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Quantitative assessment of normal and potentially premalignant epithelium at different levels of human colorectal crypts

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Summary. The present study uses morphometric techniques to assess whether altered differentiation patterns exist in PPM which might reflect its pre-malignant status. Samples were obtained from resected malignant lesions of large bowels of 10 Chinese patients. Normal (N) samples were biopsied from the margins of each resected large bowel. Potentially premalignant (PPM) mucosae were obtained from within 2 cm of the margins of the malignant lesions. Tissues were processed for histological examination and using strict criteria, colorectal crypts were divided into basal (B), intermediate (I) and surface (S) segments. Interactive digitisation of sections from each group was used to generate the following morphometric parameters in each segment: nuclear profile circularity indices (NSF and NCI); nuclear numerical density (N_A and N_V); the degree of deviation of the major nuclear axis in relation to the epithelial-connective junction (AGDMAX); cell height (CH); the distance between nuclear apex to cell apex (DNACA); the distance between cell base to nuclear apex (DCBNA); stratification index (SI) - the ratio of DCBNA and CH; and the volume density of mucous vacuoles in the reference epithelium ($V_{VMV,EP}$). In comparisons of different segments within groups, the nuclei at the S segment of N and PPM crypts were more irregular and less circular in shape than nuclei from other segments. There was a shift of nuclear profile shape (NSF and NCI) from circular to ellipsiodal between B and S segments. In comparisons of similar segments between groups, no significant nuclear shape changes were detected in nuclei of PPM crypts when compared with nuclei in similar segments of N crypts and the pattern of nuclear shape alterations resembled those of normal crypts. In comparisons of different segments within groups of N and PPM crypts, AGDMAX. DNACA, DCBNA, CH and SI parameters demonstrated that epithelial cells at the I segments have more centrally positioned nuclei with the tallest epithelial height when compared with epithelial cells in other segments of both crypts. In B segments, nuclear N_A and N_V were almost double those of other segments in both N and PPM crypts, with marked reductions in these parameters between B and I segments. $V_{VMV,EP}$ was significantly highest in the I segment and significantly lowest in the S segment of both groups. Both N and PPM crypts showed similar trends in $V_{VMV,EP}$ within the crypt segments but when comparing similar segments between both crypts, a significant difference was detected only between S segments. The alterations of nuclear shape and packing densities, orientation and mucous content in N crypts were similarly expressed in PPM crypts and distinct differences in numerical density (N_V) and stratification index existed in crypts between these two groups when comparing similar segments. All values in PPM were consistently lower when compared with N crypts. These preliminary observations may represent a subtly altered state of cellular differentiation in PPM which may be a reflection of early preneoplastic transformation.

Key words: Human, Colorectum, Morphometry, Differentiation, Precancer, Crypts

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Introduction

The premalignant nature of "Transitional Mucosa" or "TM" (Williams, 1985; Boland and Kim, 1987) remains unresolved. To avoid confusion with histological terms used to describe the mucosa of the urinary tract, we prefer to use the term "Potentially Premalignant Mucosa" or "PPM". There are a number of morphological and histochemical alterations in PPM (Shamssudin and Trump, 1981; Filipe, 1984) which have lent support to its premalignant nature but other investigators have refuted their exclusivity and they have been reported in mucosae accompanying other lesions of the large bowel (Franzin et al., 1981; Listinsky and Riddell, 1981; Sawady et al., 1991).

Morphometry has been utilised as an objective tool in evaluating the structural changes of nuclei and cells

including their subcellular components during differentiation and maturation in the various strata of normal rodent (Gohari and White, 1984; Meyer et al., 1970; White and Gohari, 1982) and human stratified epithelia (Schroeder and Münzel-Pedrazzoli, 1970; Meyer and Schroeder, 1975; Rowden, 1975; Klein-Szanto et al., 1976; Landay and Schroeder, 1977).

A similar approach has been adopted in simple glandular epithelium of human colorectum. However, published stereological studies have been limited (Nakamura et al., 1974; Tipoe et al., 1992; Tipoe and White, 1996) and have not adequately assessed differentiation and maturation along the length of colorectal crypts. Cell kinetic, autoradiographic research and electron microscopical observations into the colorectal epithelium has identified four cell types in the crypt originating from stem cells which are located in the basal part of the crypt (Cheng and Leblond, 1974; Altmann, 1990). Active cellular proliferation occurs in the lower third of the crypt (Lipkin et al., 1963; Leblond, 1981) and the maturation process extends towards the large bowel lumen in both man (Cole and McKalen, 1961) and mouse (Tsubouchi, 1981). Structural differences which reflect cellular differentiation and maturation might thus exist at different crypt levels.

