Coagulation and angiogenic gene expression profiles are defined by molecular subgroups of medulloblastoma- evidence for growth factor-thrombin cross-talk

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ABSTRACT

Background. The coagulation system becomes activated during progression and therapy of high grade brain tumors. Triggering tissue factor (F3/TF) and thrombin receptors (F2R/PAR-1) may influence the vascular tumor microenvironment and angiogenesis irrespective of clinically apparent thrombosis. These processes are poorly understood in medulloblastoma (MB), in which diverse oncogenic pathways define at least four molecular disease subtypes (WNT, SHH, Group 3, Group 4). We asked whether there is a link between molecular subtype and the network of vascular regulators expressed in MB.

Methods. Using R2: microarray analysis and visualization platform, we mined MB datasets for differential expression of vascular (coagulation and angiogenesis) - related genes, and explored their link to known oncogenic drivers. We evaluated the functional significance of this link in DAOY cells *in vitro* following growth factor and thrombin stimulation.

Results. The coagulome and angiome differ across MB subtypes. F3/TF and F2R/PAR-1 mRNA expression are upregulated in SHH tumors and correlate with higher levels of MET receptor. Cultured DAOY (MB) cells exhibit an upregulation of F3/TF and F2R/PAR-1 following combined SHH and MET ligand (HGF) treatment. These factors cooperate with thrombin impacting the profile of vascular regulators including interleukin 1β (IL1B) and chondromodulin 1 (LECT1).

Conclusions. Coagulation pathway sensors (F3/TF, F2R/PAR-1) are expressed in MB in a subtype-specific manner, and may be functionally linked with SHH and MET circuitry. Thus coagulation system perturbations may elicit subtype/context-specific changes in vascular and cellular responses in MB.

Keywords: angiogenesis modulating agents, blood coagulation factors, medulloblastoma, molecular pathology, oncogenes

INTRODUCTION

The vascular system is engaged in brain tumor progression through natural processes such as angiogenesis, or due to intervention including surgery, chemotherapy, and radiation. Indeed, high grade brain tumors may exhibit significant hemostatic perturbations including haemorrhage and thrombosis, occurring both locally and systemically.[1, 2] This is often attributed to activation of tissue factor (F3/TF),[3] the cell-associated transmembrane receptor for the blood borne zymogen coagulation factor VII/VIIa. The TF/VIIa complex activates factor X (F10) to Xa, which directly participates in the generation of active thrombin (IIa) from prothrombin (F2).[4] While thrombin plays a central role in the formation of fibrin clots and platelet activation, all proteolytic components of this pathway (TF/VIIa, Xa, and IIa) also possess important cellular signaling functions mediated chiefly by protease activated receptors, especially F2R/PAR-1 and F2RL1/PAR-2.[5] These signals participate in the cellular sensing of clotting activity and the related activation of a 'wound healing program', which consists of growth, survival, and vascular responses.[6, 7] Thus, TF/VIIa/Xa/IIa pathway mediates activation of both F2R/PAR-1 and F2RL1/PAR-2 receptors, and resulting signals may change the expression of genes involved in other vascular processes such as inflammation and angiogenesis.[8, 9]

Cancer cells are a major source of F3/TF and PARs in the tumor mass, and their expression is controlled by changes in the tissue microenvironment and cellular genome.

Notably, mutations in tumor suppressors such as PTEN,[10] and activation of oncogenic pathways including epidermal growth factor receptor (EGFR)[11, 12] and hepatocyte growth factor receptor (MET) [13, 14] have been implicated as regulators of TF/F3, PAR1/2, and FVII. This is of interest as oncogenic networks also define the recently described molecular subtypes identified in several types of brain tumors;[15-17] hence, a corresponding variation could be expected with regard to regulation of F3/TF, PARs, and other elements of the cancer coagulome. Indeed, such variation was recently described for adult glioblastoma (GBM),[18] but has not been studied in pediatric high grade brain tumors.

Medulloblastoma (MB) is the most common primary malignant pediatric brain tumor occurring mostly in the cerebellum and arising from the neuronal lineage. The 5-year overall survival is 65-85% following aggressive therapy that includes surgical resection, radiation, and chemotherapy.[19] Long-term survivors demonstrate treatment-induced sequelae including cognitive deficits, endocrine dysfunction, and growth retardation, which reaffirm the importance of evaluating more individualized and biologically-based therapies.[19, 20] It is now understood that MB is comprised of four core molecular subtypes (WNT, SHH, Group 3, Group 4) each of which is defined by the activation of specific oncogenic pathways that determine clinical characteristics of the disease.[16, 21, 22] In addition, intrinsic events such as MYC or MYCN amplification,[21, 23-29] and upregulation of MET receptor[30, 31] influence the aggressiveness of MB tumor cells.

These events are not completely cell-autonomous and may be accompanied by altered interactions with the microenvironment, for example, hepatocyte growth factor (HGF) released from stromal cells may activate MET receptor in cancer cells.[32] Overexpression of HGF increases the formation of SHH-induced MB in mice, and inhibiting HGF activity prolongs

survival.[20] MET is predominantly overexpressed in SHH MB tumors and is associated with reduced progression free survival.[33] Whether these changes affect regulators of crucial vascular processes in MB has not been studied.

Here we show that molecular subtypes of MB exhibit distinct expression patterns of coagulation- and angiogenesis-related genes. Specifically, F3/TF and F2R/PAR-1 mRNA expression is significantly elevated in SHH MB relative to other subtypes. DAOY cells, which demonstrate properties of SHH tumors[34] and express MET receptor,[13, 14] respond to combined stimulation with HGF and SHH by upregulation of F3/TF and F2R/PAR-1 mRNA. Under these conditions, the exposure to thrombin upregulates interleukin 1β (ILB/IL1β) and downregulates chondromodulin 1 (LECT1/ChM1), a potent inhibitor of angiogenesis. Therefore, in MB, the expression of vascular regulators is not 'unspecific', but rather results from cooperation between key oncogenic growth factor pathways and coagulation factor signaling.

MATERIALS AND METHODS

In Silico mRNA Expression Analysis

Gene expression data come from public sources deposited in GEO http://www.ncbi.nlm.nih.gov/geo (GSE10327, GSE37418, GSE12992, GSE3526, GSE35493, GSE21140), or have been generated at the German Cancer Research Center DKFZ in Heidelberg (Kool et al., unpublished).[16, 35-39] The MAS5.0 algorithm of the GCOS program (Affymetrix Inc) was used for normalization of the expression data. All data have been analyzed using the R2 program for analysis and visualization of microarray data (http://R2.amc.nl).

