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Editorial

Histological measurement of tumour angiogenesis

1. Tumour angiogenesis: size matters

The study by Offersen and colleagues in this issue has added valuable insights into the interesting, but confused, area of tumour vascularity measurement [1]. Supply of oxygen, nutrients and growth factors, to name a few, is limited to a few micrometres in tissues by the diffusion coefficient. Correspondingly, growth of any structure over a few micrometres, including a tumour, demands a vascular supply. Thus, angiogenesis is a critical step in tumorigenesis. Although logical, this simple concept was overlooked until the seminal work of Folkman [2,3,14] in 1989, based on a mouse pancreatic tumour that followed a hyperplasia–neoplasia sequence [3,14]. Stromal angiogenesis immediately preceded, and was required for, the progression from hyperplasia to neoplasia [3,14]. That indicated that tumorigenesis depends as much on the host response as epigenetic abnormalities. While mice are not men, and their tumours were hardly typical pancreatic adenocarcinomas, the study underlined the central importance of angiogenesis in tumorigenesis.

Now, an enormous and increasing number of pro- and anti-angiogenic mediators have been characterised (reviewed in Ref. [4]). Pro-angiogenic mediators include vascular endothelial growth factor (VEGF), interleukin-8 (IL-8), tissue factor, thymidine phosphorylase/platelet-derived endothelial cell growth factor (TP/PDECGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and angiogenin. Expression of many of these is stimulated by the transcription factor Hypoxia-Inducible-Factor-1-alpha (HIF-1α), activated by hypoxia (reviewed in Ref. [5]). Anti-angiogenic factors include angiostatins and endostatin [4]. Although VEGF has been particularly implicated in tumour angiogenesis in numerous studies, angiogenesis *in vivo* is likely to be the result of the actions of multiple angiogenic factors [2]. Currently, animal tumour models are inhibited by novel selective VEGF receptor tyrosine kinase inhibitors, but definitive evidence in man awaits clinical trials. There are also potentially important antitumour effects of angiogenesis that have not been explored in detail. Tumour microvessels may also be required for the recruitment of T-lymphocytes, natural killer cells, macrophages or other antitumour leucocyte subsets.

So, is tumour vascularity prognostic? Although this would be a quantifiable histological marker [2], there are also reasons why it is not. Any tumour must be adequately vascularised to attain a detectable size, and since tumour vascularity is a state it may not reflect either angiogenesis or the dependence on angiogenesis [2]. Thus, microvessel density may not always predict the response to anti-angiogenic therapy [2]. Although an increasing literature has associated tumour vascularity with prognosis, there are many apparently conflicting results, probably because the area is bedevilled with methodological variations precluding direct comparisons [2].

2. Methods matter

There are now some 1000 papers on the prognostic value of angiogenesis in malignant tumours (PubMed, too many to cite individually). However, it is clear from these that it matters how, where and when angiogenesis is measured [2].

2.1. Location

For example, many tumours have a high content of collagenous tissue that is metabolically inert and will need less nutritional support. These often have a growing edge of malignant cells in which vascularisation is likely to have a relatively important role. This leads to 'hotspot' measurement of microvascular densities that in some studies outperforms average measurements for prognosis [2]. Thus, average microvascular densities are not equal to peak microvascular densities.

2.2. Identification

Similarly, authors have employed measurement of tumour microvessels using a variety of stains (haematoxylin and eosin (H&E), immunostaining with anti-von-Willibrand factor (VWF), anti-CD31 and anti-CD34). These are not equivalent. Immunostaining detects more microvessels than H&E stains. VWF is expressed by mature endothelium so anti-CD34 and anti-CD31 detect more actively growing microvessels. Indeed, this discrepancy has been used to enumerate

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active angiogenesis [6]. CD105 (endoglin, a transforming growth factor β (TGFβ) receptor) is selectively expressed by angiogenic vessels and appears to be a better marker of active angiogenesis than CD31, CD34 or VWF [6].

2.3. Method of measurement

Importantly, the study by Offersen and colleagues in this issue [1] compares two counting methods (Chalkley versus what they term microvascular density (MVD)) and several staining methods. This is an extremely useful development since it gets away from the exclusive use of one method or another and addresses which method provides more precise information. Confusingly, MVD and Chalkley counts are often used synonymously in the literature. As the study indicates, methods are *not* equivalent. This is particularly important since Chalkley counting has been cited in a consensus document [7,13]. Chalkley counting is based on counting intersections with an eyepiece graticule and has been reported in approximately 100–200 papers covering mesothelioma, prostate, non-small cell lung cancer (NSCLC), small-cell lung cancer (SCLC), breast carcinoma and Hodgkin's disease (PubMed). MVD measurements by other methods have been reported in approximately 330 papers covering melanoma, hepatocellular carcinoma (HCC), colorectal carcinoma and endometrial carcinoma (PubMed). Again, other methods have been used by other authors.

Graticule-based vascular morphometry methods (e.g. Chalkley) are potentially operator-dependent. Conceivably, immunostaining for endothelial markers and computer-assisted estimation of microvessel area fraction or total endothelial length may improve measurement accuracy and reproducibility [8]. Computer morphometry may measure multiple variables simultaneously and automatically. Indeed, one study of thick melanomas immunostained for anti-CD31 and measured microvessels by computer-assisted morphometry showing prognostic value [9]. It will be helpful to directly compare eyepiece and computer-assisted techniques.

3. Angiogenesis: measurement matters

Measurement of tumour angiogenesis may become a helpful addition to clinical histopathological examination of tumour biopsies (or more probably) tumour resections. There is a need for more objectification and quantification of histopathological grade, possibly by automated morphometry [10]. Vascularisation may become a robust prognostic quantitative aid in the routine evaluation of tumours as initially suggested in 1972 [11], especially with further refinement, e.g. CD105 immunostaining and computer-assisted analysis. Tumour angiogenesis is likely to acquire therapeutic significance. Several anti-angiogenic strategies have now entered phase II or phase III trials. There will be a requirement for more careful targeting of therapy and more careful measurement of angiogenesis [2,12]. Just as not all tumour angiogenesis measurement is equal, not all tumour angiogenesis therapy is equal and a more tailored approach to both may serve cancer patients better.

In conclusion, I think that the study by Offersen and colleagues [1] has highlighted the need for more studies comparing different methods of prognostic tumour vascularity measurement.

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