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The Different Faces
of Angiogenesis in Neuroblastoma

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1. Introduction

Neuroblastoma represents one of the most frequent solid tumour of childhood, arising along the sympathetic nervous system, most frequently in the adrenal gland. Clinically, neuroblastoma is an heterogeneous entity and prognosis vary widely according to different biological variables (such as DNA ploidy and MYCN amplification), patient age, anatomical location and tumour stage at diagnosis (Maris et al., 2002; Schwab et al., 2003). Unfortunately, the majority of patients show systemic disease at the time of diagnosis, with rapid tumour progression and fatal outcome. According to this clinical heterogeneity, the International Pathology Classification System has identified a broad spectrum of different histological features, ranging from undifferentiated/poorly differentiated to differentiating/fully differentiated lesions, with progressive increase in the Schwannian component and differentiation of neuroblastic cells towards a mature ganglionic phenotype (Shimada et al., 1999; Cohn et al., 2009). Of note, as others paediatric tumours, neuroblastoma displays the capacity to undergo spontaneous regression and/or differentiation into benign fully mature lesions, with increase of Schwannian stroma and differentiating/differentiated neuroblasts, that biologically resemble ganglion cells, directly correlated with tumour maturation and better prognosis (Ambros et al., 2002; Haas et al., 1988). Indeed, differentiating and well-differentiated neuroblastic lesions with low mitosis/karyorrhexis index are classified by International Neuroblastoma Pathology Classification consensus as tumours associated with a better prognosis and a higher rate of cure (Peuchmaur et al., 2003; Shimada et al., 1999).

The role of angiogenesis in tumour growth and progression represents a major subject in modern oncology. It is generally accepted that angiogenesis promotes tumour growth and is essential for invasion of surrounding tissues and metastasis. Angiogenesis is tidily regulated by the balance of numerous pro-angiogenic and anti-angiogenic factors. Tumours that fail to recruit new blood vessels remain dormant while tumours characterized by prominent neo-angiogenesis growth faster and display an aggressive behaviour (Carmeliet et al., 2003; Ribatti et al., 1999). A large variety of pro-angiogenic factors have been identified so far to play an important role in the induction of angiogenesis in neuroblastic lesions, including vascular endothelial growth factor (VEGF), interleukin-8 (IL-8), fibroblast growth factor-2 (FGF-2), and angiopoietins (Eggert et al., 2000; Ribatti et al., 1998). High vascular index in

neuroblastoma have been demonstrated to correlate with poor prognosis, suggesting a tide correlation between aggressive tumour behaviour and active angiogenesis (Katzenstein et al., 2000). These observations point out to an important role of promising emerging anti-angiogenic strategies for the treatment of neuroblastoma, a lesion characterized by a high degree of vascularity (Pastorino et al., 2009; Marimpietri et al., 2007; Ribatti et al., 2005; Rossler et al., 2008). In this review the recent findings in neuroblastoma angiogenesis as well as the most recent advances in the development of novel anti-angiogenetic approaches in the treatment of neuroblastoma will be discussed.

However, although it has been well established that angiogenesis in neuroblastoma correlates with tumour progression, advanced stages and worst prognosis (Carmeliet et al., 2003), little is known about the role of angiogenesis in the maturation phase of undifferentiated neuroblastomas towards a more differentiated phenotype. As previously described, neuroblastic lesions show a wide spectrum of histological variability that reflects different steps of tumour maturation, whose molecular mechanisms are not still fully understood. Recent findings have suggested that the same molecular pathways driving the development of normal neuroectodermal-derived tissues might also be involved in neuroblastoma maturation (Christiansen et al., 2000; Hoehner et al., 1998). Between these, angiogenesis plays a key role during neural differentiation, exerting a trophic activity (Ribatti et al., 2007; Kitlinska et al., 2005). In line with these observations, recent findings have shown that specific angiogenetic pathways play a crucial role during the maturation phase of neuroblastic tumours and contribute to neuroblast maturation (Poliani et al., 2007).

Finally, it has been suggested that cross-talk between Schwann cells and neuroblasts influences the biology and clinical behaviour of neuroblastic tumours (Kwiatkowski et al., 1998). Schwannian stroma rich tumours have been shown to have low vascularity, suggesting that Schwann cells may influence neuroblast biology by producing soluble factors capable of inducing neuroblast differentiation and inhibiting tumour neo-angiogenesis (Ambros et al., 1996).