Cells and Growth/Coagulation Factor Treatment

DAOY cells were kindly provided by Dr. Nada Jabado (Montreal Children's Hospital, McGill University Health Center, 2012), and have previously been characterized and validated.[40] These cells were maintained in AMEM (alpha minimal essential medium; Multicell) supplemented with 1% penicillin/streptomycin (Invitrogen) and 10% fetal bovine serum (Wisent). DAOY cells (55,000/well) were seeded in a 6-well plate and the following day they were serum-starved overnight in basal growth medium. Subsequently, the cells were stimulated with HGF (50 ng/ml) and SHH (1 ug/ml) alone or in combination, and collected after 7 hours[41] for analysis of F3/TF and F2R/PAR-1 mRNA expression. To assess changes in gene expression downstream of F2R/PAR-1 activation, following 24 hours stimulation with HGF and SHH alone or in combination, cells were stimulated with thrombin (1 unit/ml) for an additional 24 hours[42] before collection.

RNA Preparation and Expression Profiling

RNA was extracted using the RNeasy Plus Mini Kit (Qiagen) and quantified using Nanodrop. RNA (1 ug) was reverse transcribed using the RT² First Strand Kit (SABiosciences). Using the RT² SYBR Green PCR Master Mix (SABiosciences), the Human Angiogenesis RT² Profiler™ PCR Array (SABiosciences) was used to assess changes in the expression of 84 angiogenesis-related genes. Genes that displayed more than one melting peak curve (non-specific amplification) were removed from further consideration.

Quantitative Real-time PCR

RNA was reverse transcribed using the QuantiTect Reverse Transcription Kit (Qiagen). cDNA was amplified using the LightCycler480 SYBR Green I Master Mix (Roche). F3/TF, plasminogen activator inhibitor-1 (SERPINE1/PAI-1), and β-actin primer sets (QuantiTect primer assays) were also purchased from Qiagen. The following are primer sets purchased from Integrated DNA Technologies (IDT): F2R/PAR-1 sense 5'-GCA ACA AAT GCC ACC TTA GAT CC-3' and antisense 5'-GAG ACT AAT CTG TAT TCA GTT AAC CCA CTT-3'; interleukin-8 (IL8) sense 5'-CTG CGC CAA CAC AGA AAT TAT TGT AAA GC-3' and antisense 5'-AAA CTT CTC CAC AAC CCT CTG CAC-3'; ILB/IL1β sense 5'-AGA AGT ACC TGA GCT CGC CA-3' and antisense 5'-CTG GAA GGA GCA CTT CAT CTG T-3'; GAPDH sense 5'-TGC ACC ACC AAC TGC TTA GC-3' and antisense 5'-GGC ATG GAC TGT GGT CAT GAG-3';[43] and LECT1/ChM1 sense 5'-CAG GCG CTG GAA GAC TGA ATA-3' and antisense 5'-CTT TCC CCT TCC TGC TGA TGA T-3'. Real time PCR was performed on a LightCycler480 (Roche), and relative mRNA levels were quantified from the

standard curve for each primer set and normalized to β-actin and GAPDH using the LightCycler480 Software Release 1.5.0.

Statistical analysis

Heatmaps of mRNA expression data were generated directly in R2 (Supplementary Figures) or using MultiExperiment Viewer (MeV) version 4.9. Statistical analyses were performed using GraphPad Prism 6. All data were analyzed using a one- or two-way ANOVA (Kruskal-Wallis test for nonparametric analyses) as appropriate. The factors were tumor subgroup, growth factor treatment, and coagulation factor treatment. The post-hoc Dunn's (nonparametric) or Tukey's Honestly Significant Difference (HSD) tests were used to determine the significance of main effects. The nonparametric Spearman coefficient was calculated for the correlation between F3/TF or F2R/PAR-1 and MET mRNA expression for the human data. F3/TF or F2R/PAR-1 was assigned as the independent variable, x, and MET as the dependent variable, y. All data were generally expressed as mean ± standard error of the mean (SEM), and the level of significance was set as $p \leq 0.05$.

RESULTS

Selective upregulation of F3/TF, F2R/PAR-1, and MET transcripts in the SHH subtype of medulloblastoma

To determine whether the expression of coagulation factors in MB is random, or linked to the specific oncogenic circuitries activated in subtypes of cancer cells, we surveyed human mRNA expression profiles annotated for the main molecular subgroups of the disease: WNT, SHH, Group 3, and Group 4 (Fig. 1, Supplementary Fig. 1). We first evaluated the mRNA expression levels of 38 effectors of the coagulation and fibrinolytic systems in 424 patient specimens and an additional 31 samples of fetal and adult cerebellum (Supplementary Fig. 1). Notably, several coagulation factors were ectopically expressed in MB tumors. While there were many similarities, we observed that coagulation factor X (F10) is significantly upregulated in WNT tumors compared to every other molecular subgroup in addition to the normal fetal (but not adult) cerebellum. The WNT subgroup uniquely displays upregulated levels of protein C receptor (PROCR) compared to all other tumors, but not to normal cerebellum. SHH, Group 3, and Group 4 tumors demonstrate reduced expression of PROCR compared to normal fetal cerebellum. The expression of fibrinogen-like 2 (FGL2) prothrombinase was most abundant in WNT and SHH tumors compared to those in Group 3 and Group 4; the latter groups also have lower FGL2 levels compared to the normal cerebellum. Notably, Group 4 demonstrates striking diversity in the expression of coagulation-related genes, which may suggest the existence of vascular subtypes within this patient population (Supplementary Fig. 1).

F2R/PAR-1 expression was higher in SHH tumors (and fetal cerebellum) compared to all other subgroups and adult cerebellum. Levels of F3/TF were also upregulated in the SHH

subgroup. Interestingly, in comparison to the fetal cerebellum, WNT, Group 3, and Group 4 tumors exhibit dramatically lower levels of tissue factor pathway inhibitor (TFPI) (Supplementary Table 1). As levels of coagulation factors have been shown to correlate with age,[44-46] we compared mRNA levels for F3/TF and F2R/PAR-1 across the different age groups of MB patients, but no age-related differences were found (Fig. 2). It is unclear why levels of some ectopic factors (F10, FGL2, PROCR, TFPI) in MB resembled adult, but not fetal cerebellum.

