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Blood vessel morphometry in human colorectal lesions

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Summary. Neovascularisation in tumours of different cell origins has been well documented qualitatively. In this report, we have assessed vascular architecture in different pathological lesions of the colorectum by quantifying blood vessel parameters in order to detect subtle morphological changes using objective methods.

Colorectal tissue samples were obtained from resected large bowels containing malignant tumours. Biopsies were taken from defined sites in the resected specimen and were classified as normal (N), potentially premalignant mucosa (PPM), adenomatous polyp (P) and adenocarcinoma (ADCA). All tissues were fixed in modified Karnovsky's fixative for 4 hrs and postfixed in 1% OsO_4 for 1 hr. Samples were processed for EM under standardized procedures and embedded in Epon. 0.5 µm semithin sections from five patients per group were stained with toluidine blue. A multi-stage systematic sampling procedure was adopted. The inner outlines of all blood vessels in the lamina propria (LP) were digitised using a Zeiss VIDAS Image Analyzer at a final magnification of x1,050. The area of the reference (LP) was also measured. No attempt was made to distinguish between the different types of vessel. The morphometric blood vessels parameters quantified were volume density (V_v), numerical density (N_A), length density (L_V) and mean transverse sectional area (A).

Statistically significant differences in Vv and A were detected between all groups except between N and PPM and between P and ADCA. No significant differences in N_A and L_V were present in any group comparisons. The mean values of all parameters were the highest in ADCA. Our results suggest that vasodilatation occurred in order to provide an increased supply of nutrients to support active growth and division of the transforming cells. Such vasodilatation might also reflect the inflammatory response to the presence of actively growing malignant cells since activated immune cells are able to release vasoactive substances.

Key words: Angiogenesis, Morphometry, Human, Blood vessels, Colorectum, Cancer

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Introduction

Neovascularisation or angiogenesis is the process whereby new capillary proliferation occurs. It occurs in the host stroma and new blood vessels migrate towards the tumour cell mass. In vivo and in vitro experiments have documented that new capillary formation from the host tissue is necessary for tumours to sustain continuous growth (Folkman, 1974; Gullino, 1978). Experimental evidence has suggested the presence of a humoral mediator which activates angiogenesis in the host tissue in solid tumours. Tumors separated from a host stroma by Millipore filters induce angiogenesis (Warren et al., 1972) and the active component, known as "Tumour Angiogenesis Factor" or "TAF", has been purified and induces angiogenesis (Folkman, 1990). Furthermore, work done by Gullino's group indicated that acquisition of angiogenic properties may be an early marker for preneoplastic cells (Gullino, 1977; Brem et al., 1978). Cartilage and aorta contain inhibitors that neutralise the angiogenic effect of TAF in vivo and significantly inhibited the growth of vascular endothelial cells in vitro (Brem and Folkman, 1975; Kuettner et al., 1977). Subsequently, more angiostatic factors have been discovered, including combinations of heparin and corticosteroids (Choay et al., 1983; Crum and Folkman, 1984) and platelet factor 4 (Sharpe et al., 1990; Maione et al., 1991).

Neovascularisation in a variety of tumours of different cellular origins has been well documented qualitatively. The present work has assessed vascular architecture in different pathological lesions of the colorectum by quantifying blood vessel parameters in order to detect subtle morphological changes using objective methods.

Materials and methods

I. Collection of samples from resected human large bowels

Specimens were obtained from resected large bowels with malignant lesions from Chinese patients who were confined at the Department of Surgery, Queen Mary Hospital, Hong Kong during the period of October 1988

to March 1990. Our study had a total of 10 patients (6 females and 4 males) with a mean age of 65.40 (s.d.±10.98). The resected part of the large intestine was opened up by an incision along its length immediately after removal and was washed thoroughly with tap water and placed flat on top of a white board. Because of the requirements of the surgical procedure, specimens were devascularised for approximately 10 to 15 minutes prior to removal from the patient's abdominal cavity. The different mucosal lesions were identified and biopsies were performed.

Biopsies of normal (N) samples were taken from the proximal resection margins of grossly normal mucosa, not less than 5 cm from the edges of the malignant lesions. Potentially premalignant mucosa (PPM) was obtained from within 2 cm of the edges of the malignant lesions. Multiple samples were also taken from the malignant lesions (ADCA), avoiding regions of tissue necrosis. Pedunculated polyps (P) were also biopsied if they were several in number. Five patients were selected for the N, PPM and ADCA groups and another 5 patients for the P group. These samples were processed for electron microscopy (EM). The distribution of specimens from colorectal samples used in the morphometric analyses is summarised in Table 1.