These observations indicate a different role of different angiogenetic pathways in neuroblastoma, with some of them mimicking physiological steps leading to maturation of vasculature in developing normal neuroectodermal-derived tissues, thus contributing to the maturation stage of neuroblastic tumours, and others that sustain tumour neo-angiogenesis and contribute to tumour aggressiveness and progression.

In conclusions, this review will focus on the most recent advances in the field of angiogenesis in neuroblastic lesions keeping in mind that understanding the mechanisms of angiogenesis will provide the basis for a rational and targeted approach to the development of specific treatments targeting tumour angiogenesis or promoting neuroblastic maturation in patients affected by neuroblastoma.

2. Role of angiogenesis in tumour growth and progression

During solid tumour development, an avascular phase precede the fully active vascular phase. Assuming that tumour development is dependent on angiogenesis and that this depends on the release of angiogenic factors, the acquisition of an angiogenic ability can be considered as an expression of progression from neoplastic transformation to tumour growth and metastasis (Ribatti et al., 1999). The avascular phase appears to correspond to a small colony of neoplastic cells that reaches a steady state before proliferation and

acquisition of an invasive behaviour. In fact, dormant tumours can been found during autopsies of individuals who died of causes other than cancer (Black & Welch, 1993). Moreover, in situ carcinomas are a frequent finding in individuals aged 50 to 70 years who died of trauma, but are diagnosed in only 0.1% of patients during life. Malignant tumours can grow beyond the critical size of 2 mm at their site of origin by exploiting the host's pre-existing vessels. These finding support the notion that only a very small subset of dormant tumours enters the vascular phase and rapidly progress.

The role of angiogenesis in tumour growth has become evident thanks to a series of studies demonstrating that tumour progression is clearly related to the degree of angiogenesis, as seen in different types of tumours, including neuroblastoma. In fact, high vascular density and active angiogenesis in neuroblastoma have been shown to correlate with poor prognosis and tumour progression (Ribatti et al., 2004).

Tumour angiogenesis is strictly linked to the switch in the balance between positive and negative regulators, and mainly depends on the release by neoplastic cells of specific growth factors for endothelial cells, that stimulate the growth of the host's blood vessels or the down-regulation of natural angiogenic inhibitors. In normal tissues, vascular quiescence is maintained by the dominant influence of endogenous angiogenic inhibitors over angiogenic stimuli. This switch depends on increased production of one or more positive regulators of angiogenesis, such as vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), IL-8, placental growth factor (PlGF), transforming growth factor-β (TGF-β), platelet derived growth factor (PDGF), pleiotrophins and others. These factors can be directly produced by tumour cells, mobilized from the extracellular matrix, or released from host cells recruited to the tumour. The switch clearly involves more than a simple up-regulation of angiogenic activity and has thus been regarded as the result of the net balance between positive and negative regulators (Ribatti et al., 2007).

Considerable differences exist between normal and tumour vasculature. Tumour endothelial cells may divide up to 50 times more frequently than normal endothelial cells and are qualified by peculiar molecular and morphological features, mostly related to an immature phenotype. This immaturity of tumour vessels led H. Dvorak to define a tumour as "a wound that never heals" (Dvorak, 1986).

Moreover, although the tumour vasculature originates from the host vessels engaging similar angiogenetic mechanisms, the organization may differ dramatically depending on the tumour type and location and tumour-associated blood vessels display many structural and functional abnormalities (Ribatti et al., 2006). Their unusual leakage, rapid growth and remodelling capacity, along with the expression of distinctive surface molecules, mediate the dissemination of neoplastic cells into the bloodstream and contribute to maintain the tumour microenvironment. Similar to normal blood vessels, tumour vasculature consists of endothelial cells, pericytes and their enveloping basement membrane. Common features, regardless of their origin, size and growth pattern, include the absence of an hierarchy, the formation of large-caliber sinusoidal vessels and a markedly heterogeneous density.

A complex interrelationship has also been described between tumour hypoxia and tumour angiogenesis. Hypoxia in tumours develops both as chronic hypoxia, resulting from long diffusion distances between tumour vessels, and/or acute hypoxia, resulting from a transient collapse of tumour vessels. Most of the solid tumours contain a hypoxic microenvironment, a condition that is associated with poor prognosis and resistance to

treatment. Of note, the production of important angiogenic factors, such as FGF-2, VEGF, TGF-β , TNF- β and IL-8, is regulated by hypoxia. Accordingly, VEGF-mRNA expression is rapidly and reversibly induced by exposure of cultured endothelial cells to low PO_2 (Levy et al., 1995).