Thus we observed that F3/TF and the thrombin receptor (F2R/PAR-1) are selectively elevated in SHH tumors compared to all other molecular subgroups of MB (Fig. 3A and B). Interestingly, this upregulation is paralleled by increased expression of MET (Fig. 3C); furthermore, both F3/TF ($r= 0.3779$, $p<0.0001$) and F2R/PAR-1 ($r= 0.3002$, $p<0.0001$) are positively correlated with levels of MET (data not shown). To exclude the possibility that stromal cells contribute to F3/TF and F2R/PAR-1 expression, we also analyzed mRNA levels of lineage markers of endothelial (PECAM1/CD31), hematopoietic (PTPRC/CD45), and myeloid-derived cells (ITGAM/CD11b); however, we found that overall there were no significant differences among the MB subgroups in this regard (Supplementary Fig. 2). This suggests that tumor cells themselves are responsible for differences in F3/TF and F2R/PAR-1 expression among MB subgroups.

F3/TF and F2R/PAR-1 expression are induced by HGF and SHH in cultured medulloblastoma cells

The link between TF/F3 levels and MET in samples of SHH-type MB is intriguing as MET is a known regulator of F3/TF expression and activity in MB cell lines.[13, 14] In SHH MB patients, MET expression parallels a more aggressive course of the disease,[33] but the role of this pathway in the regulation of the thrombin receptor and responses to thrombin have not been studied. To examine whether the expression of F3/TF and F2R/PAR-1 are causally linked to MET pathway activation, we chose DAOY cells, which exhibit a hedgehog-related gene expression signature and can be regarded as a model of SHH MB.[34] Indeed, HGF (MET ligand) treatment of DAOY cells triggered a marked upregulation of F3/TF mRNA, but had no effect on F2R/PAR-1 (Fig. 4). While SHH treatment *per se* was relatively inconsequential in this setting, it triggered a notable increase in the level of F2R/PAR-1 mRNA in the presence of HGF (Fig. 4). Thus the response of DAOY cells to MET/SHH activators recapitulate the endogenous F3/TF and F2R/PAR-1 mRNA upregulation pattern present in the human MB dataset. These results suggest that HGF and SHH pathways may cooperate in controlling the expression of F3/TF and F2R/PAR-1 genes in MB.

Oncogenic growth factor pathways regulate angiogenesis-related gene expression in medulloblastoma subtypes.

Thrombin is often activated in the tumor microenvironment and may trigger the expression of genes regulating growth, inflammation, and angiogenesis.[47] Since MB tumors demonstrate a subtype-specific profile of F3/TF and F2R/PAR-1 expression, we surmised that cellular responses to thrombin may exhibit corresponding differences and affect angiogenesis-related genes.

We chose to first explore this possibility *in silico* by assessing the expression of 77 angiogenesis-related factors (Fig. 5, Supplementary Fig. 3) of which 22 we found to exhibit significant subtype-specific differences in MB (Supplementary Table 2). For example, the WNT subgroup displays the highest levels of 9/18 proangiogenic factors (ANG, ANPEP, FGF2, MDK, MMP14, MMP2, NRP1, PTGS1, TGFA); but also some anti-angiogenic factors (SERPINF1, TIMP2). In Group 3 different proangiogenic factors are elevated (ANGPT2, FN1, TGFB1, TGFBR1, VEGFA), but also some anti-angiogenic factors are increased (S1PR1, SERPINF1, TIMP2, TIMP3). Group 4 tumors exhibit the highest mRNA levels of the TEK (Tie-2) angiopoietin receptor, but reduced expression of most anti-angiogenic factors (S1PR1, SERPINF1, TIMP3). Interestingly, Group 4 once again demonstrates significant heterogeneity in angiogenesis factor expression between individual samples suggesting coexistence of possible 'vascular' subtypes (Supplementary Fig. 3).

In SHH tumors several proangiogenic factors are upregulated, some of which uniquely to this disease subgroup (EPHB4, SPHK1, TGFB2). Interestingly, low levels of ANGPT2 (angiopoietin 2, the natural antagonist of ANGPT1) and high levels of the angiopoietin receptor (TEK) may suggest deregulation of this pathway in SHH MB tumors. SHH tumors differ from the WNT subgroup due to less prominent expression of endogenous anti-angiogenic factors.

Given that pediatric and adult MB are genetically disparate,[48] it is not surprising that there is a significant and robust (greater than 2-fold) difference in expression of angiogenesis-related genes across ages, and within specific molecular subgroups. Interestingly, SHH tumors most frequently display age-dependency in their angiome profile (Supplementary Fig. 4).

Oncogenic growth factor pathways cooperate with thrombin in regulating angiogenesis-related genes.

To understand whether there is a functional link between the MB coagulome and angiome we have analysed the profiles of angiogenic transcripts in DAOY cells in the presence or absence of relevant growth factors and thrombin. Indeed, we found that there were at least 5 classes of angiogenesis-related genes that are thrombin-regulated in MB cells (Table 1, Fig. 5B). Class 1 genes (EDN1, IL1B, IL6) have been reported as thrombin targets in non-tumor cells.[49-53] Class 2 includes MMP9, which is induced by thrombin and a F2R/PAR-1 agonist in non-CNS cancer cells.[54] Class 3 are confirmed thrombin and F2R/PAR-1 downstream targets in cancer cell lines including brain tumor cells (IL8, SERPINE1).[47] Class 4 includes all those genes (ANG, CCL11, CDH5, CXCL10, EDN1, EGF, IGF1, IL1B, IL6, IL8, KDR, LECT1, MMP9, SERPINE1) that are differentially regulated by thrombin in the context of growth factor signaling activation in our hands. Of those, class 5 (ANG, CCL11, CDH5, CXCL10, EGF, IGF1, KDR, LECT1) includes thrombin-regulated genes whose modulation has not been previously reported and may depend on the concomitant activation of growth factor signaling pathways (Supplementary Table 3).

