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Microtubule-targeting agents in angiogenesis: Where do we stand?

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Abstract

Angiogenesis is a key event of tumor progression and metastasis and hence a target for cancer chemotherapy. Therapeutic strategies focused on angiogenesis include the discovery of new, targeted anti-angiogenic agents and the re-evaluation of conventional anti-cancer drugs. Here, we review the most recent studies investigating the molecular and cellular mechanisms responsible for the anti-angiogenic activity of microtubule-targeting agents (MTAs). These agents include some of the most widely used and effective antitumor drugs that are also among the most anti-angiogenic. In addition, we summarize the latest results of pre-clinical and clinical studies involving MTAs administered at low metronomic doses and in anti-angiogenic combination strategies. Finally, we discuss the future development of these agents, their clinical potential and their limitations.

Keywords: Microtubule; Microtubule-targeting agents; Endothelial cells; Angiogenesis; Metronomic chemotherapy

1. Introduction

The formation of a functional vascular network through angiogenesis is a key event associated with tumor growth and cancer progression. Like normal tissues, tumors require an adequate supply of oxygen and nutrients and an efficient way to eliminate waste products of metabolism. Tumor blood vessels play an additional, critical role in metastasis, which is a major cause of treatment failure and cancer relapse. The dependence on angiogenesis for tumor progression has been demonstrated for a wide variety of human cancers including bladder, brain, breast, cervix, colon, lung, prostate and testis (Miller et al., 2001), and has led to the emergence of new therapeutic strategies aiming to specifically target this process. This approach was first suggested in 1971 by Judah Folkman (Folkman, 1971). In 2004, more than three decades of extensive, worldwide research later, a specific anti-angiogenic drug, the humanized anti-VEGF-A monoclonal antibody bevacizumab (Avastin®), was approved for the treatment of metastatic colorectal cancer in combination with standard chemotherapy (Hurwitz et al., 2004).

In addition to the discovery and development of novel anti-angiogenic molecules currently under clinical evaluation (Kerbel and Folkman, 2002; Eichhorn et al., 2004), the pioneering studies of Folkman and co-workers have led to the re-examination of "classical" chemotherapeutic drugs. Thus, it has been shown that many of the most effective chemotherapeutic drugs affected not only tumor cells but also the endothelial compartment of the tumors (Miller et al., 2001). Among cytotoxic agents exhibiting anti-angiogenic properties, microtubule-targeting agents (MTAs) were among the most effective (Miller et al., 2001; Hayot et al., 2002; Wang et al., 2003). These compounds, which can be classically subdivided into microtubule (MT)-stabilizing (e.g. taxanes) and MT-depolymerizing agents (e.g. *Vinca* alkaloids), are already used in the clinic for the treatment of a wide variety of human cancers, including breast, lung, ovarian and prostate as well as hematologic malignancies and childhood cancers. Although their anti-angiogenic activity was first demonstrated over a decade ago in in vitro studies (Ettenson and Gotlieb, 1992; Belotti et al., 1996; Vacca et al., 1999) and confirmed in several in vivo models (Belotti et al., 1996; Klauber et al., 1997; Lau et al., 1999; Vacca et al., 1999; Vacca et al.,

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2002; Hotchkiss et al., 2002), the complex mechanism of their anti-angiogenic action has only begun to be elucidated (Broxterman and Georgopapadakou, 2005). The most interesting anti-angiogenic effects of MTAs have been observed in vitro using very low concentrations and often long-term exposure (Belotti et al., 1996; Vacca et al., 1999; Wang et al., 2003; Grant et al., 2003; Bocci et al., 2002). In addition, these anti-angiogenic effects can also be achieved in vivo by administering MTAs at low and non-cytotoxic doses, well below the maximum tolerated doses, and as frequently as possible, in so-called metronomic schedules (Hanahan et al., 2000; Browder et al., 2000; Kerbel and Kamen, 2004; Munoz et al., 2005). This kind of therapeutic schedule – currently evaluated in several clinical trials – allows the targeting of activated endothelial cells in tumors. Theoretically, such a schedule reduces the toxic side effects as well as the development of drug resistance and it could lead to increased antitumor efficacy (Klement et al., 2002; Kerbel and Kamen, 2004; Munoz et al., 2005). However, as is typical for newly introduced treatments, the initial period of enthusiasm was followed by a period of disillusionment, due to the emergence of anti-angiogenic resistance (Bender et al., 2004; Miller et al., 2005a,b).