There are also increasing evidences that stromal cells as well as inflammatory cells within the tumour, such as lymphocytes, neutrophils, macrophages and mast cells, cooperate with endothelial and cancer cells in promoting angiogenesis, by mean of the production of different growth factors and proteases (Parket et al., 2000).

Finally, it is increasingly recognized that oncogenes, such as mutated RAS or SRC, may also contribute to tumour angiogenesis by enhancing the production of pro-angiogenic factors, such as VEGF and inhibitors of angiogenesis such as thrombospondin-1 (TSP-1) (Rak et al., 1995; Ellis et al., 1998). In line with these observations it has been demonstrated that down-regulation of RAS-oncogene in a melanoma driven by doxycycline-inducible ras led to tumour regression within 12 days (Tang et al., 2005) and that cells expressing low levels of RAS remain dormant and non-angiogenic, whereas cells expressing high levels of RAS produce a full-blown tumours with marked up-regulation of TSP-1 levels (Watnick et al., 2003).

3. Role of angiogenesis in the maturation phase of neuroblastic lesions

As described previously a correlation between angiogenesis and poor outcome in human neuroblastomas have been demonstrated (Eggert et al., 2000; Ribatti et al., 2001; Ribatti et al., 2002; Ribatti et al., 2004). However, little is known about the role of angiogenesis in the maturation of undifferentiated neuroblasts towards a mature ganglionic phenotype. In fact, as others paediatric tumours, neuroblastoma displays the capacity to undergo spontaneous regression in infants and/or differentiation into benign ganglioneuroma in older patients (Pritchard et al., 1994; Maris et al., 2002). This peculiarity reflects the wide spectrum of histological variability within the neuroblastic tumours, ranging from undifferentiated to differentiating or fully differentiated lesions (Figure 1, Panel A). Moreover, along with young age (<18 months) and localized disease, differentiation has been strongly correlated to a better prognosis (Ambros et al., 1996). Spontaneous or treatment-induced maturation characterizes a subgroup of neuroblastomas and constitutes the basis for maturation targeting therapies, such as retinoic acid treatment (Ferrari-Toninelli et al., 2010; Matthay et al., 1999; Mora et al., 2004; Giannini et al., 2000). Nevertheless, the molecular mechanisms that drive maturation of neuroblastic lesions are still poorly understood. Schwannian stroma cells have been claimed to be implicated in differentiation of neuroblastomas (Coco et al., 2005; Ambros et al., 1996) as well as a large variety of transcriptional factors and different genes involved in neural development and differentiation of normal neuroectodermal-derived tissues (Hoehner et al. 1998; Kitlinska et al., 2005; Ferrari-Toninelli et al., 2010; Christiansen et al., 2000; Koppen et al., 2008). Gene expression profiles studies recently allowed to identify genes differentially expressed between undifferentiated and differentiated neuroblastic lesions. These studies led to the identification of different molecular patterns of differentiation, ranging from undifferentiated pre-adrenergic/adrenergic neuroblastomas with frequent MYCN amplification to cholinergic-committed or fully cholinergic neuroblastomas that may reflect a fully differentiated phenotype (Bourdeaut et al., 2009).

Angiogenesis has been described to plays a key role during neural differentiation, exerting a trophic activity and promoting the maturation of vasculature in developing normal neuronal tissue (Kitlinska et al., 2005). We have recently described that the human carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), a transmembrane glycoprotein that belongs to the carcinoembryonic antigen (CEA) gene family, is peculiarly expressed in the microvessels among differentiating neuroblastic/ganglion cells and is related to the tumour maturation (Poliani et al., 2007). CEACAM1 exerts a large variety of biological functions, including a strong pro-angiogenic role during the activation phase of angiogenesis, being expressed in the developing immature blood vessels of both in newly normal formed vessels and in different tumours (Wagener et al., 2000; Volpert et al., 2002). Microvascular endothelial cells over-expressing CEACAM1 show up-regulation of various angiogenic factors, including VEGF, angiopoietins and IL-8, indicating that CEACAM1 expression in endothelial cells switches them to an angiogenic phenotype, a mechanism active during the formation of new vessels in physiological conditions such as wound healing and embryonic development, but silent in endothelial cells of large mature quiescent vessels (Wagener et al., 2000). Accordingly, CEACAM1 has been found to be expressed transiently in the microvessels of the developing central nervous system (Sawa et al., 1994), suggesting a role in the induction of capillary formation during tissue development rather than in the maintenance of mature vasculature. Interestingly, CEACAM1 have been also found to be expressed in microvessels of the normal adrenal gland, particularly during fetal development (Figure 1, Panel B). We have investigated the role of CEACAM1/VEGF-mediated angiogenesis in neuroblastic tumours at different stages of maturation, demonstrating that CEACAM1 is transiently expressed in microvessels among differentiating neuroblast/ganglion cells whereas it is completely absent in poorly differentiated/undifferentiated tumours as well as in fully mature ganglioneuromas. Interestingly, strong VEGF expression was observed in differentiating neuroblast/ganglion cells adjacent to CEACAM1 positive microvessels (Figure 1, Panel C). In contrast, CEACAM1 is not expressed in poorly differentiated/undifferentiated neuroblastomas, characterized by high levels of VEGF and VEGFR (FLK-1), in keeping with several studies reporting the association of VEGF expression with tumour aggressiveness and a poor prognosis (Backman et al., 2002; Eggert et al., 2000) and suggesting that in poorly differentiated/undifferentiated lesions VEGF expression is independent from CEACAM1 and may follow different molecular pathways. Accordingly, we have also demonstrated that conditioned medium from human neuroblastoma SH-SY5Y cell lines collected at different stages of differentiation caused a progressive in vitro up-regulation of CEACAM1 expression in human umbilical vein endothelial cells (Poliani et al., 2007).