Among the most notable changes independently validated by Q-PCR was the selective upregulation of ILB/IL1β by thrombin in MB cells pre-treated with HGF and SHH (Figure 6). Thrombin also exerted a potent suppressive effect on the gene LECT1, which encodes chondromodulin 1 (ChM1), the angiogenesis inhibitor[55] and stemness marker,[56], growth inhibitor of cancer cells (Fig. 6).[57] Interestingly, LECT1/ChM1 is also significantly downregulated by thrombin in the absence of HGF. We also observed changes in the expression

of interleukin 8 (IL8) and plasminogen activator inhibitor-1 (SERPINE1/PAI-1) described previously (Fig. 6).[47]

Overall, these observations support the notion that there is a link between oncogenic pathways that drive different subgroups of MB, coagulation system receptors associated with them, and related changes in thrombin regulation of the tumor angiome.

DISCUSSION

Although clinically evident thromboembolism is rare in pediatric brain tumor patients,[58] MB cells inevitably come into contact with coagulation system effectors due to vascular permeability, angiogenesis, surgical intervention, and disruption of tissue microanatomy following chemotherapy and radiation. The coagulation system is programmed to respond to these vascular challenges and to intersect with inflammatory and angiogenic components of the wound healing continuum.

The main question raised in our study was whether MB cells/subtypes are genetically and distinctively 'pre-programmed' to modulate vascular changes, or whether such changes and their effectors are altered due to common and 'unspecific' microenvironmental perturbations. Our study reveals the former to be the case through several novel observations.

First, we found that in a large cohort of MB patients the genes regulating two major (and interlinked) components of the vascular compartment, coagulation and angiogenesis, are deregulated in a manner dependent on the molecular disease subtype. Notably, F3/TF and F2R/PAR-1 are selectively upregulated in the SHH subgroup, while the significant expression of

ectopic (extrahepatic) coagulation factor X (F10) as well as PROCR are selectively associated with the WNT subtype of MB. PROCR (also known as endothelial protein C receptor, EPCR) is elevated in lung carcinoma samples and its expression is correlated with aggressive clinical status and high microvessel density.[59] Although the PROCR ligand, activated protein C (APC), can reduce clotting potential, PROCR is reported to act as an essential adaptor protein in ternary complex (TF/VIIa/Xa) signaling via F2R/PAR-1,[9] and its inhibition in cultured lung carcinoma cells reduced cell growth and migration.[60] Interestingly, FGL2 is upregulated in both WNT and SHH tumors; this protein acts as a direct and independent activator of prothrombin.[61] In addition, FGL2 is upregulated in several human malignancies, and its inhibition in hepatocellular carcinoma cells impedes tumor growth and angiogenesis.[62] Remarkably, activation of SHH and MET pathways, a crucial step in the progression of SHH MB,[16, 33] also resulted in upregulation of F3/TF and F2R/PAR-1 mRNA in cultured MB cells (DAOY). This documents a new link between tumor genetics and coagulome in MB.

We reasoned that the selective upregulation of thrombin receptor mRNA (F2R/PAR-1) downstream of MET and SHH pathways may result in a subtype-related shift in responses of MB cells to thrombin, which is a known regulator of clotting, angiogenesis, and inflammation.[42, 47] In agreement with these predictions, we observed hitherto unrecognized qualitative and quantitative changes in cellular sensitivity to thrombin in cultured MB cells, especially as a function of their growth factor environment. Notably, HGF- and SHH-stimulated MB cells exhibited a selective upregulation of ILB/IL1β in response to thrombin exposure. Interestingly in dendritic cells thrombin-stimulated F2R/PAR-1 activates sphingosine kinase 1 (SPHK1) leading to sphingosine-1-phosphate (S1P) receptor (S1PR3)-mediated upregulation of ILB/IL1β.[53] Whether such mechanisms operate in the context of SHH MB remains to be studied, but SPHK1

is upregulated in SHH MB (not shown). ILB/IL1β is expressed in solid tumors and may act in an autocrine or paracrine manner to stimulate invasion, proliferation, angiogenesis and inflammation.[63, 64]

Inflammation has a profound impact on therapeutic strategies, especially those targeting the vascular tumor stroma and angiogenesis. These are largely based on an anti-VEGF backbone[65] and display only transient effectiveness, in part because of the recruitment of immature myeloid cells that bypass the requirement for VEGF production. It has been proposed that ILB/IL1β may contribute to this 'evasive' process.[66] In pediatric brain tumors anti-VEGF therapy, such as bevacizumab, demonstrates only limited efficacy [67-69] and the role of coagulation and inflammation pathways deserves further studies.

Thus our findings may have several translational implications. First, it may be informative to assess the local and systemic activation of the coagulation system in CNS tumors, regardless of clinically evident coagulopathy, and in view of the biological effects of coagulation system effectors. Second, studies involving stroma-targeted agents (e.g. anti-angiogenics) should, perhaps, be designed and evaluated (stratified) with some attention given to molecular subtype-specific pro-coagulant, proangiogenic, and pro-inflammatory characteristics of the disease. Third, it may be worth considering a more comprehensive evaluation of tumor stroma in MB as described recently in breast cancer.[70] Fourth, as surgery, chemotherapy, and anti-angiogenic therapies can increase the risk of thromboembolism,[2, 71] in general, it is possible that 'unspecific' activation of the coagulation system by surgery and other factors may have subtype-specific and biologically meaningful consequences in lesions where appropriate coagulation receptors are expressed. For example, it may be informative to correlate these responses in SHH tumors with relapse or metastasis following therapy. Furthermore,

conventional (anticoagulants) and unconventional agents targeting the coagulation system (e.g. F2R/PAR-1 antagonists) could be considered as adjunctive or supportive therapeutics in the context of specific MB subtypes.

In conclusion, we would like to note that the coagulation system represents the first line of tissue and vascular responses to injury and disease including cancer, and a greater understanding of its involvement in pediatric CNS tumors, especially MB subtypes, may open new diagnostic and therapeutic opportunities.

ADDENDUM

Concept and design (E. D'Asti, J. Rak); analysis and/or interpretation of data (E. D'Asti, M. Kool, J. Rak); critical writing and revising of intellectual content (E. D'Asti, M. Kool, S.M. Pfister, J. Rak), and final approval of the version to be published (E. D'Asti, M. Kool, S.M. Pfister, J. Rak).

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DISCLOSURE OF CONFLICT OF INTERESTS

The authors state that they have no conflict of interest.

REFERENCES

1 Brat DJ, Van Meir EG. Vaso-occlusive and prothrombotic mechanisms associated with tumor hypoxia, necrosis, and accelerated growth in glioblastoma. *Laboratory investigation; a journal of technical methods and pathology*. 2004; **84**: 397-405. 10.1038/labinvest.3700070.