The aims of the present study are to compare nuclear and cellular epithelial alterations at defined levels of colorectal crypts between normal (N) and potentially premalignant mucosa (PPM) using morphometric and stereological techniques with the objective of determining whether structural alterations occur in PPM which might be indicative of its premalignant status.

Materials and methods

I. Sample collection

Specimens were obtained from tumour-bearing resected large bowels from Chinese patients. Each resected bowel was incised along its length immediately, washed thoroughly and samples of tissue which appeared grossly normal (N) were obtained not less than 5 cm from the proximal resection margins of the malignant lesions. Samples of potentially premalignant (PPM) mucosa were removed from within 2 cm of the edges of the malignant lesions, avoiding necrotic areas. Material from a total of ten patients was obtained for histological processing. Colorectal sample distribution is summarised in Table 1.

Table 1. Distribution of specimens from the colorectum.

SITE	RECTUM	SIGMOID	CAECUM	ASCENDING COLON	DESCENDING COLON
No.	3	3	2	1	1

Mean age: 65.10±12.80; male: 4; female: 6

II. Histological processing

All tissue samples were routinely processed for histology under strictly standardised schedules. Samples were immersion fixed in 10% neutral buffered formalin for 18 to 20 hrs, washed with running tap water and dehydrated with increasing grades of alcohol. They were cleared in chloroform, infiltrated and embedded with paraffin wax.

Serial 4 μm sections separated by 20 μm were cut using a Reichert-Jung 1130/Biocut microtome and several sections were selected randomly for haematoxylin and eosin (H&E) staining. Two sections per block were used in the final sampling procedure (Table 2).

III. Morphometric sampling and measurements

Each glandular crypt of N and PPM was divided into (B) basal, (I) intermediate and (S) surface segments (Fig. 1). Epithelial cells were classified as either goblet or non-goblet cells. No further attempt was made to classify the non-goblet cells as absorptive and endocrine cells because of the identification difficulties imposed by using H&E stained sections. The basal segment (B) was defined superiorly by the lowest part of the crypt lumen and inferiorly by the lowest region of the epithelial cells nearest to the muscularis mucosae. The surface segment (S) contained those epithelial cells lining the colorectal lumen and which lay superficial to the most luminal aspect of the lamina propria. The intermediate segment (I) represented the region between B and S crypt segments. A multi-stage stratified systematic random sampling procedure (Mayhew and Gundersen, 1996) was adopted (Table 2). Initially, the first longitudinally cut crypt on the left hand side of the section was chosen. The actual measuring procedure commenced by choosing the first field from the S segment and then the section was moved to the next alternate field towards the basal part of the crypt. This was repeated into the next consecutive longitudinally sectioned crypt until a total number of 180 cells had been counted for the three segments, comprising 60 epithelial cells per crypt. The

Table 2. Sampling procedures.

GROUPS	No. PATIENTS	No. BLOCKS/ PATIENT	No. SECTIONS/ PATIENT	SEGMENTS/ BLOCK	No. FIELDS/ SEGMENT	CELLS/ SEGMENT	TOTAL No. CELLS
N	10	1	2	3	6	60	1800
TM	10	1	2	3	6	60	1800

total number of fields per section was 18. A total of 180 epithelial cells from 2 sections was obtained per patient representing the 3 segments of the crypt and thus a total of 1800 epithelial cells were quantified from a total of 10 patients..

Interactive morphometric measurements were made using a Zeiss VIDAS image analyser linked to a dedicated Zeiss microscope (Universal 3 Model) with an oil immersion objective to which was attached a black and white camera (JVC TK5310). The final magnification of x2,165 was the optimal magnification which provided a large sample area with unambiguously identifiable nuclear and cellular profiles. Primary parameters were obtained by tracing component profiles and in some cases, these were used to derive secondary parameters (Table 3). Primary parameters comprised AGMAX, the maximum angle between the major axis (a) and the basement membrane. Similarly, the areas of the epithelial reference (A_{EP}) and mucous vacuoles (AMV) were digitised. The distances between the apical part of the nucleus to the apical plasma membrane (DNACA), between basement membrane to apical part of the nucleus (DCBNA) and between the apical plasma membrane to the basement membrane (CH) were also measured. Mean values of all morphometric parameters were obtained immediately after completing measurements for each patient. The minimal sample size of each morphometric parameter for individual patients was calculated at ±5% error using the progressive mean plot analysis (Williams, 1977).