Overall, our data indicate a crucial role of CEACAM1/VEGF mediated angiogenesis during the maturation phase of neuroblastic tumours, mimicking physiologic events leading to maturation of vasculature in developing neuroectodermal-derived tissues. Endothelial up-regulation of CEACAM-1 activate angiogenesis via increased expression of VEGF in neuroblastic cells triggering an angiogenic cascade that promote and maintain VEGF induced angiogenesis. This feature could be involved in the complex mechanism driving the switch from immature to differentiating forms of neuroblastomas. Conversely, in poorly differentiated/undifferentiated neuroblastomas the VEGF sustained angiogenesis does not reproduce physiological steps, it is associated with tumour aggressiveness and may involve other molecular pathways.
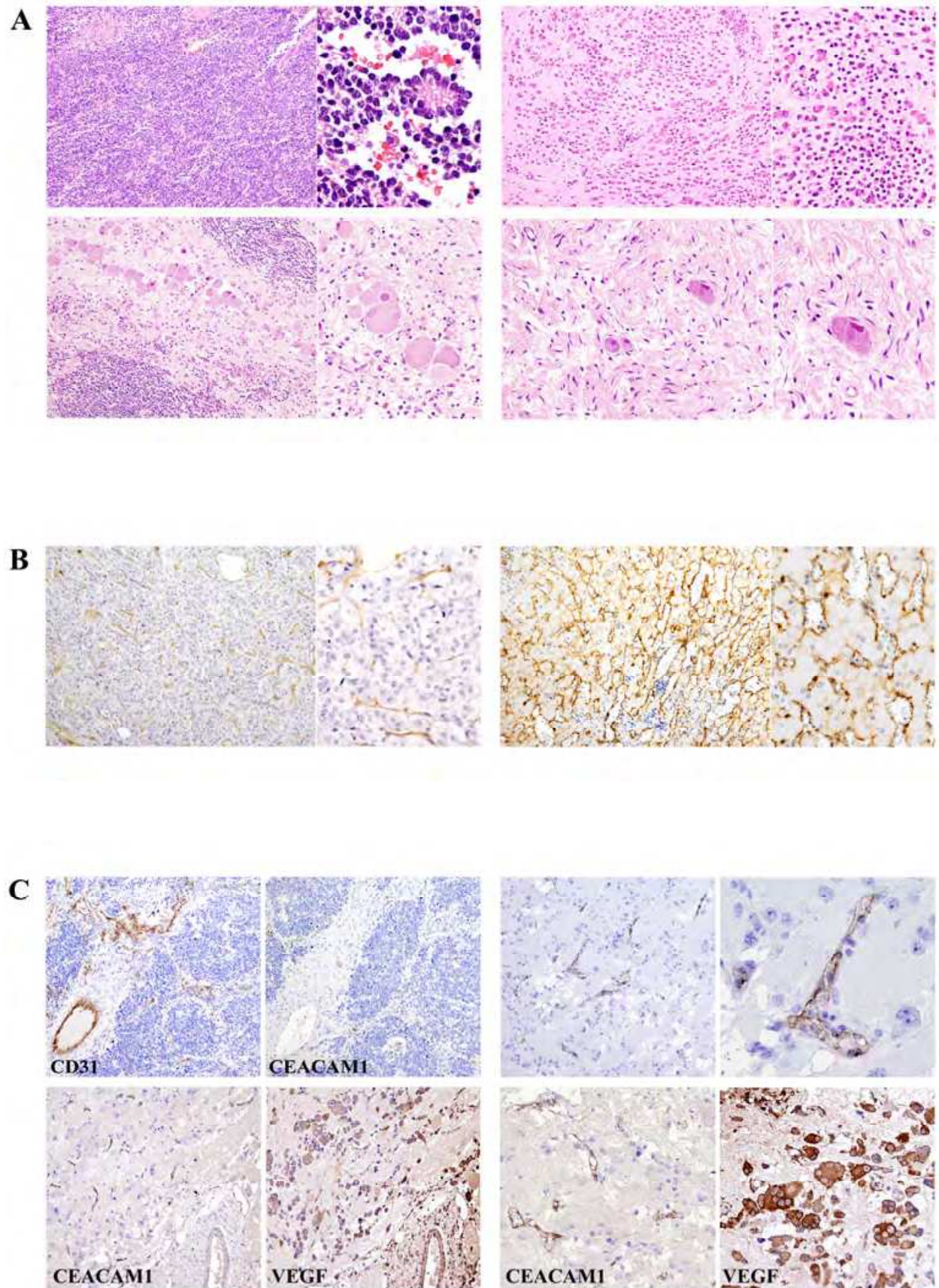
Fig. 1. Histological variants of neuroblastic tumours and CEACAM1/VEGF expression.