2 Semrad TJ, O'Donnell R, Wun T, Chew H, Harvey D, Zhou H, White RH. Epidemiology of venous thromboembolism in 9489 patients with malignant glioma. *Journal of neurosurgery*. 2007; **106**: 601-8. 10.3171/jns.2007.106.4.601.

3 Khorana AA. Cancer and coagulation. *American journal of hematology*. 2012; **87 Suppl 1**: S82-7. 10.1002/ajh.23143.

4 Mackman N. Role of tissue factor in hemostasis, thrombosis, and vascular development. *Arteriosclerosis, thrombosis, and vascular biology*. 2004; **24**: 1015-22. 10.1161/01.atv.0000130465.23430.74.

5 Ruf W, Disse J, Carneiro-Lobo TC, Yokota N, Schaffner F. Tissue factor and cell signalling in cancer progression and thrombosis. *Journal of thrombosis and haemostasis : JTH*. 2011; **9 Suppl 1**: 306-15. 10.1111/j.1538-7836.2011.04318.x.

6 Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *The New England journal of medicine*. 1986; **315**: 1650-9. 10.1056/nejm198612253152606.

7 Kasthuri RS, Taubman MB, Mackman N. Role of tissue factor in cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2009; **27**: 4834-8. 10.1200/jco.2009.22.6324.

8 Camerer E, Huang W, Coughlin SR. Tissue factor- and factor X-dependent activation of protease-activated receptor 2 by factor VIIa. *Proceedings of the National Academy of Sciences of the United States of America*. 2000; **97**: 5255-60.

9 Disse J, Petersen HH, Larsen KS, Persson E, Esmon N, Esmon CT, Teyton L, Petersen LC, Ruf W. The endothelial protein C receptor supports tissue factor ternary coagulation initiation complex signaling through protease-activated receptors. *The Journal of biological chemistry*. 2011; **286**: 5756-67. 10.1074/jbc.M110.201228.

10 Rong Y, Post DE, Pieper RO, Durden DL, Van Meir EG, Brat DJ. PTEN and hypoxia regulate tissue factor expression and plasma coagulation by glioblastoma. *Cancer research*. 2005; **65**: 1406-13. 10.1158/0008-5472.can-04-3376.

11 Milsom CC, Yu JL, Mackman N, Micallef J, Anderson GM, Guha A, Rak JW. Tissue factor regulation by epidermal growth factor receptor and epithelial-to-mesenchymal transitions: effect on tumor initiation and angiogenesis. *Cancer research*. 2008; **68**: 10068-76. 10.1158/0008-5472.can-08-2067.

12 Magnus N, Garnier D, Rak J. Oncogenic epidermal growth factor receptor up-regulates multiple elements of the tissue factor signaling pathway in human glioma cells. *Blood*. 2010; **116**: 815-8. 10.1182/blood-2009-10-250639.

13 Provencal M, Labbe D, Veitch R, Boivin D, Rivard GE, Sartelet H, Robitaille Y, Gingras D, Beliveau R. c-Met activation in medulloblastoma induces tissue factor expression and activity: effects on cell migration. *Carcinogenesis*. 2009; **30**: 1089-96. 10.1093/carcin/bgp085.

14 Provencal M, Berger-Thibault N, Labbe D, Veitch R, Boivin D, Rivard GE, Gingras D, Beliveau R. Tissue factor mediates the HGF/Met-induced anti-apoptotic pathway in DAOY medulloblastoma cells. *Journal of neuro-oncology*. 2010; **97**: 365-72. 10.1007/s11060-009-0041-z.

15 Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, Alexe G, Lawrence M, O'Kelly M, Tamayo P, Weir BA, Gabriel S, Winckler W, Gupta S, Jakkula L, Feiler HS, Hodgson JG, James CD, Sarkaria JN, Brennan C, Kahn A, Spellman PT, Wilson RK, Speed TP, Gray JW, Meyerson M, Getz G, Perou CM, Hayes DN. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer cell*. 2010; **17**: 98-110. 10.1016/j.ccr.2009.12.020.

16 Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, Bouffet E, Clifford SC, Hawkins CE, French P, Rutka JT, Pfister S, Taylor MD. Medulloblastoma comprises four distinct molecular variants. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2011; **29**: 1408-14. 10.1200/jco.2009.27.4324.

17 Picard D, Miller S, Hawkins CE, Bouffet E, Rogers HA, Chan TS, Kim SK, Ra YS, Fangusaro J, Korshunov A, Toledano H, Nakamura H, Hayden JT, Chan J, Lafay-Cousin L, Hu P, Fan X, Muraszko KM, Pomeroy SL, Lau CC, Ng HK, Jones C, Van Meter T, Clifford SC, Eberhart C, Gajjar A, Pfister SM, Grundy RG, Huang A. Markers of survival and metastatic potential in childhood CNS primitive

neuro-ectodermal brain tumors: an integrative genomic analysis. *The lancet oncology*. 2012; **13**: 838-48. 10.1016/s1470-2045(12)70257-7.

18 Magnus N, Gerges N, Jabado N, Rak J. Coagulation-related gene expression profile in glioblastoma is defined by molecular disease subtype. *Journal of thrombosis and haemostasis : JTH*. 2013; **11**: 1197-200. 10.1111/jth.12242.

19 Remke M, Ramaswamy V, Taylor MD. Medulloblastoma molecular dissection: the way toward targeted therapy. *Current opinion in oncology*. 2013; **25**: 674-81. 10.1097/cco.0000000000000008.

20 Binning MJ, Niazi T, Pedone CA, Lal B, Eberhart CG, Kim KJ, Laterra J, Fults DW. Hepatocyte growth factor and sonic Hedgehog expression in cerebellar neural progenitor cells costimulate medulloblastoma initiation and growth. *Cancer research*. 2008; **68**: 7838-45. 10.1158/0008-5472.can-08-1899.

21 Kool M, Korshunov A, Remke M, Jones DT, Schlanstein M, Northcott PA, Cho YJ, Koster J, Schouten-van Meeteren A, van Vuurden D, Clifford SC, Pietsch T, von Bueren AO, Rutkowski S, McCabe M, Collins VP, Backlund ML, Haberler C, Bourdeaut F, Delattre O, Doz F, Ellison DW, Gilbertson RJ, Pomeroy SL, Taylor MD, Lichter P, Pfister SM. Molecular subgroups of medulloblastoma: an international meta-analysis of transcriptome, genetic aberrations, and clinical data of WNT, SHH, Group 3, and Group 4 medulloblastomas. *Acta neuropathologica*. 2012; **123**: 473-84. 10.1007/s00401-012-0958-8.

