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Prospects for therapeutic inhibition of neuroblastoma angiogenesis

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Abstract

Despite aggressive therapy, survival for advanced stage neuroblastoma remains poor with significant long-term morbidity in disease survivors. High-risk disease features are strongly correlated with tumor vascularity, suggesting that angiogenesis inhibitors may be a useful addition to current therapeutic strategies. However, challenges include the well-known clinical heterogeneity and embryonal origins of this disease, which suggests a complex regulation of neovascularization that may be distinct from epithelial-derived carcinomas. We will review what is understood about angiogenesis-related signaling in neuroblastoma. In particular, we will present evidence that angiogenesis-related molecules are differentially expressed in primary neuroblastomas in a pattern suggesting promotion of a pro-angiogenic phenotype in high-risk tumors and an anti-angiogenic phenotype in low-risk tumors. We will also discuss a variety of vascular inhibition strategies that have been used in neuroblastoma preclinical models including specific inhibition of vascular endothelial growth factor (VEGF) and methionine aminopeptidase 2 (MetAP2). Recent observations that the combination of angiogenesis inhibitors with conventional chemotherapy provides synergy without additive toxicity, suggests the potential use of angiogenesis inhibitors as an adjunct between cycles of conventional cytotoxic therapy. Further identification of critical angiogenic signaling pathways and evaluation of specific inhibitors in preclinical neuroblastoma models should provide justification for future selection and evaluation of angiogenesis inhibitors in clinical trials for high-risk neuroblastoma patients.

Keywords: Neuroblastoma; Angiogenesis; VEGF; MetAP2; Angiogenesis inhibition

1. Introduction

Neuroblastoma is a common pediatric solid tumor for which the prognosis is variable and largely dependent in on measurable tumor biologic features [1]. While neuroblastoma may have the highest rate of spontaneous regression of any human cancer and patients with localized disease may be successfully treated with surgery alone or with minimal therapy, over half of neuroblastoma patients present with metastatic disease and/or adverse tumor-specific biologic feature such

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as *MYCN* amplification. Despite intensive multimodal therapy, these 'high-risk' patients have a cure rate of less than 40% [2], and children who survive often experience significant long-term morbidity as a result of receiving dose-intensive therapy at a young age [3]. Novel therapeutic approaches that will both improve patient survival and decrease treatment-related toxicity are clearly needed.

Tumor vascularity is correlated with an aggressive phenotype in neuroblastoma suggesting that antiangiogenic therapy might be a useful addition to current high-risk neuroblastoma treatment strategies. High-risk neuroblastomas are highly vascular, and while some controversy exists [4,5], most authors agree that tumor vascularity is strongly correlated with adverse prognostic features including the presence of metastases at diagnosis, *MYCN* amplification, unfavorable histopathology and poor survival probability [6]. In contrast, localized tumors with favorable biological features are often less vascular and have a rich stromal component composed largely of nonmalignant Schwann cells which have recently been shown to produce potent antiangiogenic molecules [7,8], Thus, neuroblastoma vascularity is intrinsically related to the underlying tumor biology as well as tumor-host interactions.

Unlike adult solid malignancies that generally arise in fully differentiated tissues, neuroblastoma and the other embryonal malignancies of childhood likely arise during normal tissue development. Thus, the fundamental processes of providing a blood supply for the growing primary tumor mass may be inherently different. We speculate that neuroblastoma neovascularization may rely more on blood vessel co-option rather than true angiogenesis. This is an important distinction because of the growing body of evidence that the molecular regulation of these processes are different [9,10]. In order to prioritize development of antiangiogenic compounds, delineation of the most clinically relevant angiogenic signaling pathways intrinsic to neuroblastoma, and the most efficient ways to inhibit them is needed. The most appropriate use of antiangiogenic strategies as potential adjuncts to current treatment regimens can then be determined.

2. Evidence of differential expression of angiogenesis-related genes in neuroblastoma

2.1. Neuroblastoma angiogenesis is regulated by potentially many pro-angiogenic cytokines

Signaling through the vascular endothelial growth factor (VEGF) family of receptors plays a crucial role in both physiologic and pathologic angiogenesis [11]. In neuroblastoma, VEGF is expressed in the majority of cell lines and primary tumors [12,13]. Neuroblastoma cells secrete VEGF, and neuroblastoma cell-conditioned media stimulates growth of human umbilical vein endothelial cells in vitro that can be inhibited by treatment with a monoclonal antibody to VEGF [14]. In vivo, treatment of a neuroblastoma xenograft model with anti-VEGF reagents results in decreased tumor vascularity [15]. The VEGF receptor 2 (VEGFR2) is co-expressed with VEGF in many neuroblastoma cell lines and primary tumors suggesting the presence of an additional autocrine growth loop [12]. However, although VEGF is clearly important in neuroblastoma, treatment of a xenograft neuroblastoma model with anti-VEGF antibody causes only partial tumor growth inhibition, and although decreased vascularity is observed, angiography shows cooption of host vasculature that likely serves as a source of blood supply during initial tumor growth, suggesting VEGF blockade is not sufficient to inhibit angiogenesis and that multiple angiogenic factors play a role in neuroblastoma [15].

Several pro-angiogenic factors, including VEGF, have been shown to be differentially expressed in primary neuroblastomas in a pattern suggesting promotion of an angiogenic phenotype in high-risk tumors. Eggert and colleagues have shown that the VEGF, VEGFB, VEGFC, basic fibroblast growth factor (FGF2), ANGPT2 transforming growth factor alpha (TGFA) and platelet derived growth factor (PDGF) genes are more highly expressed in advanced staged tumors as compared to low stage tumors, and that the expression of PDGFA was inversely correlated with patient survival in a small series of patient tumor samples [16]. Edreich-Epstein and colleagues have demonstrated that the endothelial integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are expressed in the neuroblastoma stromal cell compartment and expression levels correlate with advanced disease features [17]. In addition, the matrix