Panel A: neuroblastic lesions show a wide spectrum of different histological variants reflecting different maturation phases, ranging from undifferentiated/poorly differentiated neuroblastomas with neuronal rosettes (upper left images), differentiating lesions with larger cells acquiring a ganglionic phenotype (upper right images), mixed lesions (ganglioneuroblastomas) with both immature and fully mature ganglionic cells (lower left panels) and fully differentiated lesions with mature ganglion cells and progressive increase in the Schwannian component (lower right panels). *Panel B:* CEACAM1 is only weakly expressed in the microvessels of normal adult adrenal gland (left images) while strong and diffuse expression is present in the developing microvessels of normal fetal adrenal gland (right images). *Panel C:* Serial sections from a representative case of undifferentiated neuroblastoma showing no expression of CEACAM1 in the tumour vessels that stain positive for CD31 (upper left images). On the contrary, differentiating neuroblastomas show CEACAM1-positive microvessels adjacent to differentiating neuroblastic/ganglion cells (upper right images). Serial tissue sections from a representative case of differentiating neuroblastoma show CEACAM1-positive microvessels adjacent to VEGF-positive differentiating neuroblastic cells (lower images, on the right a detail at higher magnification).

4. Role of Schwann cells in angiogenesis

As previously described, neuroblastic lesions exhibits a large variety of histological features reflecting the different steps of tumour maturation, ranging from immature neuroblastoma, with small undifferentiated neuroblasts with scarce Schwannian stroma, to progressively maturing neuroblastomas composed of larger neuroblastic/ganglion-like cells with abundant Schwannian stroma. Diagnostic categories of Schwannian stroma-rich/dominant tumours include mature and maturing ganglioneuroma and ganglioneuroblastoma of intermixed and nodular type. Categories of Schwannian stroma-poor neuroblastoma tumours include differentiating, poorly differentiated and undifferentiated (Shimada et al., 1999). Importantly, the increasing presence of Schwannian stroma and differentiating/differentiated neuroblasts, that biologically resemble ganglion cells, directly correlates with tumour maturation and better prognosis, with the exception of nodular ganglioneuroblastoma, (Peuchmaur et al., 2003; Nagoshi et al., 1992).

Interestingly, in addition to a more differentiated phenotype, Schwannian stroma rich tumours also display low vascularity (Meitar et al., 1996). A correlation between low vascular density and absence of microvascular proliferation in Schwannian stroma-rich tumours have been demonstrated (Peddinti et al., 2007). On the contrary, high vascular index and abnormal blood vessels have been associated to clinical aggressiveness in Schwannian stroma-poor neuroblastoma tumours (Meitar et al., 1996; Peddinti et al., 2007).