A) Volume density of mucous vacuoles in the epithelium

The volume density (V_V) of mucous vacuoles (MV) in the epithelium (EP) was determined by the ratio of the sum of the areas of the mucous vacuoles (A_{MV}) contained in the defined epithelial area (A_{EP}) and the sum of the areas of the reference epithelium and was calculated by

$$V_{VMV,\,EP} = \frac{\Sigma A_{MV}}{\Sigma A_{EP}}$$

B) Nuclear stratification index and orientation

The nuclear stratification index (SI) was derived from ratios of linear measurements of the nucleus in relation to its apical and basal membranes (DNACA, DCBNA and CH). SI (Eide, 1986) was determined by the ratio of DCBNA to CH and was computed by

$$SI = \frac{DCBNA}{CH}$$

Nuclear orientation (AGDMAX) was defined as the relationship between the maximum angle of the nucleus with respect to the epithelial-connective tissue junction. In this case, all measurements were performed in such a way that the epithelial-connective tissue junction of the cell was orientated parallel to the X axis of the digitising pad.

C) Nuclear profile circularity indices

The nuclear circularity index (NCI) was obtained by dividing the nuclear profle perimeter (PERIM) of an equivalent circle derived from the measurement of nuclear area which was given by $2 \times (A_N \times \pi)^{1/2}$ by the actual measured nuclear perimeter i.e.

$$NCI = \frac{2\sqrt{A_N \cdot \pi}}{PERIM}$$

The nuclear profile shape factor (NSF) was derived from the following relationship:

$$NSF = \frac{4 \cdot \pi \cdot A_N}{(PERIM)^2}$$

Both parameters are indicators of the degree of deviation of a nuclear profile from its equivalent circle.

D) Numerical Density

The nuclear numerical density per unit area of epithelial segment (NA, no./μm^2), was determined by

$$N_A = \frac{N_P}{A_{EP}}$$

N_P is the total number of nuclear profiles per epithelial segment and A_{EP} is the mean area of the epithelial segment of reference. N_V (no./μm^3), the numerical density per unit volume of epithelial segment, was determined by

$$N_V = \frac{N_A}{D + t}$$

This formula is a variant of that of Dehoff and Rhines (1961) after Abercrombie (1946), where N_A is the number of nuclear profiles per unit area observed in sections of thickness t and D is the mean corrected nuclear diameter. Our preliminary estimates of D were between 6.0 μm and 7.5 μm. Section thickness effects are not corrected if D>12t. Since t was assumed to be 4 μm, the calculation for NV has to take into account the "Holmes effect" to avoid overestimation of numerical density (Holmes, 1921).

Table 3. Morphometric and steorological parameters.

PRIMARY PARAMETERS	SECONDARY PARAMETERS
PERIM*	NCI, NSF
DNACA, DCBNA	N_A, N_V
CH, AGDMAX	$V_{VMV,\,EP}$
A_{MV}, A_{EP}	SI

*: data published in Tipoe and White (1996).

IV. Statistical analyses

Data from each patient used to calculate each morphometric parameter were pooled to obtain a single value representing each patient and then values were pooled to obtain a single mean and standard error of the mean for each group. The distribution of the data was tested by chi-square goodness of fit. If the data were not normally distributed, logarithmic transformation was performed in order to make the data suitable for statistical analysis. Two-way and one-way ANOVAs followed by a Duncan's multiple range test (Duncan, 1955) were performed using the SPSS PC software package.

Results

I. Qualitative results

The detailed morphological appearances of normal and potentially premalignant mucosa of the human colorectum are found in our other published work (Tipoe et al., 1992; Tipoe and White, 1996). Normal colorectal epithelium comprised simple tubular glandular crypts when sectioned along their long axes. Each crypt possessed two crypt columns of simple epithelium with a lumen between them (Fig. 2). Cells from the basal segment appeared low columnar in shape and their nuclear profiles were irregular and contained relatively low amounts of cytoplasm. The frequency of mucous cells appeared low in this segment. The epithelial cells