II. Electron Microscopy

A) Electron Microscopically processing procedures

The mucosa was stripped and separated from the submucosa and external muscular layer using fine scissors prior to dicing. This step was only applicable to normal and potentially premalignant mucosae. Samples from other sites were diced into small pieces and immersed immediately in modified Karnovsky's solution (2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M Sorensen phosphate buffer at pH 7.40 with a total osmomality of 650 to 690 milliosmol/kg) for 4 hours at room temperature. Tissue samples were washed in phosphate buffer with two changes and postfixed with 1% osmium tetroxide (Sigma Chemical Company, St. Louis, USA) in phosphate buffer for 1 hour. Tissue samples were washed again in phosphate buffer with two changes before dehydration. The electron microscopical processing was carried out predominantly using a Reichert-Jung "Lynx" automated microscopy tissue processor (C. Reichert AG. Hernalser Hauptstrasse 219. A- 1170, Austria) beginning with dehydration and ending with infiltration. Dehydration began with 70% alcohol with two changes of 15 minutes each, 80% and 90% alcohol for 15 minutes each, 95% alcohol with three changes of 15 minutes each and 100% alcohol with three changes of 30 minutes each. Subsequently, the samples were immersed in propylene oxide (Merck, West Germany) with two changes of 10 minutes each. Infiltration was performed by immersing the tissues in a 1:1 (v:v) ratio of Epon 812 and propylene oxide for two hours at 25 ºC, before being immersed in pure Epon for 24 hours. All samples were embedded in flat TAAB embedding moulds using a dissecting microscope.

B) Preparation of semithin sections

Semithin sections of 0.5 µm thickness were cut using a Reichert-Jung Ultracut E ultramicrotome (C. Reichert AG. Hernalser Haupstrasse 219. A-1170, Austria). TAAB glass strips of 8 mm thickness were used to make glass knives using a LKB Broma 7800 Knifemaker (S-16, 25 Bromma 1, Sweden). Two semithin sections per block were sectioned by a glass knife. Sections were floated on deionized water on top of a slide which was heated to 100 ºC. Water evaporated in a few minutes and sections adhered to the slide surface. The slide was cooled for 15 minutes and then the sections were stained with 0.5% toluidine blue (TAAB) for 2 minutes. Subsequently, sections were washed with deionized water to remove excess stain, dried on a hot plate then mounted using a cover slip and "Permount" mounting medium (Fisher Scientific, Fair Lawn, New Jersey, USA).

III. Stereological Procedures

A nested or stratified systematic random sampling procedure (Gundersen and Osterby, 1981; Mayhew et al., 1982; Gupta et al., 1983) was employed in all groups. A summary of the sampling procedure used in this study is shown in Table 2. All blood vessels contained within the lamina propria were measured, excluding those blood vessels beyond the superficial boundary of the muscularis mucosae.

Using 0.5 µm toluidine blue stained semithin sections, the inner boundaries of the blood vessels were traced as was the reference area in which these blood vessels were contained. Measurements were carried out interactively using a Zeiss VIDAS image analyser comprising a Zeiss Axiophot microscope fitted with a JVC TK5310 black and white camera using x40 objective with a final magnification of x1,050 on the monitor. No attempt was made to distinguish between

Table 1. Distribution of specimens from the colorectal sample.

SITE	N, PPM and ADCA Groups	P Group
Rectum	2	2
Sigmoid colon	1	1
Caecum	1	1
Descending colon	1	1
	n=5	n=5

Table 2. Stratified sampling procedure.

GROUPS	N	PPM	P	ADCA
No. patients	5	5	5	5
No. blocks/patient	2	2	2	2
No. sections/block	1	1	1	1
No. fields/section	5	5	5	5

arteries, veins and capillaries within the reference area. For the purpose of the investigation, blood vessels were defined as those structures lined by endothelial cells which might contain erythrocytes.

The volume density of the blood vessels in the lamina propria ($V_{VBV, LP}$) was obtained by the relationship

$$V_V = A_A \text{ (Weibel, 1979)}$$

$$\text{i.e. } V_{VBV, LP} = \frac{A_{BV}}{A_{LP}},$$

where A_{BV} is the area of the blood vessel and A_{LP} is the area of the lamina propria. The numerical density of the blood vessels per unit area (N_A) was determined by:

$$N_A = \frac{N_{BV}}{A_{LP}}$$

where N_{BV} is the total number of blood vessels.

Length density was derived using the following formula (Weibel, 1979)

$$L_V = 2 \times N_A$$

Mean transverse sectional area (A) was obtained according to the following relationship:

$$A = \frac{V_V}{L_V}$$

IV. Computation and statistical evaluation of data

All data used to calculate each morphometric parameter were pooled to obtain a single value for each patient. Values of each morphometric parameter were pooled to obtain a single mean and standard deviation for each group for that particular morphometric parameter. The normal distributions of the data were tested by chi-square goodness of fit. When necessary, logarithmic transformations of the data were performed in order to make the data suitable for statistical analysis. One-way analysis of variance (ANOVA) was performed followed by a Scheffe pairwise comparison test in order to detect specific differences between groups using the SPSSPC package (Norusis, 1990).