22 Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, Eberhart CG, Parsons DW, Rutkowski S, Gajjar A, Ellison DW, Lichter P, Gilbertson RJ, Pomeroy SL, Kool M, Pfister SM. Molecular subgroups of medulloblastoma: the current consensus. *Acta neuropathologica*. 2012; **123**: 465-72. 10.1007/s00401-011-0922-z.

23 Aldosari N, Bigner SH, Burger PC, Becker L, Kepner JL, Friedman HS, McLendon RE. MYCC and MYCN oncogene amplification in medulloblastoma. A fluorescence in situ hybridization study on paraffin sections from the Children's Oncology Group. *Archives of pathology & laboratory medicine*. 2002; **126**: 540-4. 10.1043/0003-9985(2002)126<0540:mamoai>2.0.co;2.

24 Cho YJ, Tsherniak A, Tamayo P, Santagata S, Ligon A, Greulich H, Berhoukim R, Amani V, Goumnerova L, Eberhart CG, Lau CC, Olson JM, Gilbertson RJ, Gajjar A, Delattre O, Kool M, Ligon K, Meyerson M, Mesirov JP, Pomeroy SL. Integrative genomic analysis of medulloblastoma identifies a molecular subgroup that drives poor clinical outcome. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2011; **29**: 1424-30. 10.1200/jco.2010.28.5148.

25 Ellison DW, Dalton J, Kocak M, Nicholson SL, Fraga C, Neale G, Kenney AM, Brat DJ, Perry A, Yong WH, Taylor RE, Bailey S, Clifford SC, Gilbertson RJ. Medulloblastoma: clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups. *Acta neuropathologica*. 2011; **121**: 381-96. 10.1007/s00401-011-0800-8.

26 Ellison DW, Kocak M, Dalton J, Megahed H, Lusher ME, Ryan SL, Zhao W, Nicholson SL, Taylor RE, Bailey S, Clifford SC. Definition of disease-risk stratification groups in childhood medulloblastoma using combined clinical, pathologic, and molecular variables. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2011; **29**: 1400-7. 10.1200/jco.2010.30.2810.

27 Pfister S, Remke M, Benner A, Mendrzyk F, Toedt G, Felsberg J, Wittmann A, Devens F, Gerber NU, Joos S, Kulozik A, Reifenberger G, Rutkowski S, Wiestler OD, Radlwimmer B, Scheurlen W, Lichter P, Korshunov A. Outcome prediction in pediatric medulloblastoma based on DNA copy-number aberrations of chromosomes 6q and 17q and the MYC and MYCN loci. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2009; **27**: 1627-36. 10.1200/jco.2008.17.9432.

28 Zitterbart K, Filkova H, Tomasikova L, Necesalova E, Zambo I, Kantorova D, Slamova I, Vranova V, Zezulkova D, Pesakova M, Pavelka Z, Veselska R, Kuglik P, Sterba J. Low-level copy number changes of MYC genes have a prognostic impact in medulloblastoma. *Journal of neuro-oncology*. 2011; **102**: 25-33. 10.1007/s11060-010-0289-3.

29 Korshunov A, Remke M, Kool M, Hielscher T, Northcott PA, Williamson D, Pfaff E, Witt H, Jones DT, Ryzhova M, Cho YJ, Wittmann A, Benner A, Weiss WA, von Deimling A, Scheurlen W,

Kulozik AE, Clifford SC, Peter Collins V, Westermann F, Taylor MD, Lichter P, Pfister SM. Biological and clinical heterogeneity of MYCN-amplified medulloblastoma. *Acta neuropathologica*. 2012; **123**: 515-27. 10.1007/s00401-011-0918-8.

30 Tong CY, Hui AB, Yin XL, Pang JC, Zhu XL, Poon WS, Ng HK. Detection of oncogene amplifications in medulloblastomas by comparative genomic hybridization and array-based comparative genomic hybridization. *Journal of neurosurgery*. 2004; **100**: 187-93. 10.3171/ped.2004.100.2.0187.

31 Li Y, Lal B, Kwon S, Fan X, Saldanha U, Reznik TE, Kuchner EB, Eberhart C, Laterra J, Abounader R. The scatter factor/hepatocyte growth factor: c-met pathway in human embryonal central nervous system tumor malignancy. *Cancer research*. 2005; **65**: 9355-62. 10.1158/0008-5472.can-05-1946.

32 Comoglio PM, Giordano S, Trusolino L. Drug development of MET inhibitors: targeting oncogene addiction and expedience. *Nature reviews Drug discovery*. 2008; **7**: 504-16. 10.1038/nrd2530.

33 Faria CC, Dubuc AM, Golbourn BJ, Diaz RJ, Agnihotri S, Luck A, Sabha N, Leadly M, Reynaud D, Ermini L, Wu X, Remke M, Rasmaswarmy V, Northcott PA, Pfister S, Croul S, Kool M, Korshunov A, Smith CA, Taylor MD, Rutka JT. Effective targeting of the HGF/c-MET pathway in medulloblastoma. *2013 Pediatric Neuro-Oncology Basic and Translational Research Conference*. Fort Lauderdale, Florida, 2013.

34 Wang X, Venugopal C, Manoranjan B, McFarlane N, O'Farrell E, Nolte S, Gunnarsson T, Hollenberg R, Kwiecien J, Northcott P, Taylor MD, Hawkins C, Singh SK. Sonic hedgehog regulates Bmi1 in human medulloblastoma brain tumor-initiating cells. *Oncogene*. 2012; **31**: 187-99. 10.1038/onc.2011.232.

35 Kool M, Koster J, Bunt J, Hasselt NE, Lakeman A, van Sluis P, Troost D, Meeteren NS, Caron HN, Cloos J, Mrsic A, Ylstra B, Grajkowska W, Hartmann W, Pietsch T, Ellison D, Clifford SC, Versteeg R. Integrated genomics identifies five medulloblastoma subtypes with distinct genetic profiles, pathway signatures and clinicopathological features. *PloS one*. 2008; **3**: e3088. 10.1371/journal.pone.0003088.

