

Neoplastic Non-Angiogenic Growth by Means of Vascular Co-Option

Francesco Pezzella^{*} and Kevin Gatter

NDCLS, Radcliffe Department of Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK

^{*}Corresponding author: Pezzella F, Nuffield Division of Clinical Laboratory Science, John Radcliffe Hospital, Oxford OX3 9DU, UK, E-mail: francesco.pezzella@ndcls.ox.ac.uk.

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Summary

The creation of the term angiogenesis goes back to 1787 [1] and the role of vessels in cancer has been studied since. In 1971 Folkman [2] introduced the hypothesis, until now widely accepted, that tumour growth is angiogenesis dependent [3]. However, the discovery that cancer can also grow without angiogenesis, by co-opting pre-existing vessels both in humans [4-7] and in mice [8] has demonstrated that this is not always the case. The observation that cancer cells can exploit pre-existing vessels provides a new aspect of the interaction between host and tumors, sheds new light on the biology of the latter and has implications for resistance to antiangiogenic drugs and development of new vascular targeting strategies.

Introduction

The availability of oxygen and nutrients supplied by the vasculature is critical for cancer growth and the role played by the blood vessels has been a long standing object of many studies. The introduction of the concept of angiogenesis goes back as far as 1787 [1] and in 1939 Ide et al described that tumour implants in the ears of rabbits were accompanied by formation of new capillaries [9] leading to the idea that angiogenesis is necessary to support tumours. At the same time instead several histopathologists maintained the point that both pre-existing and newly formed vessels were co-existing in tumours and, sometime, only pre-existing vessels could be observed [10]. However it was not until thirty years later that the subject started to be systematically studied by Judah Folkman [11] following the path indicated by Ide.

The case for angiogenesis dependence

In 1971 Folkman published a seminal paper [12] in which the idea that “the growth of solid neoplasm is always accompanied by neovascularization” was put forward. This hypothesis was mostly based on “in vitro” and animal models [13] with experiments conducted in avascular sites, such as the cornea of a rabbits [14], regarded as classic proof of concept. Subsequent work on mice has not only confirmed the need for angiogenesis but also shown that its induction is an early event [15].

Immunohistochemical studies of human *in situ* breast [16] and cervical [17] carcinomas have demonstrated the enhanced presence of micro vessels in the underlying basal membranes at this early stage inferring that angiogenesis may represent an essential intermediate phase between *in situ* and infiltrating carcinomas [15]. A formal classification of intratumour vessels in human tumours maintained

that they were all newly formed [18]. The direct correlation between microvessel density and outcome [19] further strengthened the idea of a link between angiogenesis and tumour growth although such an association has been subsequently strongly questioned [20].

Recently it has been concluded that induction of angiogenesis is an hallmark of cancer as it is necessary to address the needs of tumour cells for oxygens and nutrients and clearance of catabolic products [21].

Non-angiogenic tumours

An increasing body of evidences has uncovered an added layer of complexity: the possibility that some malignant tumours grow in the absence of neo-angiogenesis by co-opting the pre-existing vasculature.

This observation was first made in clinically detected non-small cell lung carcinomas where four distinct patterns of growth and vascularization were described [22-24]. Three of these patterns have in common the destruction of normal lung architecture, the recruitment of tumour-associated stroma and new vessel formation as they grow in an organ-like fashion [21]. In the fourth non-angiogenic pattern, the immunostainings for endothelial markers highlight the alveolar vessels, entrapped by the neoplastic cells filling the alveolar spaces and growing by co-opting the existing vessels. Soot in macrophages is commonly seen alongside these vessels indicating that they are pre date the appearance of the tumour. Tumours with both angiogenic and non-angiogenic areas are also commonly seen [5]. Clinicopathological correlation showed that stage I pT1N0 non-angiogenic tumours actually had a worst overall and disease free survival, compared with the angiogenic, with microvessel density having no prognostic value [25,26]. Furthermore also lung metastases were found to grow in a non-angiogenic fashion [4,27] even when the primary tumour was angiogenic [2000] raising doubts about the idea that angiogenesis is always linked to progression of disease and showing that the angiogenic switch can be reversed during disease progression. This type of non-angiogenic growth in the lung had been previously described as “intra alveolar” [10] and the first description is accredited to a paper from 1861 [28] although, not surprisingly, the relevance to the problem of angiogenesis was not discussed.