Results

I. Qualitative observations

A) Normal (N) and Potentially Premalignant Mucosae (PPM)

The normal colorectal epithelium possessed simple glandular crypts of tubular appearance when sectioned perpendicular to the luminal axis (Fig. 1A). Each gland was composed of two crypt columns of simple epithelium with a lumen between them. The epithelial cells were predominantly columnar in shape and were either absorptive or mucous cells. The mucous cells were highly concentrated within the crypt columns and diminished substantially at the luminal aspect of the crypt.

The PPM demonstrated similar architectural features to the normal epithelium except for the presence of elongated and branched tubular glands (Fig. 1C). Three of five PPM lesions showed glandular hyperplasia and elongated crypts and one out of three were minimally branched. The remainder showed normal morphology. In the elongated glands, the epithelial cells appeared crowded. Mucous cells appeared in considerably greater numbers when compared with the normal epithelium.

The lamina propria of N was located around the colorectal glands and extended as far as the muscularis mucosae. It was composed of loose connective tissue which contained a moderate number of infiltrating leucocytes, predominantly lymphocytes, but with occasional neutrophils and some mast cells. The normal stroma contained venules, small arterioles and capillaries which were often filled with red blood cells (Fig. 1B). The lymphatics were difficult to distinguish from capillaries not filled with red blood cells. The lamina propria of PPM also contained infiltrating lymphocytes and occasional neutrophils and mast cells. The relative frequency of leucocytes was similar to that observed in the normal stroma. Venules, small arterioles and capillaries were also present in the connective tissue stroma. The blood vessels in the lamina propria of PPM appeared to be similar in size and frequency to their normal counterpart (Fig. 1D).

B) Colorectal Adenoma and Adenocarcinomas

Five pedunculated adenomas were included in the present study. One of the five had severe epithelial dysplasia, three showed moderate dysplasia and one was mildly dysplastic. Four were tubular adenomas and one of the five was a tubulovillous adenoma. In adenomatous epithelium, a "picket-fence" appearance of the nuclei or pseudostratification (Fig. 2A) was observed and the degree of pseudostratification varied from mild to severe. Three out of five adenocarcinomas were moderately differentiated and all of them were Duke's stage B. Two of the five were poorly differentiated adenocarcinomas and one was Dukes stage B and the remainder were Dukes' stage C. The epithelium of the adenocarcinomas showed enlarged and pleomorphic cells (Fig. 2C). The degree of cellular differentiation based upon nuclear morphology and the frequency of mucous cells varied substantially. Mucous cells were either plentiful or almost completely absent as in poorly differentiated cells. The number of mitotic cells per high power field (x100) was generally much higher in malignant epithelium when compared with normal epithelium.

In adenomas and adenocarcinomas, the connective tissue stroma showed variable amounts of inflammatory