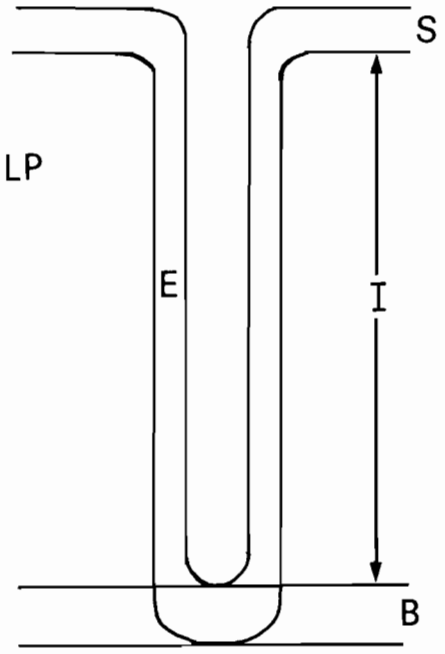
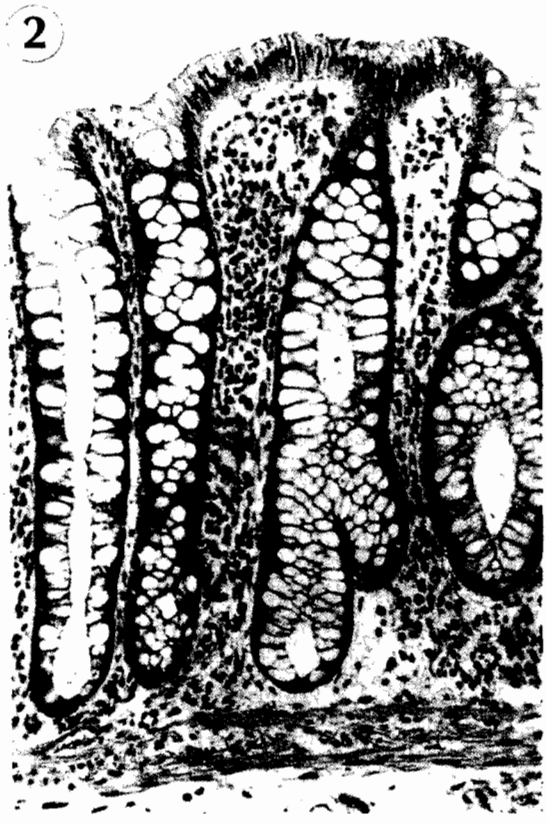


Fig. 1. A diagram of colorectal epithelium showing the different segments of the crypt. The simple colorectal gland is surrounded by loose connective tissue lamina propria (LP). S: surface segment; I: intermediate segment; B: basal segment. E: epithelium.



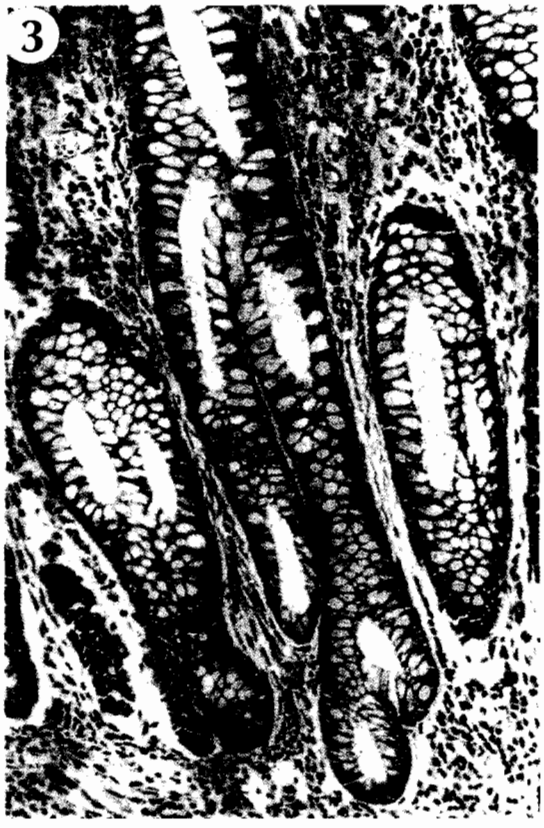


Fig. 2. Normal colorectal epithelium showing branched and unbranched crypts. x 650

Fig. 3. Potentially premalignant epithelium showing elongated and branched crypts. x 650

of the I segment were mostly all columnar in shape and mucous cells was more numerous when compared with the B segment. The majority of the nuclei were elliptical in profile, with their long axes perpendicular to the basement membrane zone, and were basally located.