If some neoplastic cells are able to grow in a non-angiogenic way by co-opting pre-existing vessels, this type of tumour growth may be expected to be seen in some other organs, and indeed this is the case in the liver. Vermeulen and colleagues [7,29] described three different patterns of hepatic metastatic growth of colorectal and breast adenocarcinomas. In two types, the desmoplastic and the pushing, the architecture of the liver parenchyma was not preserved and an angiogenic tumour is present. However, in the replacement growth pattern, the metastatic cells infiltrate the liver parenchyma without any disturbance of the pre-existing liver structure. As in the lung, liver

metastases can have a pure or mixed pattern of growth with some patients who have multiple metastases displaying both “pure” angiogenic and non-angiogenic tumours [30]. The patients with non-angiogenic metastases appeared to have a slower but still aggressive disease as they had a better prognosis at 24 but not at 60 months [31]. The incidence of these liver patterns of metastases depends on the tissue of origin: breast, pancreatic and urothelial secondaries have a prevalent non-angiogenic growth pattern [32] while only one third of the colorectal metastases grows in this fashion. A second non-angiogenic growth pattern, in which the neoplastic cells colonised the sinusoid rather than replace the hepatocytes, has also been described [33].

The possibility that some brain tumours, namely glioblastoma multiforme, could be not entirely angiogenesis dependent was first raised in 1994 [34]. Since then the occurrence of vascular co-option in brain has been confirmed for glioblastoma [35] and reported also for gliomas [36,37]. Vascular co-option is now regarded as one of the reasons for the development of resistance to anti angiogenic agents in primary CNS tumours [38]. Cerebral metastases can also grow by vascular co-option [39]. Two recent studies on brain malignancies started to unravel the molecular pathways leading to vascular co-option. Serpins expression in the metastatic cells have been described as essential for this to happen by Valiente [40] while Caspani et al. highlighted the role of filopodia which are Cdc42-dependent and actin-based cytoplasmic extensions in glioblastoma multiforme [41].

Vascular co-option in mouse models

Following the description in human tumours, non-angiogenic growth by vascular co-option has been described also in animal models. The first report was from rats [8] demonstrating that glioma and mammary adenocarcinomas implanted in their brain can grow by exploiting the pre-existing vessels. This observation suggested that in the brain as well a significant non-angiogenic neoplastic growth does occur. The authors also examined a model in which the mouse lung is colonised by Lewis lung carcinoma cells that as seen in human lung are able to co-opt the pre-existing vessels. These findings have been supported by further animal models in which both gliomas [36] and metastatic melanoma [28] were growing in the brain by vascular co-option. Recently Szabo and co-workers [42] described the different anatomical phases in which cancer cells co-opt the lung alveolar vessels.

Non-angiogenic growth: why does it happen?

Different non-angiogenic patterns, intra alveolar in the lung, hepatocyte replacement in liver and parenchyma infiltration in brain, suggest that different mechanisms are at the basis of these ways of growing. We are far from understanding why some tumours do not trigger angiogenesis as not many studies have been carried out so far.

Only minor differences were found as far as necrosis is concerned, with the non-angiogenic tumours being more prone to necrosis, while chronic inflammation and fibrosis were characteristics of angiogenic tumours. No differences in microvessel density and apoptosis were observed [12]. An immunohistochemical study failed to demonstrate any major difference in the expression of markers of angiogenesis and hypoxia, the only exception being stromal thrombospondin that was almost absent in non-angiogenic but widely present in angiogenic tumours [12,13] possibly because of its anti-angiogenic activity plays a role in the vascular re modelling occurring in angiogenic cancers [43].

mRNA expression profiling by microarray studies confirmed that no differences in classic hypoxia or angiogenesis pathways could be found with the exception, again, of Thrombospondin1. An unexpected finding was instead the increased expression in non-angiogenic tumours of a set of genes linked to Oxidative Phosphorylation, suggesting the possibility of metabolic reprogramming in the non-angiogenic tumours. The second finding was the decreased level, in the same tumours, of a set of adhesion molecule genes, raising the hypothesis that diminished cell to cell contact might be associated with failure to develop a vascular infrastructure [43].