This association between abundant Schwannian stroma, neuronal differentiation and decrease vascularity had led to the hypothesis that Schwann cells may secrete soluble factors capable of inducing neuroblast differentiation and inhibit angiogenesis (Huang et al., 2000). In fact, Schwann cells have been shown to secrete potent angiogenesis inhibitors, such as secreted protein acid rich in cysteine (SPARC) and pigment epithelium-derived factor

(PEDF) (Chlenski et al., 2002; Crawford et al., 2001). Moreover, angiogenesis is inhibited in neuroblastoma xenograft model in which mouse Schwann cells were induced to infiltrate the tumour by engrafting neuroblastoma cells in the sciatic nerve of nude mice (Liu et al., 2005). Moreover, significantly higher number of cancer-associated fibroblasts have been demonstrated in Schwannian stroma-poor tumours compared to Schwannian stroma rich/dominant ganglioneuroblastomas/ganglioneuromas, consistent with the established pro-angiogenic function of cancer-associated fibroblasts (Zeine et al., 2009).

5. Novel treatments targeting angiogenesis

It has been already discussed in the previous paragraphs that high vascularity is a peculiar feature of neuroblastoma and promote tumour growth and aggressiveness. A large spectrum of angiogenic factors, including VEGF, have been reported to be expressed in neuroblastomas. Understanding the key mechanisms promoting angiogenesis in neuroblastoma represent a clue step in the development of effective anti-angiogenic strategies. The following paragraphs will aid to summarize the state of the art of the most current and experimental anti-angiogenic strategies in the treatment of neuroblastoma.

5.1 Retinoids

Retinoic acid has been shown to induce neuroblastoma maturation throughout different molecular pathways (Ferrari-Toninelli, 2010). In patients after autologous stem cell transplantation retinoic acid has been introduced as a maintenance treatment (Matthay et al., 1999). The synthetic retinoid N-(4-hydroxyphenyl) retinamide (fenretinide, HPR) inhibits human neuroblastoma cell growth through the induction of programmed cell death (Ponzoni et al., 1995). The contribution of retinoic acid in the control of tumour angiogenesis has been recently demonstrated. HPR inhibits angiogenesis induced by neuroblastoma specimens implanted onto the chorioallantoic membrane (CAM) (Ribatti et al., 2001). Moreover, retinoic acid have been also shown to induce expression of the endogenous angiogenesis inhibitor TSP-1 (Castle et al., 1992).

5.2 TNP-470

TNP-470 treatment have been shown to induce a reduction of tumour growth and microvascular density in mouse model xenograft of poorly differentiated human neuroblastoma cell line SHSY5Y. Moreover, TNP 470 treatment improved animal survival and reduced tumour growth of primary and metastatic murine neuroblastoma (Nagabuchi et al., 1997). Interestingly, TNP-470 also effectively inhibits neuroblastoma growth in animals with minimal disease treated before tumour were clinically apparent after subcutaneous injection of neuroblastoma cells. Furthermore, when TNP-470 is administered to animals with small tumours, the rate of growth is reduced, while does not significantly altered the tumour growth rate when it is administered to animals with large tumours. Moreover, TNP-470-treated tumours exhibited striking chromaffin differentiation, suggesting that by inhibiting angiogenesis, TNP-470 induces metabolic stress resulting in chromaffin differentiation (Katzenstein et al., 1999). A further study, confirmed that TNP-470 effectively inhibits neuroblastoma xenograft growth when administered as a single agent or in association with other chemotherapic agents (Shusterman et al., 2001), indicating that TNP-470 may be useful as adjuvant therapy for high-risk neuroblastoma.

5.3 Thalidomide

Kaicker et al. (2003) investigated the anti-angiogenic and anti-tumour properties of thalidomide in a xenograft model of human neuroblastoma. Indeed, thalidomide treatment did not significantly alter tumour growth as compared with control mice injected with neuroblastoma cells. However, thalidomide suppressed angiogenesis, as demonstrated both by fluorescent angiography and immunohistochemical staining, and induces apoptosis of endothelial cells in neuroblastoma xenografts (Kaicker et al., 2003).

5.4 Endostatin

Davidoff et al. (2001) developed a gene therapy approach in which the genes encoding for endostatin were delivered to murine neuroblastoma cells prior to inoculation of tumour cells into syngenic immunocompetent mice. Although the effect of either angiogenesis inhibition or immunomodulation alone resulted in only a modest delay in tumour growth, when these approaches were used in combination, prevention of the formation of appreciable tumours was effected in 63% mice (Davidoff et al., 2001). Interestingly, continuous administration of recombinant endostatin resulted even in a more significant tumour regression as compared to intermittent administration in neuroblastoma xenografts (Kuroiwa et al., 2003). Streck et al. (2004) evaluated the influence of a pre-existing primary neuroblastoma xenografts on the growth of a new second subcutaneously injected tumour, hypothesizing that an existing primary tumour could inhibit the growth of a secondary tumour, in part mediated by tumour release of endostatin. Decreased angiogenesis and increased apoptosis were seen in the secondary tumours, along with decrease of the weight of liver metastases. Although no difference in microvessel density was seen between groups, apoptosis was seen to significantly increase when the primary tumour was retained (Streck et al., 2004).