The epithelial cells located at the S segment retained their columnar shape but appeared more slender and taller and mucous vacuolation was reduced significantly when compared with epithelial cells at the I segment. Surface epithelial nuclei were more regular and ellipsoidal than intermediate nuclei and were located approximately centrally within the cells. The lamina propria supporting the colorectal glands was composed of loose connective tissue with a moderate number of infiltrating lymphocytes, plasma cells and occasional neutrophils and eosinophils. The pericryptal region immediately beneath the basement membane zone contained cells with elongated nuclei which appeared to be fibroblasts.

PPM crypts were similar in their morphology to the normal epithelium except for the presence of elongated and branched tubular glands (Fig. 3). Five out of ten PPM lesions showed glandular hyperplasia with elongated crypts and four of these were minimally branched. The others showed normal morphology without either glandular hyperplasia or branching. PPM crypts were lined with an epithelial cell monolayer composed of goblet and columnar cells. The epithelial cells appeared crowded in the elongated crypts. Nuclear morphology was similar to that described in the normal group. The concentration of goblet cells appeared highest in the I segment and lowest in the S segment and appeared considerably increased in frequency when compared with normal crypts.

Infiltrating leucocytes and blood vessels were present in the connective tissue stroma in frequencies comparable to those observed in normal mucosa.

II. Quantitative results

Quantitative data and statistical analyses are summarised in Tables 4, 5 and Tables 6-9 respectively. Generally, the majority of the morphometric parameters had higher values in N crypts when compared with PPM crypts. Comparisons between the conditions (N and PPM) were statistically significant for all morphometric parameters except NSF , NCI, AGDMAX, CH and $V_{VMV,EP}$ but the positions of the crypt were statistically significant for all parameters. However, no significant interactions were detected for any morphometric parameters by two-way ANOVA (Table 6).

The nuclear circularity index (NSF) decreased progressively between B and S segments by 17% and 20% respectively in N and PPM groups. Statistically significant differences were observed between B and S segments and between I and S segments in N but were significantly different between all crypt segments in PPM. Comparisons of NSF with similar segments of the crypts showed significant differences only between B segments. The nuclear circularity index (NCI) also decreased progressively between B and S segments by 9% and 11% respectively in N and PPM. Statistically significant differences were observed only between B and S segments in N but between all crypt segments in PPM. Comparisons of NCI with similar segments of the crypts in N and PPM groups failed to reveal significant differences between all crypt levels.

The nuclear orientation as determined by AGDMAX increased progressively between B and S segments by 11% and 16% in N and PPM respectively. Significant

Table 4. Nuclear and cellular morphometric parameters of N crypts.

SEGMENTS	PARAMETERS									
	NSF*	NCI*	AGDMAX* (degrees)	NA* (μm^{-2}) x 10^{-3}	NV* (μm^{-3}) x 10^{-3}	DNACA (μm)	DCBNA (μm)	CH (μm)	SI	$V_{vMV, EP}$
Basal	0.664±0.012	0.818±0.009	75.14±3.07	16.69±0.73	1.62±0.08	12.54±0.86	6.70±0.38	19.12±0.94	0.354±0.180	0.300±0.030
Intermediate	0.616±0.021	0.776±0.012	81.97±4.22	7.72±0.24	0.73±0.02	19.34±0.76	7.83±0.35	26.85±0.81	0.293±0.012	0.524±0.026
Surface	0.554±0.017	0.743±0.022	83.51±1.36	7.55±0.32	0.67±0.03	15.56±0.60	25.27±0.76	41.06±0.78	0.616±0.014	0.085±0.008

* Data published in J. Anat (1992) 181, pp 189-197; ± : Standard Error of Mean.

Table 5. Nuclear and cellular morphometric parameters of PPM crypts.

SEGMENTS	PARAMETERS									
	NSF*	NCI	AGDMAX (degrees)	NA (μm^{-2}) x 10^{-3}	NV (μm^{-3}) x 10^{-3}	DNACA (μm)	DCBNA (μm)	CH (μm)	SI	$V_{vMV, EP}$
Basal	0.669±0.012	0.817±0.007	76.73±5.34	20.48±1.50	2.26±0.19	15.12±1.15	6.16±0.29	21.26±1.24	0.296±0.016	0.264±0.032
Intermediate	0.608±0.007	0.775±0.005	83.66±4.76	8.69±0.56	0.92±0.07	21.41±0.93	7.15±0.31	28.44±1.12	0.252±0.008	0.535±0.019
Surface	0.532±0.025	0.724±0.019	89.36±2.13	8.37±0.46	0.82±0.06	18.28±0.97	21.50±0.73	40.72±1.35	0.529±0.012	0.129±0.015

±: Standard Error of Mean.

differences were detected only between B and S segments in both groups. Comparisons of similar segments of the crypts showed no significant differences in any of the crypt levels.