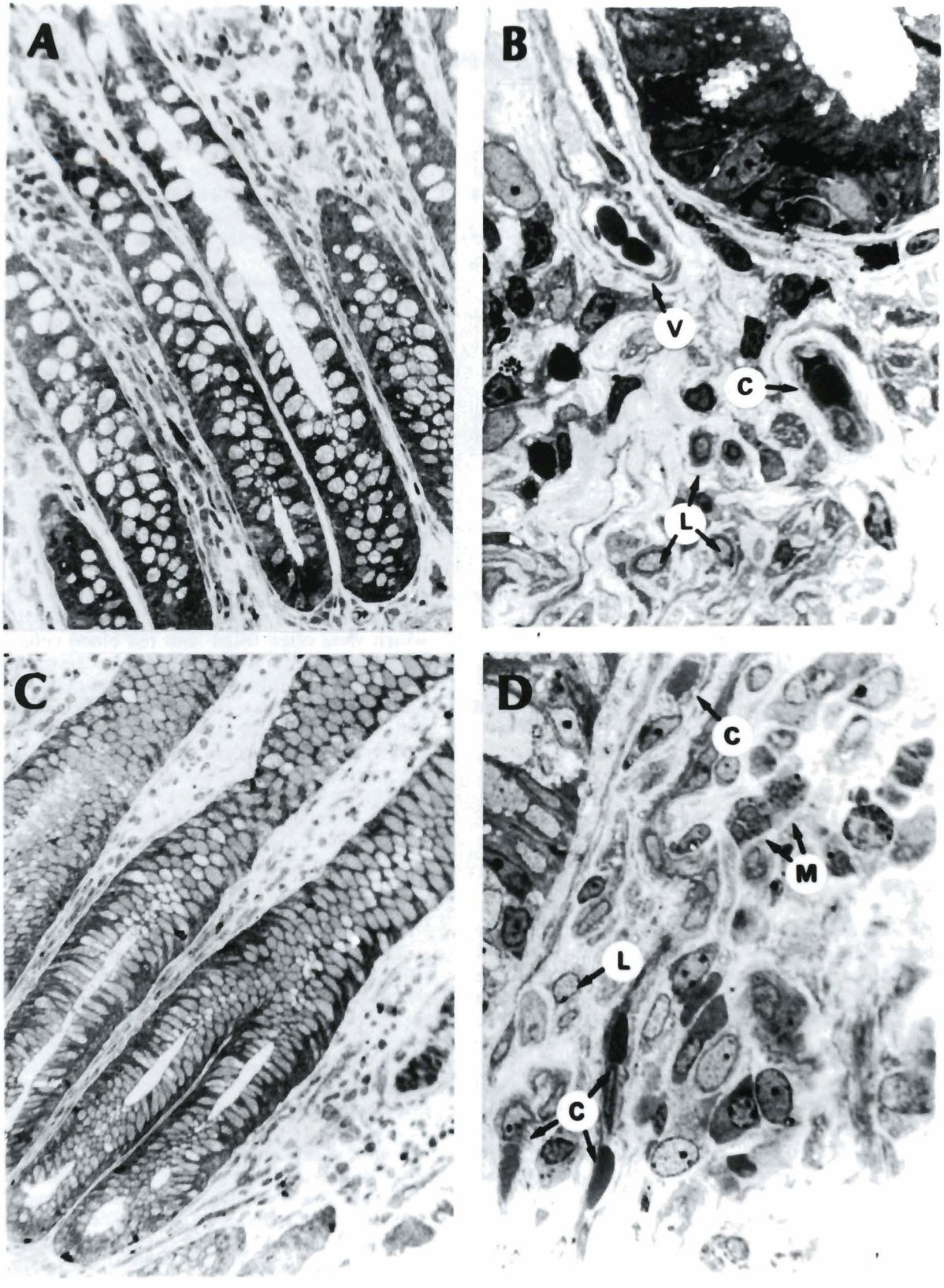

Fig. 1. A. Normal colorectal epithelium. The normal crypts show a tubular appearance when sectioned perpendicular to the luminal axis. Each gland consists of two crypt columns lined by a simple epithelium. Colorectal glands are surrounded by lamina propria. x 1,100. **B.** Higher magnification of normal colorectal epithelium showing venules (V) and capillaries (C) filled with red blood cells. The connective tissue contains a moderate amount of inflammatory cells, predominantly lymphocytes (L). x 5,450. **C.** Potentially premalignant mucosa. The glands are hyperplastic and show branching. The proportion of mucous cells is higher when compared with the normal counterpart. x 850. **D.** Higher magnification of PPM shows that the connective tissue stroma has several capillaries (C) filled with red blood cells. Some venules are also identifiable. The blood vessels are not dilated when compared with the normal counterpart x 4,400. L: lymphocytes; M: mast cells.

Table 3. Quantitative results of blood vessel parameters.

GROUPS / PARAMETERS	N	PPM	P	ADCA
$V_{VBV,LP}$ ($\mu m^3/\mu m^3$)				
1	0.015	0.019	0.067	0.130
2	0.017	0.013	0.130	0.270
3	0.020	0.016	0.099	0.110
4	0.018	0.039	0.048	0.072
5	0.019	0.023	0.060	0.100
Mean	0.018	0.022	0.081	0.140
SD	0.002	0.010	0.030	0.080
$N_{ABV,LP}$ (μm^{-2}) x 10^{-2}				
1	0.022	0.024	0.024	0.032
2	0.028	0.030	0.094	0.087
3	0.022	0.032	0.038	0.023
4	0.031	0.044	0.039	0.032
5	0.037	0.040	0.044	0.067
Mean	0.028	0.034	0.048	0.048
SD	0.006	0.008	0.027	0.027
$L_{VBV,LP}$ (μm^{-2}) X 10^{-2}				
1	0.044	0.048	0.048	0.064
2	0.056	0.060	0.190	0.174
3	0.044	0.064	0.077	0.046
4	0.062	0.088	0.078	0.064
5	0.074	0.080	0.088	0.134
Mean	0.056	0.068	0.096	0.096
SD	0.013	0.016	0.050	0.050
A(μm^2)				
1	34.09	39.58	139.58	203.13
2	30.36	21.67	68.42	158.82
3	45.45	25.40	128.57	234.04
4	29.03	44.32	61.54	112.50
5	25.68	28.75	68.18	76.92
Mean	32.92	31.94	93.26	157.08
SD	7.62	9.62	37.57	64.14

SD: standard deviation.

cells. The lamina propria contained numerous lymphocytes and neutrophils but more of the former. These cells were increased in frequency when compared with N and PPM groups. Generally, the blood vessels in the lamina propria appeared markedly dilated (Figs. 2B,D). Dilated venules and arterioles filled with numerous red blood cells were a common observation. The frequency of capillaries filled with red blood cells also increased when compared with the N and PPM groups. Aggregates of adenocarcinoma cells were sometimes seen invading the dilated venules and arterioles in adenocarcinoma lesions.