Understanding the anti-angiogenic properties of MTAs, and the specific involvement of MTs in angiogenesis, may have important clinical implications, both for the development of new anti-angiogenic drugs and for the elaboration of new therapeutic schedules and combination strategies involving MTAs. Here, we present the latest advances in elucidating the mechanisms involved in the anti-angiogenic effect of MTAs. We also summarize the first results of pre-clinical and clinical studies involving MTAs in anti-angiogenic strategies and discuss the future of these therapies, considering both the advantages and the drawbacks.

2. Unraveling the mechanism of MTAs' anti-angiogenic activity

2.1. Tumor angiogenesis

After an initial avascular state, which can last several years following first acquisition of mutations (Folkman and Kalluri, 2004), tumors enter a second phase that involves a switch to the angiogenic phenotype through the constant recruitment of new blood vessels. This angiogenic switch crucially depends on the balance between pro- and anti-angiogenic signals and is greatly influenced by hypoxia. Schematically, when the angiogenic switch turns on in tumors – due to hypoxic stress, low pH, oncogene activation and infiltration of immune cells – the secretion of pro-angiogenic factors increases, leading to the activation of neighbouring endothelial cells. This activation results in the degradation of the basement membrane (BM), migration of endothelial cells and invasion of the extracellular matrix (ECM), proliferation of endothelial cells and formation of a new capillary network (Fig. 1, top). In physiological angiogenesis, this activation phase is followed by a resolution phase, corresponding to the maturation and stabilization of the newly formed microvasculature by pericytes, inhibition of endothelial proliferation, and BM reconstitution. However, this resolution phase is generally incomplete in tumor angiogenesis, resulting in the formation of abnormal vessels developing unique features (i.e. disorganized, chaotic, heterogeneous and leaky, with irregular blood flow and irregular association with perivascular cells). Although little is known about the final steps of tumor angiogenesis, especially how vascular projections fuse with each other to form loops to enable blood to flow in newly vascularized areas, this pathological angiogenesis has been extensively investigated and reviewed (Carmeliet and Jain, 2000; Kerbel, 2000; Bergers and Benjamin, 2003).

Recent reports have demonstrated that tumor angiogenesis is also supported by the mobilization and the functional incorporation of bone-marrow-derived circulating endothelial progenitor cells (CEPs) (Rafii et al., 2002; Ribatti, 2004). In some cancers, CEP mobilization has actually been shown to correlate with tumor volume and VEGF production (Mancuso et al., 2001; Monestiroli et al., 2001) and to be required for tumor angiogenesis (Lyden et al., 2001). However, the percentage of CEPs incorporated into tumor blood vessels varies with tumor type, ranging from 95% in B6RV2 lymphoma or 55% in Lewis lung carcinoma (Lyden et al., 2001) to scattered incorporation in other cancers such as neuroblastoma and colon carcinoma (Rafii et al., 2002).

Tumor angiogenesis thus appears as a multi-step process, involving both cancer and endothelial cells, that can be inhibited at several levels. Indeed, each of these steps constitutes a putative target for developing anti-angiogenic therapeutic strategies. In this way, there is a wide variety of molecules in development or even in clinical evaluation that aim to specifically target either the activation and proliferation of endothelial cells or the degradation of BM and ECM and subsequent endothelial cell migration (Kerbel and Folkman, 2002; Eichhorn et al., 2004). It has been shown that MTAs can inhibit tumor angiogenesis at different levels and their anti-angiogenic effects can be direct or indirect, affecting endothelial or cancer cells, respectively.

2.2. Direct effects of MTAs on endothelial cells

As mentioned above, the anti-angiogenic properties of MTAs were discovered over a decade ago in in vitro studies (Ettenson and Gotlieb, 1992; Belotti et al., 1996; Vacca et al., 1999). These works showed that both MT-stabilizing agents, such as paclitaxel, and MT-depolymerizing agents, such as vinblastine, can inhibit several functions of endothelial cells involved in angiogenesis: proliferation, migration, capillary-like structure formation on Matrigel™, and degradation of BM and/or secretion of metalloproteases (Belotti et al., 1996; Vacca et al., 1999). These anti-angiogenic properties were then confirmed (Ribatti et al., 2003; Pasquier et al., 2004) and extended to most of the MTAs, including docetaxel (Vacca et