The numerical densities, N_A and N_V, decreased progressively between B and S segments of the crypts in N and PPM. In N, values of N_A were lower by 23%, 13% and 11% and of NV by 40%, 26% and 22% when compared with PPM group in B, I and S segments of the crypts respectively. N_A and N_V showed significant differences between B and I segments and between B and S segments in both groups. When comparing N_A with similar segments of the crypts between N and PPM, significant differences were detected only in the B segments whereas N_V was significantly different in comparisons between all crypt segments.

Table 6. Results of statistical analysis of N and PPM crypts.

PARAMETERS	CONDITIONS D.F. (1,59) F ratio	Probability	POSTIONS D.F. (2,59) F ratio	Probability	INTERACTION D.F. (2,59) F ratio	Probability
NSF	0.35	NS	27.65	p<0.001	0.33	NS
NCI	0.38	NS	18.05	p<0.001	0.28	NS
AGDMAX	0.98	NS	4.03	p<0.05	0.21	NS
N_A	8.99	P<0.01	127.70	P<0.001	2.43	NS
N_V	24.77	P<0.001	89.65	P<0.001	5.69	NS
DNACA	11.33	P<0.002	26.80	P>0.001	0.08	NS
DCBNA	16.05	P<0.001	697.33	P<0.001	6.47	NS
CH	1.68	NS	194.91	P<0.001	0.75	NS
SI	29.78	P<0.001	268.27	P<0.001	1.43	NS
$V_{VMV,EP}$	0.86	NS	78.94	P<0.001	1.35	NS

Two-way ANOVA; NS: Not Statistically Significant.

Table 7. Results of statistical analysis of N crypts.

SEGMENTS	PARAMETERS									
	NSF	NCI	AGDMAX	N_A	N_V	DNACA	DCBNA	CH	SI	$V_{VMV, EP}$
Basal	*	*	*	*	*	*	*	*	*	*
Intermediate	*	* *	* *	*	*	*	*	*	*	*
Surface	*	*	*	*	*	*	*	*	*	*

One-way ANOVA followed by Duncan Multiple Range Test; overlap of (*) down column indicates that the group comparisons are not statistically significant at 95% confidence interval.

Table 8. Results of statistical analysis of PPM crypts.

SEGMENTS	PARAMETERS									
	NSF	NCI	AGDMAX	N_A	N_V	DNACA	DCBNA	CH	SI	$V_{VMV, EP}$
Basal	*	*	*	*	*	*	*	*	*	*
Intermediate	*	*	* *	*	*	*	*	*	*	*
Surface	*	*	*	*	*	*	*	*	*	*

One-way ANOVA followed by Duncan Multiple Range Test; overlap of (*) down column indicates that the group comparisons are not statistically significant at 95% confidence interval.

Table 9. Results of statistical analysis of N and PPM crypts.

SEGMENTS	PARAMETERS									
	NSF	NCI	AGDMAX	N_A	N_V	DNACA	DCBNA	CH	SI	$V_{VMV, EP}$
B vs B	*	ns	ns	*	*	ns	ns	ns	*	ns
I vs I	ns	ns	ns	ns	*	ns	ns	ns	*	ns
S vs S	ns	ns	ns	ns	*	*	*	ns	*	*

Duncan multiple range tes for comparison between similar crypt segments; *: statistically significant; ns: not statistically significant.

DNACA increased between B and S segments with the highest values being found in the I segments in N and PPM. DNACA in the I segments increased by 54% in N and by 42% in PPM. Moreover, there was a progressive increase in the values of DCBNA and CH between B and S segments in both groups. DNACA, DCBNA and CH showed statistically significant differences between all crypt segments in N and PPM groups. Comparisons of DNACA and DCBNA with similar crypts segments showed a significant difference only between S segments. When comparing CH with similar segments of the crypts between N and PPM groups, no significant differences were observed in any of the crypt levels. SI increased between B and S segments in N and PPM groups by 74% and 79% respectively, with the lowest values being in the I segments. SI was significantly different between all segments in N and PPM groups. When comparing similar segments of the crypts between N and PPM, significant differences were detected between all crypt levels.