II. Quantitative results

Blood vessel parameters data of each patient from the different groups are shown in Table 3, and Table 4 shows the results of statistical analysis.

A) Volume density of blood vessels

The volume density of blood vessels per unit area of lamina propria ($V_{VBV,LP}$) increased progressively approximately seven-folded between N and ADCA. Statistically significant differences were detected in

Table 4. Results of statistical analysis.

PARAMETERS / GROUPS	$V_{VBV,LP}$	$N_{ABV,LP}$	$L_{VBV,LP}$	A
N vs PPM	NS	NS	NS	NS
N vs P	p<0.001	NS	NS	p<0.01
N vs ADCA	p<0.001	NS	NS	p<0.001
PPM vs P	p<0.001	NS	NS	p<0.01
PPM vs ADCA	p<0.001	NS	NS	p<0.001
P vs ADCA	NS	NS	NS	NS

One-way ANOVA followed by Scheffe test. NS: not statistically significant; N: normal; PPM: potentially premalignant; P: polyp; ADCA: adenocarcinoma.

comparisons between N and P, between N and ADCA, between P and PPM and between ADCA and PPM by one-way ANOVA and Scheffe tests.

B) Numerical and Length Densities

Both numerical density ($N_{ABV,LP}$) of blood vessels per unit are of LP and length density ($L_{VBV,LP}$) of blood vessels per unit volume of LP increased by 71% between N and ADCA with the highest values in P and ADCA. However, statistically significant differences were not detected between any of the groups by one-way ANOVA and Scheffe tests.

C) Mean Transverse Sectional Area

The mean transverse sectional area of blood vessels (A) increased markedly approximately four-fold between N and ADCA with the lowest value of A being in PPM. Comparisons between groups of A by one-way ANOVA and Scheffe tests showed significant differences between N and P, between N and ADCA, between P and PPM and between ADCA and PPM.

Discussion

PPM is known as "Transitional Mucosa" or TM in the literature. The epithelium of TM was shown to demonstrate histochemical and morphological alterations (Saffos and Rhatigan, 1977; Shamsuddin et al., 1981; Filipe, 1984). The high iron diamine/alcian blue technique showed a predominance of sialomucin in TM and of sulphomucin in normal colorectal epithelium. TM glands were hyperplastic and showed branching and distortion. These authors considered these histochemical and structural findings intermediate between those present in normal and carcinoma and thus considered TM to be premalignant. However, this hypothesis was refuted by others who found that TM was often associated with other types of lesions such as squamous cell carcinomas, malignant melanomas, colonic lymphomas, solitary ulcer syndrome and normal colons resected for traumatic injuries (Isaacson and Attwood, 1979; Franzin et al., 1981; Listinsky and Riddell, 1981). They attributed such changes as being secondary responses to existing lesions rather than premalignant