36 Robinson G, Parker M, Kranenburg TA, Lu C, Chen X, Ding L, Phoenix TN, Hedlund E, Wei L, Zhu X, Chalhoub N, Baker SJ, Huether R, Kriwacki R, Curley N, Thiruvenkatam R, Wang J, Wu G, Rusch M, Hong X, Becksfort J, Gupta P, Ma J, Easton J, Vadodaria B, Onar-Thomas A, Lin T, Li S, Pounds S, Paugh S, Zhao D, Kawauchi D, Roussel MF, Finkelstein D, Ellison DW, Lau CC, Bouffet E, Hassall T, Gururangan S, Cohn R, Fulton RS, Fulton LL, Dooling DJ, Ochoa K, Gajjar A, Mardis ER, Wilson RK, Downing JR, Zhang J, Gilbertson RJ. Novel mutations target distinct subgroups of medulloblastoma. *Nature*. 2012; **488**: 43-8. 10.1038/nature11213.

37 Fattet S, Haberler C, Legoix P, Varlet P, Lellouch-Tubiana A, Lair S, Manie E, Raquin MA, Bours D, Carpentier S, Barillot E, Grill J, Doz F, Puget S, Janoueix-Lerosey I, Delattre O. Beta-catenin status in paediatric medulloblastomas: correlation of immunohistochemical expression with mutational status, genetic profiles, and clinical characteristics. *The Journal of pathology*. 2009; **218**: 86-94. 10.1002/path.2514.

38 Roth RB, Hevezi P, Lee J, Willhite D, Lechner SM, Foster AC, Zlotnik A. Gene expression analyses reveal molecular relationships among 20 regions of the human CNS. *Neurogenetics*. 2006; **7**: 67-80. 10.1007/s10048-006-0032-6.

39 Birks DK, Donson AM, Patel PR, Sufit A, Algar EM, Dunham C, Kleinschmidt-DeMasters BK, Handler MH, Vibhakar R, Foreman NK. Pediatric rhabdoid tumors of kidney and brain show many differences in gene expression but share dysregulation of cell cycle and epigenetic effector genes. *Pediatric blood & cancer*. 2013; **60**: 1095-102. 10.1002/pbc.24481.

40 Ranger A, McDonald W, Bauman GS, Del Maestro R. Effects of surgical excision and radiation on medulloblastoma cell invasiveness. *The Canadian journal of neurological sciences Le journal canadien des sciences neurologiques*. 2009; **36**: 631-7.

41 Hartmann W, Koch A, Brune H, Waha A, Schuller U, Dani I, Denkhaus D, Langmann W, Bode U, Wiestler OD, Schilling K, Pietsch T. Insulin-like growth factor II is involved in the proliferation control of medulloblastoma and its cerebellar precursor cells. *The American journal of pathology*. 2005; **166**: 1153-62. 10.1016/s0002-9440(10)62335-8.

42 Caunt M, Hu L, Tang T, Brooks PC, Ibrahim S, Karpatkin S. Growth-regulated oncogene is pivotal in thrombin-induced angiogenesis. *Cancer research*. 2006; **66**: 4125-32. 10.1158/0008-5472.can-05-2570.

43 Willems E, Mateizel I, Kemp C, Cauffman G, Sermon K, Leyns L. Selection of reference genes in mouse embryos and in differentiating human and mouse ES cells. *The International journal of developmental biology*. 2006; **50**: 627-35. 10.1387/ijdb.052130ew.

44 Andrew M, Mitchell L, Vegh P, Ofosu F. Thrombin regulation in children differs from adults in the absence and presence of heparin. *Thrombosis and haemostasis*. 1994; **72**: 836-42.

45 Kurachi K, Zhang K, Ameri A, Huo J, Atoda H, Kurachi S. Genetic and molecular mechanisms of age regulation (homeostasis) of blood coagulation. *IUBMB life*. 2000; **49**: 189-96. 10.1080/713803620.

46 Ariens RA, Coppola R, Potenza I, Mannucci PM. The increase with age of the components of the tissue factor coagulation pathway is gender-dependent. *Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis*. 1995; **6**: 433-7.

47 Albrektsen T, Sorensen BB, Hjorto GM, Fleckner J, Rao LV, Petersen LC. Transcriptional program induced by factor VIIa-tissue factor, PAR1 and PAR2 in MDA-MB-231 cells. *Journal of thrombosis and haemostasis : JTH*. 2007; **5**: 1588-97. 10.1111/j.1538-7836.2007.02603.x.

48 Korshunov A, Remke M, Werft W, Benner A, Ryzhova M, Witt H, Sturm D, Wittmann A, Schottler A, Felsberg J, Reifenberger G, Rutkowski S, Scheurlen W, Kulozik AE, von Deimling A, Lichter P, Pfister SM. Adult and pediatric medulloblastomas are genetically distinct and require different algorithms for molecular risk stratification. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2010; **28**: 3054-60. 10.1200/jco.2009.25.7121.

49 Hoffman M, Cooper ST. Thrombin enhances monocyte secretion of tumor necrosis factor and interleukin-1 beta by two distinct mechanisms. *Blood cells, molecules & diseases*. 1995; **21**: 156-67. 10.1006/bcmd.1995.0018.

50 Pillitteri D, Bassus S, Boller K, Mahnel R, Scholz T, Westrup D, Wegert W, Kirchmaier CM. Thrombin-induced interleukin 1beta synthesis in platelet suspensions: impact of contaminating leukocytes. *Platelets*. 2007; **18**: 119-27. 10.1080/09537100600800792.

51 McLaughlin JN, Mazzoni MR, Cleator JH, Earls L, Perdigoto AL, Brooks JD, Muldowney JA, 3rd, Vaughan DE, Hamm HE. Thrombin modulates the expression of a set of genes including thrombospondin-1 in human microvascular endothelial cells. *The Journal of biological chemistry*. 2005; **280**: 22172-80. 10.1074/jbc.M500721200.

52 Sower LE, Froelich CJ, Carney DH, Fenton JW, 2nd, Klimpel GR. Thrombin induces IL-6 production in fibroblasts and epithelial cells. Evidence for the involvement of the seven-transmembrane domain (STD) receptor for alpha-thrombin. *Journal of immunology (Baltimore, Md : 1950)*. 1995; **155**: 895-901.