$V_{VMV,EP}$ was markedly reduced between B and S segments by 72% and 51% in both N and PPM groups respectively, with the highest value being in the I segment. $V_{VMV,EP}$ showed substantial increases of 73% in N and 108% in PPM between B and I segments. Significant differences were detected between all segments in both groups. Comparisons with similar crypt segments between N and PPM showed a significant difference only between S segments.

Discussion

The premalignant status of lesions which we have termed PPM is still unknown, although histochemical and morphological alterations have been documented in PPM (Filipe, 1984; Boland and Kim, 1987). These may be non-specific (Isaacson and Attwood, 1979) or reactive changes to the presence of malignant lesions (Sawady et al., 1991) and additional convincing data are required to substantiate these alterations as primary premalignant changes. This study used morphometric methods to establish whether structural variations exist within the differentiating and maturing epithelial cells of PPM crypts and to compare them with those of N crypts. No comparative quantitative studies have yet documented epithelial cells variations during differentiation and maturation between normal and PPM colorectal crypts.

Based on the quantitative data of NSF and NCI, nuclear profiles at the B segments of N and PPM crypts were more circular than those at the S segments of the crypts. The progressive increase of the a/b ratio and **a** of the nucleus (Tipoe and White, 1996) between B and S segments in N and PPM crypts as well as doubling in the values of **a** compared to **b** provided more evidence that nuclei at the S segments were more ellipsiodal than B segment nuclei. Considering nuclear PERIM in conjunction with NSF, NCI and a/b ratio, the nuclei at the S segment of N and PPM crypts were more irregular, less circular in shape with longer boundaries than nuclei located at the B and I segments. The nuclear volumetric variations and the shift of nuclear profile shape from circular to ellipsiodal between B and S segments might relate to an increase in functional and metabolic requirements of cells at the B and I segments of the N and PPM crypts, which were probably more active in cell division and differentiation. As documented by autoradiographic, cell kinetic and cell proliferation marker (Ki67) studies (Cole and McKalen, 1961; Chang and Leblond, 1971; Tsubouchi, 1981; Wright and Alison, 1984; Sahin et al., 1994), epithelial cells at the S segments showed diminishing cell division and activity and were destined to be exfoliated. The changes occurring in nuclear shape might also be involved in the active formation and arrangement of chromatins within the nucleus at a given stage of the cell cycle (Kamel et al., 1990).

Although there were significant differences of NSF and NCI between some crypt segments in N and between all crypt segments in PPM, generally no significant differences were detected between similar segments in either groups. This suggests that no significant nuclear profile shape changes occurred in the nuclei of PPM crypts when compared with nuclei in similar segments of N crypts and that the pattern of nuclear profile shape alterations during cellular differentiation and maturation in the defined levels of the crypt resembled those of normal crypts.

One of the features of cellular atypia is the loss of nuclear polarity or orientation (Underwood, 1990). The morphometric parameters AGDMAX, DNACA, DCBNA and SI were used to characterise the shift in the position of the nucleus within the cell. AGDMAX provided information about the change of nuclear position in relation to the horizontal plane within the cell whereas DNACA, DCBNA, CH and SI parameters are related to shifts of nuclear position in relation to the vertical axis of the cell. Eide (1986) has shown that SI and nuclear size in colorectal adenomas were significantly higher than the in normal mucosa. In his study, the values of DCBNA, CH and SI were comparable with the values of similar parameters in the S segment of N and PPM groups of our study. However, the aggregated values of these parameters for the three crypt levels were lower in the present study when compared with Eidés work. AGDMAX only altered significantly during cellular differentiation and migration along the N and PPM crypts between B and S segments. The nuclei at the S segments of both crypts were significantly more perpendicular in position in relation to the epithelial-connective tissue junction (ECJ) when compared with nuclei at the B segments. Values of AGDMAX, DNACA, DCBNA, CH and SI parameters demonstrated that epithelial cells from S segments have more centrally positioned nuclei with the tallest epithelial height when compared with the epithelial cells in any of the segments in the N and PPM crypts. This

displacement of the nuclei towards the ECJ was probably caused by the accumulation of mucous vacuoles in the apical part of the cells present within the I segments which comprised predominantly goblet cells as also qualitatively described by Shamsudin et al. (1982). Values of SI and its associated parameters were probably a reflection of the presence of goblet cells rather than of other types of cell within the crypts. Likewise the secretion of mucous from the goblet cells at the S segments of the crypts into the lumen of the large bowel might allow more space within the cell, thus permitting the more central positioning of the nuclei. Such observations were consistent with the qualitative descriptions made on histological photomicrographs. SI was significantly different between all crypts when making comparisons between similar segments in N and PPM crypts which implied that SI might be a useful objective descriptor of altered nuclear orientation or polarity in N and PPM crypts. Loss of nuclear orientation is one of the main criteria used for grading adenomas (Kozuka, 1975; Gabbert and Höhn, 1980). The present work documented distinct variations in nuclear position in B and S segments of N and PPM crypts during cellular differentiaton and maturation as well as between similar segments of both crypts.