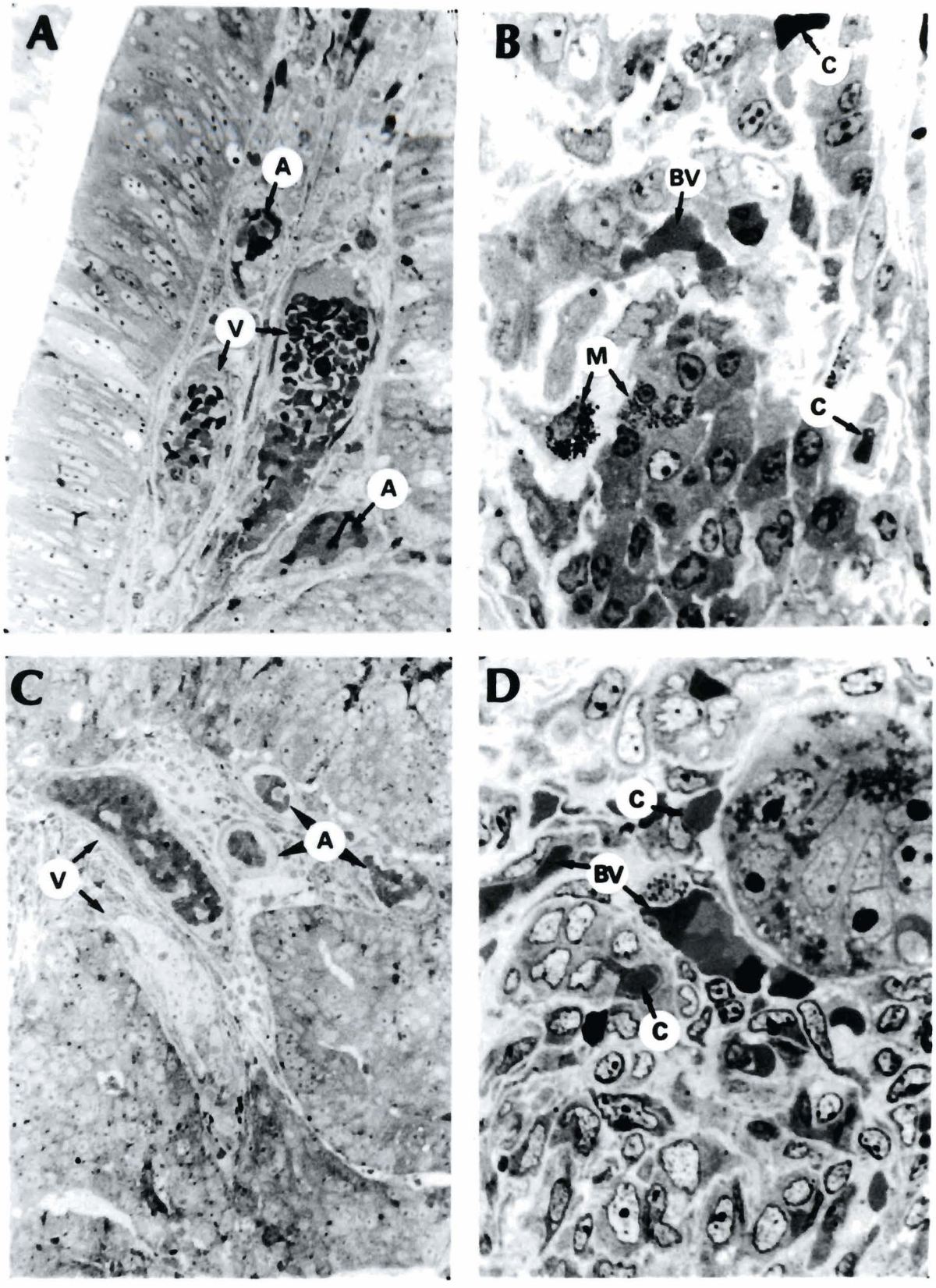

Fig. 2. A. Adenomatous epithelium. The epithelium shows varying degrees of pseudostratification. The nuclei are enlarged with prominent nucleoli x 1,750. V: venules; A: arterioles. **B.** Higher magnification of the connective tissue stroma shows infiltrating lymphocytes with occasional mast cells. The dilated blood vessels (BV) are mostly small diameter arterioles and venules filled with red blood cells. Capillaries (C) are higher in frequency when compared with the normal counterpart. x 4,400. M: mast cells. **C.** Adenocarcinomatous epithelium. The epithelium shows enlarged and pleomorphic nuclei. The degree of pseudostratification is marked x 1,100. V: venules; A: arterioles. **D.** Generally, the blood vessels (BV) in the connective tissue stroma are markedly dilated with a higher frequency of capillaries (C) when compared with the normal counterpart. There is a high concentration of lymphocytes in the connective tissue stroma. x 4,400

changes. We have documented quantitatively evidence of hyperplasia in both normal looking and morphologically hyperplastic glands of PPM (Tipoe et al., 1992; White et al., 1992). We have designated TM as potentially premalignant mucosa until more evidence is available to substantiate its premalignant status.

PPM lesions are suspected of being premalignant but our quantitative assessment of the blood vessel alterations does not suggest that angiogenesis has occurred. PPM showed no significant differences in any of the blood vessel parameters when compared with the normal counterpart. This may still be consistent with the avascular phase of angiogenesis as proposed by Folkman (1974), where the sustenance of the epithelial cells occurs by diffusion prior to capillary proliferation from preformed vessels. Furthermore, the dilatation of blood vessels occurring in the adenomas, which are considered to be premalignant lesions, may be suggestive of the early vascular phase of angiogenesis, whereas dilatation in the ADCA group may represent the late phase of angiogenesis. In the present report, this was demonstrated by an increase in volume density and mean transverse sectional area of 268% and 92% respectively between PPM and P groups. Likewise, there were also seven and four fold increases in the volume density and mean transverse sectional area of blood vessels respectively in ADCA when compared with the N group. All of these increases between groups were statistically significant. Our blood vessel parameter data have suggested that the principal event during malignant transformation was an increase in the volume density. This was brought about by an increase in the mean transverse diameter of individual blood vessels in the P and ADCA groups rather than in the number or length densities. The numerical density (N_A) progressively increased between N and ADCA, although it was not statistically significant.

