An update of new targets for cancer treatment: vessels and matrix

R. Bicknell

Introduction
The identification and validation of targets for therapeutic drugs lies at the very heart of pharmacological research. Angiogenesis, or more specifically tumour initiated growth of new vessels, has been an active area of cancer research for several years. Indeed, there are currently more drugs in cancer clinical trials that are anti-angiogenics than those that fit into any other mechanistic category. Despite this, none has yet reached the clinic and there have been some setbacks along the way. Nevertheless, there remains a prevailing mood of optimism concerning the future for anti-angiogenic drugs. In addition, recent developments in direct targeting of drugs to existing tumour vessels has led to a resurgence of interest in this complimentary approach. The purpose of this article is to provide a brief overview of where we stand with anti-angiogenic therapy and to outline the recent advances in vascular targeting. For extensive reviews the reader is referred to Yancopoulos et al. [1], Ferrara and Alitalo [2] and Oehler and Bicknell [3].

Anti-angiogenesis
It is now well known that angiogenesis describes the growth of new blood vessels. It is equally well known that abrogation of this vascular development leads to inhibition of tumour growth. Indeed, in some cases removal of an endothelial survival factor such as vascular endothelial growth factor (VEGF) can lead to loss of existing tumour vessels and tumour regression [4]. The latter observation contributed to the idea of single-agent anti-angiogenic treatment for cancer. This has recently been reported with chemical inhibitors of the tyrosine kinase activity of the VEGF receptor such as ZD4190 [5]. In the last 10 years, one of the difficulties workers in the area of tumour angiogenesis have had to face is which of the many angiogenic factors are key players in tumour angiogenesis. Screening of clinical samples has highlighted some, most notably VEGF, thymidine phosphorylase and interleukin-8 [6, 7]. Other angiogenic peptides induced by hypoxia are also likely to play a significant role, an example of which is adrenomedullin [8, 9] that has recently received much interest and is thought to mediate tamoxifen-induced endometrial angiogenesis and cancer [10].

Basic research into tumour angiogenesis has documented two primary stresses that initiate gene expression programmes leading to angiogenesis. One is hypoxia [11] and the other oxidative stress, or presence of oxygen free radicals (not to be confused with hyperoxia or an excess of oxygen) [12]. Virtually all tumours are in a state of both hypoxia and oxidative stress, the two phenomena are not mutually exclusive but exist at the same time. Many studies have shown that both hypoxia [13] and oxidative stress [14] initiate gene expression profiles in tumour cells that include a variety of angiogenic factors. In some cases, such as VEGF, they are induced by both stresses, in others by only one, for example IL-8 by oxidative stress but not hypoxia [15]. Gene induction by hypoxia is mediated via the transcription factor HIF (hypoxia inducible factor). The oxygen sensor has recently been identified as a prolyl 4-hydroxylase [16–18]. In the presence of molecular oxygen, prolyl 4-hydroxylase adds an oxygen radical to a proline residue of HIF. This initiates ubiquitination of HIF and its subsequent breakdown in the proteasome. In the absence of oxygen, these activities cease, the HIF level rises and transcription is initiated. The speed of this response is surprising. On restoration of oxygen, the HIF protein is degraded within just a few minutes. HIF-1 has been shown to play a protective role for the cell in response to redox manipulation and glucose deprivation [19]. The point of describing all this is to show that there exist many targets with which to block this response and so reduce tumour growth. For example, by blocking the action of HIF directly [20, 21] or by reducing the level of iron (an essential cofactor for prolyl 4-hydroxylase) in the cell by using antibodies against the transferrin receptor. Likewise, a major contributor to the oxidative stress appears to be the presence of strongly reducing sugars such as deoxyribose-1-phosphate generated by the action of the angiogenic enzyme thymidine phosphorylase on thymidine released by cell death and breakdown of DNA [15]. Thymidine phosphorylase provides another promising target and indeed an inhibitor has shown both anti-tumour activity [22] and potent suppression of metastasis [23]. Alternatively, we can attempt to block oxidative stress directly by, for example, N-acetylcysteine that has been shown to have anti-tumour activity [24].

