of normal amounts of E-cadherin (Supplementary Table 3). No reduction in desmocollin expression was found in the only previous study of desmosomes in colorectal cancer (Collins et al, 1990). The most likely explanation for the discrepancy lies in the antibodies used. We have been careful to use antibodies specific for individual Dsc isoforms. Collins et al (1990) carried out their study before all of the desmocollin isoforms had been discovered and their antibody may have reacted with more than one isoform.

Increased amounts of message encoding both Dsc1 and Dsc3 were detected in cancer specimens. The mechanism by which this occurs is not clear. All seven DC genes are clustered in the same region of chromosome 18q (Hunt et al, 1999), and their expression may be linked (North et al, 1996; King et al, 1997). Induction of Dsc1 and Dsc3 RNA could be a compensatory response to the loss of Dsc2 protein. In a similar way, loss of Dsc1 protein from the

RNA expression profile

Semiquantitative RT–PCR was used to determine whether loss of Dsc2 protein was accompanied by loss of RNA. Four pairs of matched samples, not previously examined, were used. Transcription of Dsc2 was not altered in any of the four tumours (Figure 3A). However, both Dsc1 (3/4) and Dsc3 (3/4) mRNA was clearly upregulated. To quantify changes in desmocollin expression, we examined a further seven samples by quantitative RT–PCR (Figure 3B). Similar results were found to those obtained using semiquantitative RT–PCR. Thus, although an increase in Dsc2 message was clearly upregulated. To quantify changes in desmocollins (i.e. there was ~2-fold difference in expression levels between tumour samples and matched controls) (Figure 3B). In no case was...
epidermis of knockout mice results in large (>-20-fold) increases in Dsc2 RNA (M Chidgey, unpublished). The mechanism whereby an increase in Dsc1 and Dsc3 message levels could result from a loss of Dsc2 protein (while Dsc2 mRNA remains unchanged) remains to be determined.

The changes in desmoscin expression profile were similar in both sporadic and colitis-associated colorectal cancer. These changes could significantly reduce the adhesion between colonic epithelial cells resulting in an increased propensity of the cells to proliferate, invade surrounding tissues and undergo metastasis. Switching of desmoscin isofoms could also affect the localisation of cytoplasmic desmosomal constituents, and indeed a redistribution of γ-catenin (plakoglobin) from the membrane to the cytoplasm does occur in both sporadic adenocarcinoma (Kolligs et al, 2000) and adenocarcinoma arising on a background of colitis (our unpublished data). The effects of this redistribution are unknown, but one downstream consequence of γ-catenin signalling is strong activation of the oncogene c-myc (Kolligs et al, 2000). It is also possible that disturbances in desmoscin expression profile could affect β-catenin signalling. Increased β-catenin transcripational activity has been detected in both Dsc1 null mice (Merritt et al, submitted), and in transgenic mice that exhibit disturbances in the normal balance between desmoscin isofoms in the epidermis (Hardman et al, 2005). Increased β-catenin signalling (as a result of APC mutation) is a common causative event in colorectal cancer, and it is conceivable that DC switching could contribute to β-catenin dysregulation and so play a contributory role in the initiation of the early stages of colorectal tumorigenesis.

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