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LETTERS TO THE EDITOR

High endothelial cell proliferation index and high microvessel density in vascular hotspots suggest an active angiogenic process in human colorectal adenocarcinomas

Sir – We read with interest the recent publication by Pritchard *et al.* (1995) on the vascular patterns in human colorectal cancer, suggesting that only a weak angiogenic response is induced by invasive carcinomas of the large intestine. Vascularity is assessed by morphometry in ten equally spaced fields of 0.14 mm^2 per region in different tumoral and peritumoral regions and in the adjacent mucosa of sections immunostained with the QB/end/10 antibody. In our opinion, several points of this study need further clarification.

In the peripheral tumour regions, vascularity is reported to be 1.6 and 1.9 times higher than in the adjacent normal mucosa for poorly and moderately differentiated carcinomas respectively. We have found that microvessel counts of highly vascular areas or vascular hotspots in colorectal tumour tissue also exceed counts in the adjacent mucosa by a factor of 1.7 (Vermeulen *et al.*, 1995*a*). These hotspots were predominantly encountered in the peripheral tumour regions. Although the results of both studies are not entirely comparable, given the different quantitative parameters, vascularity is found to be higher in colorectal tumour tissue compared with the adjacent mucosa.

In the centre of colorectal carcinomas, an equal or lower vessel density is reported by Pritchard *et al.* for moderately and poorly differentiated tumours respectively compared with the adjacent mucosa. In our opinion, by analysing only vessels with a clearly visible lumen, the difference in vascularity between peripheral and central tumour regions is overestimated, given the elevated tissue pressure in the centre of less organised tumours. It might also be that small vessels without a lumen are newly developed vascular sprouts with a high mitotic activity.

We have reported on endothelial cell proliferation, tumour cell proliferation and microvessel density in colorectal adenocarcinomas only recently and have shown not only that vascular hotspots are also present in the centre of tumours, but that they represent restricted areas of high endothelial and tumour cell proliferation throughout the entire carcinoma (Vermeulen *et al.*, 1995*b*). Median Ki67-positive endothelial cell fraction was found to be 23 times higher in the vascular hotspots compared with the adjacent mucosa. In breast carcinoma, a 45-fold difference has been reported, using the same methodology (Vartanian *et al.*, 1994).

We are also convinced that the vascularity of the mucosa adjacent to the tumour tissue cannot be regarded as representative for the baseline vessel density found elsewhere in the bowel wall. Given the proximity of both tumour and stromal cells secreting potent angiogenic factors, it can be expected that vessel density is up-regulated in the adjacent mucosa. The angiogenic response to colorectal cancers might therefore be underestimated, when compared with the vasculature of the adjacent mucosa. Analysis of vessel density at a distance of the tumour or of the large intestine of organ donors can give a more precise quantification of the normal vasculature.

The vascular structure of colorectal carcinomas is thus characterised by areas of high vessel density or vascular hotspots, separated by tissue with a lower vessel content. The hotspots can be regarded as active angiogenic tumour regions. Newly formed blood vessels might, by digesting the surrounding matrix, facilitate haematogeneous tumour cell spread. A recent retrospective study on angiogenesis in 48 rectal carcinomas shows a statistically significant association between transmural penetration, angiogenesis score and survival (Saclarides *et al.*, 1994). Large studies with a sufficiently long follow-up period are needed for an accurate estimate of the prognostic value of vessel density in human colorectal adenocarcinomas. We agree with the authors that additional parameters of the angiogenic process should be co-analysed with microvessel density for their prognostic value. In the view of Gasparini *et al.* (1995), tumours expressing the 67 kDa laminin receptor are those containing active angiogenic stimuli. In a study on 171 node-negative breast carcinomas, they have shown that the joint variable laminin receptor and vascularisation is the strongest independent prognostic factor for relapse-free survival. Although hampered by some practical restraints, the value of the endothelial cell proliferation fraction can be analysed together with microvessel density in colorectal adenocarcinomas using a double immunostaining with a vascular marker (e.g. JC70) and a proliferation marker (e.g. Ki67) (Vermeulen *et al.*, 1995*b*).

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Reply to the letter from Vermeulen

Sir – Dr Peter Vermeulen and co-workers make several points about our recent morphometric study on the distribution of blood vessels in colorectal carcinomas (Pritchard *et al.*, 1995).

We reported that poorly differentiated (but not moderately differentiated) colorectal carcinomas were significantly less vascular in their centres than in their peripheral regions (Pritchard *et al.*, 1995). However, Vermeulen and co-workers argue that by only analysing vessels with a clearly visible lumen, we may have overestimated differences between peripheral and central regions of the tumour, 'given the elevated tissue pressure in the centre of less organised tumours'. I assume the suggestion is that elevated tissue pressure in the centre of tumours causes compression of vessels with disappearance of their lumens, and that this is most likely to be seen in poorly differentiated tumours. If this is a significant factor, then one might expect to see a higher density of 'vessels without lumens' in the centre of tumours than in their peripheries, particularly in poorly differentiated tumours. However, our further unpublished studies have not shown this. Analysing the same cases as described in Pritchard *et al.* (1995), we found that for poorly differentiated tumours, mean values of Lv for 'vessels without lumens' were 63.8 (s.d. 35.4) in the centre and 86.1 (s.d. 66.1) in the periphery. For moderately differentiated tumours, mean values of Lv were 59.8 (s.d. 52.8) in the tumour centre and 50.47 (s.d. 26.07) in the tumour periphery. It must also be stated that these differences did not reach statistical significance.

Vermeulen and co-workers also correctly make the point that tumour-adjacent bowel mucosa should not be considered normal for the purposes of studies on angiogenesis. For this reason, samples of normal mucosa analysed in our study were from the resection margins of bowel specimens. The tumour-adjacent host tissue we referred to included all host tissues deep to the mucosa, but did not include the mucosa itself.

I would agree that our results are not entirely comparable: while our comparisons were based on the mean value for ten randomly selected fields in each tumour region, their comparisons were mostly based on a single carefully selected field for each tumour (Vermeulen *et al.*, 1995*a*). Our main focus was on the overall differences in vascularity between different tumour regions, while theirs was on the significance of vascular 'hotspots'.

In the letter from Vermeulen and co-workers, it is stated that we found peripheral tumour regions to be more vascular than normal mucosa. In fact this depends on which vascular parameter is considered. We used three different stereological measurements: length density (Lv), surface density (Sv) and volume density (Vv). For comparison with the data described in Vermeulen *et al.* (1995), it would be most appropriate to consider the parameter Lv. On the basis of these data, we found peripheral regions of moderately and poorly differentiated carcinomas to be less vascular than normal mucosa (0.76× and 0.75× respectively). For the paremeter Sv, it is correct to say that we found the peripheries of moderately and poorly differentiated tumours to be slightly more vascular than normal mucosa (1.3× and 1.1× respectively).

In view of the high proliferation index of tumour cells and the ability of tumour cells to produce angiogenic factors, it is surprising that this difference between colorectal tumour tissue and normal mucosal tissue is so small. It is even more surprising, in view of the recent report by Vermeulen *et al.* (1995*b*), that the mean Ki67 labelling index of endothelial cells in colorectal tumour tissue is 10.6× that of adjacent colorectal mucosa. Given that colorectal tumours grow very slowly, with volume doubling times of the order of 2 years (Steel *et al.*, 1977), one might expect that this evidently increased proliferation of endothelial cells within the tumours would result in a highly vascular tumour stroma. Perhaps the explanation is that vascular destruction within tumours, or at least endothelial cell apoptosis, are significant and underestimated factors.

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