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Finally, mention should be made of naturally occurring angiogenesis inhibitors such as angiostatin and endostatin. Much publicity surrounded the original reports of these factors. Endostatin regressed xenografted tumours in mice [25] but was less effective against spontaneous tumours occurring in transgenic mice and has so far failed to show any efficacy whatsoever in man (for a discussion see Marshall [26]). This is a scenario that has been seen with anti-angiogenic drugs before and it is worth noting that drugs developed to inhibit endothelial proliferation are likely to be more effective against xenografted tumours compared with spontaneous ones, as endothelial cell proliferation is much greater in the former than the latter. It can be argued that inhibition of endothelial migration rather than proliferation would give greater efficacy. Another target that has proved disappointing in clinical practice is matrix metalloprotease (MMP). For an account of these studies see Matrisian et al. [27]. Despite much effort, no MMP inhibitor has yet been licensed for the treatment of cancer.

Vascular targeting

The concept of vascular targeting arose originally from the ideas of Juliana Denekamp who suggested that due to its different tissue environment, tumour vasculature is different to that in normal tissues [28, 29]. There now exists considerable experimental evidence supporting the idea [30]. The importance of this is that the differences may be exploited therapeutically. Despite the idea being around for many years, it proved difficult to identify molecular targets that had potential use, particularly in man. That targeting tumour vasculature with toxic drugs was an effective anti-cancer strategy was, however, proven in 1993 when Burrows and Thorpe [31] used an anti-MHC class II antibody coupled to ricin toxin to destroy tumour vasculature in experimental animal models. The experiments showed that this approach could be used to eradicate large solid tumours from the mice [31]. Since then several studies have shown the efficacy of tumour vascular targeting in model systems; however, the largest stumbling block to further progress has been failure to identify targets in human tumour endothelium. Recently two advances have led to a resurgence of interest in vascular targeting; namely, the arrival of novel low molecular weight drugs that are selectively toxic to tumour vasculature and secondly, advances that enable either direct analysis of tumour vasculature or bioinformatic approaches to identify novel targets.

Low molecular weight compounds that target the vasculature include the combretastatins that are currently in phase I trials [32]. Such compounds have been shown to be selectively toxic to tumour vasculature by disrupting the tubulin cytoskeleton. However, unlike taxanes and vinca alkaloids that also disrupt the tubulin cytoskeleton, they show no peripheral neuropathy or neurotoxicity following chronic dosing [33]. The next few years will reveal the potential of such drugs.

Identification of new tumour endothelial targets has been achieved by the isolation of tumour endothelial cells, followed by construction of a library of genes (for example a SAGE library) that can then be compared to one from endothelium isolated from normal tissue [34, 35]. Alternatively, bioinformatic techniques to identify such genes may be used. For example, we have developed simple techniques that enable the discovery of novel tissue specific genes using computing and the rich source of sequence data that lies in the public databases. This technique has identified several new endothelial-specific genes but does not predict whether they are going to be present on tumour vasculature [36]. However, a simple screen can soon reveal this, as we found for a gene called magic roundabout (ROBO4) [37]. The identification of magic roundabout as a tumour endothelial-specific gene was of considerable interest to us for several reasons. It is on the cell surface and so ideal for targeting. It is a homologue of the axon guidance receptor roundabout (Figure 1), a family of genes previously thought to be restricted to neuronal tissue and

![Diagram of Roundabout and Magic Roundabout](image)
lastly, it appeared to be a developmental gene as it is not expressed in any adult tissue. Another such gene is delta4 that is also endothelial specific and another only found on tumour endothelium in the adult [38]. These and other recently identified genes provide exciting new targets. The future will undoubtedly see vascular targeting combined with other therapies, such as anti-angiogenesis or gene therapy [39], to achieve improved therapies.

References

33. Horner SA, Gould S, Noakes JP et al. ZD6126 showed no evidence of peripheral neuropathy or neurotoxicity following chronic intravenous dosing in the rat. AACR 2002; 269 (Abstr 1338).
37. Huminiecki L, Gorn M, Suchting S et al. Magic roundabout is a new member of the roundabout receptor family that is endothelial specific and expressed at sites of active angiogenesis. Genomics 2002; 79: 547–552.