5.5 Angiostatin

Pre-clinical use of angiostatin in neuroblastoma has been studied in a gene therapy approach using a recombinant adenovirus encoding the human angiostatin kringle 1-3 directly fused to human serum albumin HSA (Adk3-HSA). However, in human neuroblastoma xenograft models, intravenous injection of this vector showed no delay in tumour growth when compared to tumours treated with the control viral vector (Joseph et al., 2003).

5.6 Thrombospondin

ABT-510, a peptide derivative of TSP-1, significantly suppressed the growth of neuroblastoma xenografts established from two different MYCN-amplified cell lines. In combination with the histone deacetylase inhibitor valproic acid, ABT-510 inhibited more effectively the growth of xenografts compared to single-agent treatment (Yang et al., 2007).

5.7 Bortezomib

Bortezomib is a selective and reversible inhibitor of proteasome that shows a potent antitumor activity and that has been shown to inhibit proliferation and colony formation of neuroblastoma cell lines in a time- and dose-dependent manner. Moreover, bortezomib has been also shown to inhibit angiogenesis in CAMs stimulated by conditioned medium from

either neuroblastoma cell lines, xenografts and primary biopsy specimens. (Brignole et al., 2006)

5.8 Combined therapies

Combined vinblastine and rapamycin therapy displayed synergistic inhibition of human neuroblastoma-related angiogenesis (Marimpietri et al., 2007). A significant inhibition of tumour growth and microvessel density was obtained in neuroblastoma-bearing mice when treated with vinblastine or rapamycin throughout the down-modulation of both VEGF and VEGFR-2 expression, as shown also by human neuroblastoma biopsy specimens in the CAM assay. (Brignole et al., 2006). The antitumor activity of bortezomib in combination with fenretinide has also been considered. The single compounds were able to induce a dose-dependent inhibition of cell proliferation, but significant enhanced of the anti-proliferative effects has been demonstrated for the drugs used in combination. Bortezomib and fenretinide in association triggered increased apoptosis and significantly increase survival. Histologic examination and CAM assay of the primary tumours showed that combined therapeutic activity was strictly associated to anti-angiogenic mechanisms (Pagnan et al., 2009).

5.9 Anti-VEGF and anti-VEGF receptor-2 antibodies

In a murine model of human neuroblastoma the monoclonal antibody against VEGF partially suppresses tumour growth (Kim et al., 2001). In a further study, topotecan, either in association with anti-VEGF treatment or alone, have been shown to significantly suppress neuroblastoma xenograft growth in comparison with controls or anti-VEGF treated mice (Kim et al., 2002b). Combined topotecan and anti-VEGF treatment significantly inhibited rebound tumour growth. Moreover, high-affinity blockade of VEGF, using the VEGF-TRAP, a composite decoy receptor based on VEGFR-1 and VEGFR-2 fused to an Fc segment of IgG1, dramatically decreases tumour vasculature in a xenograft model of neuroblastoma (Kim et al., 2002b). Pre-clinical studies using bevacizumab, an inhibitor of VEGF, in neuroblastoma demonstrated a significant reduction in tumour growth without major toxicity (Segerstrom et al., 2006). A further study demonstrated that bevacizumab induces alterations in tumour vessels that, in turn, allows improved delivery and efficacy of chemotherapy (Dickson et al., 2007). Continuous treatment with low dose of vinblastine, a novel monoclonal anti-VEGFR-2 antibody (DC101) or both agents together have also been investigated(Klement et al., 2000). Both DC101 and low-dose vinblastine treatment individually resulted in significant, but ultimately transient, xenograft regression and decrease of tumour vascularity. Remarkably, the combination therapy resulted in a full and sustained regression of large established tumours, without any consequent increase in host toxicity or any signs of acquired drug resistance during the course of treatment which lasted more than 6 months. Activity of DC101 was also shown in a neuroblastoma cell line over-expressing MYCN, in which tumour growth delay was increased by simultaneous irradiation (Gong et al., 2003).