We chose toluidine blue stained semithin sections for analysis because of the improved resolution available using 0.5 μm sections. Our data have to be interpreted cautiously because the criteria for identification of blood vessels using toluidine blue stained semithin sections were biased towards blood vessels of larger size. Smaller blood vessels, particularly capillaries without red blood cells, were difficult to identify and to distinguish from lymphatics or venules. This might affect significantly the outcome of the numerical density (N_A) since the main event in angiogenesis is capillary proliferation. Data obtained might result in understimation of the values of N_A and L_V in the premalignant and malignant lesions. Furthermore, our samples size for each group was small in number and mean values have high standard deviations reflecting wide variations between patients, particularly in P and ADCA groups. Presently, we are performing immunocytochemical work using monoclonal anti-vimentin (clone V9, Sigma Co.) antibody which can localise vimentin in endothelial cells of blood vessels and thus can improve the identification of capillaries. This work will involve a larger sample size from each group.

The so-called 'normal' colorectal epithelia appeared to have normal morphology. However, subtle microscopical changes might have occurred which were undetectable by quantitative estimation of toluidine blue stained semithin sections. The presence of the inflammatory cell infiltrate might be indicative of early pathological change in tumor bearing bowels. Our future studies will improve the sample quality and size and will quantify normal tissue from patients without evidence of neoplastic disease.

Mlynek et al. (1985) found that N_A was significantly lower in normal colorectal tissues than in carcinomas. Wakui et al. (1992) found that the volume density of blood vessels in the tumour mass ($V_{VBV,T}$) of low and intermediate grades of prostatic carcinoma without bone metastases was similar to that found in the normal counterpart. However, $V_{VBV,T}$ of high grade primary prostatic carcinomas had values which were similar to those of prostatic carcinoma with bone metastases but which were higher than normal tissues. They speculated that high grade prostatic carcinoma possessed the potential for tumour metastasis as blood capillary growth might facilitate entry of the tumor cells into the blood vessels, increasing the chance of metastasis. Weidner et al. (1992) found that all patients with breast carcinoma having more than 100 microvessels per x200 field (microvessel density count - MDC) had tumour recurrence within 33 months, compared with less than 5% of the patients with breast carcinoma having an MDC of 33 or fewer microvessels per x200 fields. They concluded that MDC is a measure of tumour angiogenesis which is associated with metastasis and may be a prognostic indicator. Srivastava et al. (1986) also found a high degree of correlation between vascularity of the tumour base and tumour thickness in melanoma.

In stratified oral epithelium of experimentally treated hamster cheek pouch, the volume, length and numerical densities as well as the mean transverse sectional area of the blood vessels significantly increased in chemically induced oral neoplasia (CION) when compared with untreated mucosa (White and Al-Azzawi, 1983). The blood vessel parameters of CION were characterized by increases in vascular volume density as result of both increased frequency and increased individual size. Increases in the volume and numerical densities of blood vessels were reported to be good prognosticators of spread and survival in prostatic (Wakui et al., 1992) and breast (Weidner et al., 1992) carcinomas respectively.

The present work reported significant increases in volume density but not significant increases in the numerical density in the P and ADCA groups when compared with their normal counterpart. However, the mean transverse sectional area of blood vessels increased significantly in P and ADCA groups, suggesting that vasodilatation occurred in order to provide an increased supply of nutrients to sustain active growth and division of transforming cells. However, such vasodilatation might also reflect the inflammatory response to the presence of actively growing malignant cells since activated immune cells are capable of

releasing vasoactive substances. All previous studies have used conventional stains where identification of smaller blood vessels was difficult and the estimates of the blood vessels parameters were underestimated. Better characterisation of the blood vessels could be made in future studies by using immunostaining e,g., monoclonal antibodies to vimentin using the ABC immunoperoxidase technique (Wakui et al., 1992), to identify precisely and to classify further the different types of blood vessels within the lamina propria or connective tissue stroma. This will provide more information with regard to the extent of increase in the different types of blood vessel in the tumor stroma as well as avoiding underestimation of counting due to failure in identification of the smaller blood vessels.

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References

Brem H. and Folkman J. (1975). Inhibition of tumor angiogensis mediated by cartilage. J. Exp. Med. 141, 427-439.

Brem S.S., Jensen H.M. and Gullino P.M. (1978). Angiogenesis as a marker of preneoplastic lesions of the human breast. Cancer 41, 239-244.

Choay J., Petitou M., Lormeau J.C., Sinay P., Casu B. and Gatti G. (1983). Structure-activity relationship in heparin: a synthetic pentasaccharide with high affinity for anti-thrombin III and eliciting high factor X activity. Biochem. Biophys. Res. Commun. 116, 492-499.

Crum R. and Folkmann J. (1984). Anti-angiogenesis by steroids without glucocorticoid or mineralocorticoid activity in the presence of heparin. J. Cell Biol. 99, 158a.

Filipe M.I. (1984). Transitional mucosa. Histopathology 8, 707-708.

Folkman J. (1974). Tumor angiogenesis. Adv. Cancer Res. 19, 331-358.