In animal models, inactivation of P53 has been found to lead to resistance to anti angiogenic drugs by increasing the ability of the cells to survive in hypoxia [44]. As pilot study on a small number of lung cancers suggested a higher incidence of P53 mutations in non-angiogenic tumours [13] this could be another mechanism by which neoplastic cells could grow in an hypoxic environment without triggering neo angiogenesis

The evidence so far suggest that the type of vascularization associated with a tumor reflects the biology of the malignant cells on one side and the anatomical structure of the organ on the other. It has been recently started to be demonstrated that blood vessels provide not only oxygen and nutrients, but that they also secrete a large and diverse number of cytokines and growth factors in a paracrine manner [called “angiocrines”], which affect/stimulate tumor growth. It could well be that both angiogenic and non-angiogenic pattern of growth are, at least in part, dictated by the angiocrine activity of the vessels involved. [23]

Finally, whatever the site of non-angiogenic growth, the possibility that antiangiogenic factors are blocking the triggering of vascular growth should be also investigated.

Blood vessels and anti angiogenic treatment

As predicted when non-angiogenic tumours were first described [5], vascular co-option is now being increasingly considered as one of the likely leading causes of reduced responses or intrinsic resistance to antiangiogenic treatment [45], especially of metastases in organ such as the lungs and liver. Nevertheless, this constitutes only one piece of the response/resistance puzzle, and there are likely many reasons for the limited clinical benefits in prolonging progression free or overall survival outcomes in cancer patients receiving antiangiogenic drug treatments.

One is the underappreciated heterogeneous nature of the tumor vasculature, including the angiogenic vasculature populating tumors, and this especially applies to spontaneous tumors that have existed for a considerable period of time - perhaps years - in patients, as opposed to rapidly growing transplanted tumors in mice.

The work of Dvorak and colleagues has revealed evidence for such extensive tumor blood vessel heterogeneity and the fact that certain types of abnormal tumor blood vessels are not necessarily VEGF-dependent [46]. Moreover, antiangiogenic drug treatments may alter this morphologic and functional tumor blood vessel heterogeneity, and indeed, vessel normalization in tumors as a consequence of antiangiogenic therapy may be an example of this [47]. The nature of this heterogeneity may be strongly influenced by metastatic spread, and this may help explain why in some preclinical studies distant lung metastases are unresponsive to a drug such as sunitinib, in contrast the same tumour cell line is treated as a primary tumor mass [48]. Such results highlight one of the deficiencies of most types of conventional

mouse tumor therapy models, which rarely involve treatment of established visceral metastatic disease [49].

If on one hand the description of non-angiogenic tumours helps to explain why the clinical benefits of anti angiogenic drugs have been modest so far, on the other hand their understanding can become a tool to better use these drugs and exploit their potential to the full. The identification of how much of vascular co-option and classic angiogenesis is present in tumours will help to distinguish those which are more likely to benefit from anti-angiogenic drugs from those which are less likely. It is therefore conceivable that this approach could lead to obtain better results from drugs targeting classic angiogenesis.

Furthermore by unveiling the pathways dictating the non-angiogenic growth of cancer cells new targets for treatment could be discovered leading to develop a new class of anti-vascular drugs.

Conclusion: “Inducing angiogenesis” a hallmark too far?

The blood supply of a tumour can be provided not only by neoangiogenesis, but also by pre-existing vasculature exploited by cancer cells growing into a non-angiogenic primary or metastatic tumour. A mixed pattern of pre-existing and newly formed vessels is also commonly seen in many types of cancers. Therefore, contrary to the theory of Folkman [3], still regarded as one of Hallmark of Cancer [21], it is now well established that some tumours can grow and metastatize in absence of angiogenesis [4]. The reason for the delay in reaching a more global view of the role of blood vessels in cancer can be found in a gap between bedside and benchtop research [50-54].

The biological implications are that the triggering of hypoxia related pathways does not necessarily leads to angiogenesis, and that to target tumour blood supply directly may fail because of co-option. Following the initial modest results obtained so far with anti angiogenic drugs [45], understanding the mechanisms driving this behaviour is likely to generate new therapy approaches for these resistant tumours.

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