Numerical densities N_A and N_V were almost two times higher in the B segments when compared with other segments in N and PPM crypts. There were marked reductions in the numerical density between B and I segments. These observations conform to several existing studies (Boland et al., 1991; Sato and Ahnen, 1992; Sahin et al., 1994) which describe active cellular proliferation occurring at the basal or lower third of normal colorectal crypt. Our previous study had shown that the average number of epithelial cells per crypt (N_{CELL}) in PPM crypts were 86% higher when compared with this parameter in N crypts, indicating that hyperplasia exists in PPM crypts (White et al., 1992). Although N_A and N_V refer to the nuclear profiles per unit reference area or volume and not the actual number of epithelial cells, we believe that such an assumption is acceptable since we need to use a simple and rapid method to quantify density which can be used in routine histopathological diagnosis. The disector method is now being employed by us in order to compare the numerical density (N_V) obtained by model-based and by unbiased methods (Mayhew and Gundersen, 1996). In the present study, N_V was used after correction of the "Holmes effect", i.e. the overprojection related to section thickness t and mean corrected diameter D (Williams, 1977).

$V_{VM,EP}$ was significantly highest in the I segment and significantly lowest in the S segment in both N and PPM crypts. This finding is consistent with the qualitative descriptions in histological sections where the highest proportion of goblet cells was predominantly in the I segment where they were actively producing and secreting mucus into the lumen. The marked reduction in $V_{VMV,EP}$ at the S segment would be compatible with the reduced functional and metabolic status of the epithelial cells, the majority of which are in the exfoliative or senescent stage. Similar findings were also reported in qualitative studies (Donellan, 1965; Lorenzsonn and Trier, 1968). Both N and PPM crypts showed similar trends in $V_{VMV,EP}$ within the crypt segments but when comparing similar segments between both crypts, significant differences were only detected between S segments. This quantitative alteration may reflect early preneoplastic transformation of PPM in terms of the quality of mucin secretion as shown by Hertzog et al. (1991) who detected a high rate of inappropriate small intestine mucin antigen (SIMA) expression in the perineoplastic transitional mucosa and adjacent morphologically normal mucosa of the colorectum, indicating biochemical changes similar to colorectal cancer. SIMA is an oncofoetal antigen for the colon and is distinct from the normal large intestinal mucin antigen. Recently, Yang and Shamsuddin (1996) documented the expression of a disaccharide tumour marker Gal-GalNAc visualised by galactose oxidase Schiff sequence in mucus of colon cancer cells in experimental carcinogenesis. They also found a high proportion of human precancerous lesions and a higher percentage of colon cancers expressed this marker but this marker is no expressed in normal human large intestines. Fifty percent of the "Transitional Mucosa" or "PPM" showed a positive reaction.

In conjunction with our previous study (Tipoe and White, 1996), preliminary conclusions can be extrapolated based on our quantitative findings in N and PPM crypts. Firstly, some distinct nuclear and cellular volumetric variations exist in the defined levels of the N and PPM crypts. Our data suggest that most morphometric alterations occur between B and I segments, probably reflecting the increased functional and metabolic activity of nuclear and cellular components. Fewer changes were detected between I and S segments, suggesting that the epithelial cells at the S segment reach a state of morphological stability as they prepare for exfoliation. Although the I segment is heterogeneous in composition and included some mitotic cells (Potten et al., 1992). Secondly, the trends and patterns of changes in N crypts were similarly expressed in PPM crypts and distinct differences existed within the crypts and when comparing similar segments between these two groups. All values in PPM were consistently lower when compared with the N crypts. These initial observations may represent a subtle altered state of cellular differentiation in PPM and may be a reflection of early preneoplastic transformation. A recent study by Baytner et al. (1993) showed that mutant p53 protein product was basally located crypt stem cells nucleus in transitional mucosa adjacent to a human colon cancer using a monoclonal antibody AB3. No expression of mutant p53 nuclear protein was noted in mucosa 3-5 cm distal to the neoplasm and such findings support the contention of that a malignant field change exists in TM.

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