Folkman J. (1990). What is the evidence that tumors are angiogenesis dependent? J. Natl. Cancer Inst. 82, 4-6.

Franzin G., Scarpa A., Dina R. and Novelli P. (1981). Transitional and hyperplastic-metaplastic mucosa occurring in solitary ulcer of the rectum. Histopathology 5, 527-533.

Gullino P.M. (1977). Natural history of breast cancer. Progession from hyperplasia to neoplasia as predicted by angiogenesis. Cancer 39, 2697-2703.

Gullino P.M. (1978). Angiogenesis and oncogenesis. J. Natl. Cancer Inst. 61, 639-643.

Gundersen H.J.G. and Osterby R. (1981). Optimizing sampling efficiency of stereological studies in biology: or "Do more or less well". J. Microsc. 121, 65-73.

Gupta M., Mayhew T.M., Bedi K.S., Sharma A.K. and White F.H. (1983). Inter-animal variation and its influence on the overall precision of morphometric estimates based on nested sampling designs. J. Microsc. 131, 147-154.

Isaacson P. and Attwood P.R.A. (1979). Failure to demonstrate specificity of the morphological and histochemical changes in mucosa adjacent to colonic carcinoma (transitional mucosa). J. Clin. Pathol. 32, 214-218.

Kuettner K.E., Soble L., Croxen R.L., Marczynska B. and Hiti J. (1977). Tumour cell collagenase and its inhibition by a cartilage-derived protease inhibitor. Science 196, 653-654.

Listinsky C.M. and Riddell R.H. (1981). Patterns of mucin secretion in neoplastic and non-neoplastic diseases of the colon. Hum. Pathol. 8, 441-449.

Maione T.E., Gray G.S., Hunt A.J. and Sharpe R.J. (1991). Inhibition of tumor growth in mice by an analogue of platelet factor 4 that lacks affinity for heparin and retains potent angiostatic activity. Cancer Res. 51, 2077-2083.

Mayhew T.M., White F.H. and Gohari K. (1982). Towards economy of effort in quantitative ultrastructural pathology: efficient sampling schemes for studying experimental carcinogenesis. J. Pathol. 138, 179-191.

Mlynek M.L., van Beuningen D. and Leder L.D. (1985). Measurements of the grade of vascularisation in histological tumour tissue sections. Br. J. Cancer 52, 945-948.

Norusis M.J. (1990). SPSSX User's Guide. 2nd ed. Chicago. Illinois SPSS Inc. pp 465-474.

Saffos R.O. and Rhatigan R.M. (1977). Benign (non-polypoid) mucosal changes adjacent to carcinomas of the colon. Hum. Pathol. 8, 441-449.

Shamsuddin A.K.M., Weiss L., Phelps P.C. and Trump B.F. (1981). Colon epithelium. IV. Human colon carcinogenesis. Changes in human colon mucosa adjacent to and remote from carcinomas of the colon. J. Natl. Cancer Inst. 66, 441-449.

Sharpe R.J., Byers H.R., Scott C.F., Bauer S.I. and Maione T.E. (1990). Growth inhibition of murine melanoma and human colon carcinoma by recombinant human platelet factor 4. J. Natl. Cancer Inst. 82, 848-853.

Srivastava A., Laidler P. and Hughes L.E. (1986). Neovascularization in human cutaneous melanoma. Eur. J. Cancer. Clin. Oncol. 22, 1205-1209.

Tipoe G.L., White F.H. and Pritchett C.J. (1992). Estimation of absolute epithelial cell numbers in simple tubular glands of normal human colorectum. Acta Stereol. 11, 79-86.

Wakui S., Furusato M., Itoh T., Sasaki H., Akiyama A., Kinoshita I., Asano K., Tokuda T., Aizawa S. and Ushigome S. (1992). Tumour angiogenesis in prostatic carcinoma with and without bone marrow metastasis: a morphometric study. J. Pathol. 168, 257-262.

Warren B.A., Greenblatt M. and Kommineni V.R. (1972). Tumour angiogenesis: ultrastructure of endothelial cells in mitosis. Br. J. Exp. Pathol. 53, 216-224.

Weibel E.R. (1979). Stereological methods. In: Practical methods for biological morphometry. Vol. 1. London. Academic Press. pp 51-159.

Weidner N., Folkman J., Pozza F., Bevilacqua P., Allred E.N., Moore D.H., Meli S. and Gasparini G. (1992). Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma. J. Natl. Cancer. Inst. 84, 1875-1887.

White F.H., Tipoe G.L. and Pritchett C.J. (1992). Comparisons of mean glandular volume and number of epithelial cells per human colorectal crypt between normal epithelium and potentially preneoplastic lesions. Acta Stereol. 11, 391-398.

White F.H. and Al-Azzawi B. (1983). Vascularity in experimental oral neoplasia: a stereological approach. Acta Stereol. 2, 119-126.

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