5.10 Inhibitors of the tyrosine kinase of VEGFRs

SU5416, a specific inhibitor of VEGFR-1 and VEGFR-2, has been investigated as angiogenesis inhibitor strategy in neuroblastoma. Efficacy was increased when SU5416 was administered in combination with irradiation or chemotherapy (Backman et al., 2002).

5.11 Novel vascular targeting

Tumour vascular targeting strategies that target and disrupt the existent vessel network of growing tumours have been actively perused (Sieman et al., 2004). Between them, vascular disrupting agents (VDAs), such as ligand-targeted and/or drug-conjugated liposomes have been recently introduced (Thorpe, 2004). Pastorino et al. have described a novel strategy of achieving an anti-neuroblastoma response using a peptide-targeted formulation of liposomal doxorubicin (Pastorino et al., 2003; Pastorino et al., 2006). This approach was active against both established primary tumours and early-phase metastases and induced a selective apoptosis of tumour endothelial cells and destruction of tumour vasculature. This novel strategy markedly enhanced the therapeutic use of doxorubicin and enabled metronomic administration. A dual mechanism of action has been proposed: indirect tumour cell killing via the destruction of tumour endothelium by NGR-targeted liposomes and direct tumour cell killing via localization of liposomal doxorubicin to the tumour interstitial space. This approach has been validated by evaluating tumour vasculature in several murine xenografts of doxorubicin-resistant human cancers, including lung, ovarian and neuroblastoma (Pastorino et al., 2008). The TVT-DOX used in this study was manufactured as large-scale Good Manufacturing Practice (GMP) preparation suited to human clinical trials. Opposing to the untargeted formulation of DOXIL®/CAELYX®, which has been approved for clinical use for the treatment of ovarian cancer and other solid tumours (Northfelt et al., 1997; Gordon et al., 2000), the GMP preparation of TVT-DOX has been demonstrated to effectively target the angiogenic tumour blood vessels and thus, indirectly, kill the tumour cells. Whereas tumour endothelial cell is well accepted as a valid target for cancer therapies, pericytes have been only recently recognized as a novel target for cancer treatment. The membrane associated protease APA is expressed in pericytes associated with tumour blood vessels and its expression has been correlated with tumour progression (Schlingemann et al., 1996; Marchio et al., 2004). Recently, Loi et al (2010) have developed a novel liposomal formulation targeting APA that displays anti-tumour effects and prolongs survival in human neuroblastoma-bearing mice with a significant increase in the level of apoptosis in tumours cells and a pronounced destruction of the tumour vasculature(Loi et al., 2010).

6. Conclusion

Angiogenesis in neuroblastoma has been thoroughly studied. As other solid tumours, the major role of angiogenesis resides in tumour development, maintenance and progression. These observations have led to the development of several different therapeutic strategies aimed to disrupt tumour vascularity. However, in designing novel therapeutic approaches a role of angiogenesis in promoting the mechanisms of maturation of neuroblastic cells has also to be considered. In fact, different angiogenetic pathways are involved in neuroblastoma, some of them sustaining tumour neo-angiogenesis and contributing to tumour aggressiveness and others contributing to the maturation of neuroblastic tumours.

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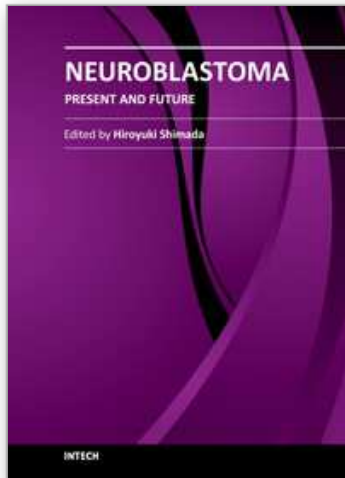
Neuroblastoma - Present and Future

Edited by Prof. Hiroyuki Shimada

Neuroblastoma, once called "enigmatic", due to "unpredictable" clinical behaviors, is composed of biologically diverse tumors. Molecular/genomic properties unique to the individual tumors closely link to the clinical outcomes of patients. Establishing risk stratification models after analyzing biologic characteristics of each case has made a great success in patient management. However, the trend of improving survival rates in neuroblastoma over the last 30 years has started to level off, and currently available treatment modalities have almost reached to their maximized intensity. Furthermore, aggressive treatment causes significant long-term morbidities to the survivors. We really need to make the next step to the level of personalized medicine with more precise understanding of neuroblastoma biology. This book includes useful data and insights from the world's experts in this field. I believe this book can make an excellent contribution to all the investigators working hard and fighting for the children stricken by this disease.

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