53 Niessen F, Schaffner F, Furlan-Freguia C, Pawlinski R, Bhattacharjee G, Chun J, Derian CK, Andrade-Gordon P, Rosen H, Ruf W. Dendritic cell PAR1-S1P3 signalling couples coagulation and inflammation. *Nature*. 2008; **452**: 654-8. 10.1038/nature06663.

54 Radjabi AR, Sawada K, Jagadeeswaran S, Eichbichler A, Kenny HA, Montag A, Bruno K, Lengyel E. Thrombin induces tumor invasion through the induction and association of matrix metalloproteinase-9 and beta1-integrin on the cell surface. *The Journal of biological chemistry*. 2008; **283**: 2822-34. 10.1074/jbc.M704855200.

55 Hiraki Y, Inoue H, Iyama K, Kamizono A, Ochiai M, Shukunami C, Iijima S, Suzuki F, Kondo J. Identification of chondromodulin I as a novel endothelial cell growth inhibitor. Purification and its localization in the avascular zone of epiphyseal cartilage. *The Journal of biological chemistry*. 1997; **272**: 32419-26.

56 Li SS, Liu YH, Tseng CN, Chung TL, Lee TY, Singh S. Characterization and gene expression profiling of five new human embryonic stem cell lines derived in Taiwan. *Stem cells and development*. 2006; **15**: 532-55. 10.1089/scd.2006.15.532.

57 Mera H, Kawashima H, Yoshizawa T, Ishibashi O, Ali MM, Hayami T, Kitahara H, Yamagiwa H, Kondo N, Ogose A, Endo N, Kawashima H. Chondromodulin-1 directly suppresses growth of human cancer cells. *BMC cancer*. 2009; **9**: 166. 10.1186/1471-2407-9-166.

58 Athale U, Siciliano S, Thabane L, Pai N, Cox S, Lathia A, Khan A, Armstrong A, Chan AK. Epidemiology and clinical risk factors predisposing to thromboembolism in children with cancer. *Pediatric blood & cancer*. 2008; **51**: 792-7. 10.1002/pbc.21734.

59 Heng W, Mu CY, Chen C, Huang JA, Wang ZY. Endothelial cell protein C receptor (EPCR) is expressed by lung carcinoma and correlated with clinical parameters. *Clinical laboratory*. 2013; **59**: 375-80.

60 Ruf W, Mueller BM. Thrombin generation and the pathogenesis of cancer. *Seminars in thrombosis and hemostasis*. 2006; **32 Suppl 1**: 61-8. 10.1055/s-2006-939555.

61 Chan CW, Chan MW, Liu M, Fung L, Cole EH, Leibowitz JL, Marsden PA, Clark DA, Levy GA. Kinetic analysis of a unique direct prothrombinase, fgl2, and identification of a serine residue critical for the prothrombinase activity. *Journal of immunology (Baltimore, Md : 1950)*. 2002; **168**: 5170-7.

62 Liu Y, Xu L, Zeng Q, Wang J, Wang M, Xi D, Wang X, Yang D, Luo X, Ning Q. Downregulation of FGL2/prothrombinase delays HCCLM6 xenograft tumor growth and decreases tumor angiogenesis. *Liver international : official journal of the International Association for the Study of the Liver*. 2012; **32**: 1585-95. 10.1111/j.1478-3231.2012.02865.x.

63 Apte RN, Krelin Y, Song X, Dotan S, Recih E, Elkabets M, Carmi Y, Dvorkin T, White RM, Gayvoronsky L, Segal S, Voronov E. Effects of micro-environment- and malignant cell-derived interleukin-1 in carcinogenesis, tumor invasiveness and tumor-host interactions. *European journal of cancer (Oxford, England : 1990)*. 2006; **42**: 751-9. 10.1016/j.ejca.2006.01.010.

64 Voronov E, Shouval DS, Krelin Y, Cagnano E, Benharroch D, Iwakura Y, Dinarello CA, Apte RN. IL-1 is required for tumor invasiveness and angiogenesis. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; **100**: 2645-50. 10.1073/pnas.0437939100.

65 Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. *Nature*. 2011; **473**: 298-307. 10.1038/nature10144.

66 Carmi Y, Dotan S, Rider P, Kaplanov I, White MR, Baron R, Abutbul S, Huszar M, Dinarello CA, Apte RN, Voronov E. The role of IL-1beta in the early tumor cell-induced angiogenic response. *Journal of immunology (Baltimore, Md : 1950)*. 2013; **190**: 3500-9. 10.4049/jimmunol.1202769.

67 Gururangan S, Chi SN, Young Poussaint T, Onar-Thomas A, Gilbertson RJ, Vajapeyam S, Friedman HS, Packer RJ, Rood BN, Boyett JM, Kun LE. Lack of efficacy of bevacizumab plus irinotecan in children with recurrent malignant glioma and diffuse brainstem glioma: a Pediatric Brain Tumor Consortium study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2010; **28**: 3069-75. 10.1200/jco.2009.26.8789.

68 Gururangan S, Fangusaro J, Young Poussaint T, Onar-Thomas A, Gilbertson RJ, Vajapeyam S, Gajjar A, Goldman S, Friedman HS, Packer RJ, Boyett JM, Kun LE, McLendon R. Lack of efficacy of bevacizumab + irinotecan in cases of pediatric recurrent ependymoma--a Pediatric Brain Tumor Consortium study. *Neuro-oncology*. 2012; **14**: 1404-12. 10.1093/neuonc/nos213.

69 Narayana A, Kunnakkat S, Chacko-Mathew J, Gardner S, Karajannis M, Raza S, Wisoff J, Weiner H, Harter D, Allen J. Bevacizumab in recurrent high-grade pediatric gliomas. *Neuro-oncology*. 2010; **12**: 985-90. 10.1093/neuonc/noq033.

70 Finak G, Bertos N, Pepin F, Sadekova S, Souleimanova M, Zhao H, Chen H, Omeroglu G, Meterissian S, Omeroglu A, Hallett M, Park M. Stromal gene expression predicts clinical outcome in breast cancer. *Nature medicine*. 2008; **14**: 518-27. 10.1038/nm1764.

71 Ma L, Francia G, Viloria-Petit A, Hicklin DJ, du Manoir J, Rak J, Kerbel RS. In vitro procoagulant activity induced in endothelial cells by chemotherapy and antiangiogenic drug combinations: modulation by lower-dose chemotherapy. *Cancer research*. 2005; **65**: 5365-73. 10.1158/0008-5472.can-04-3156.

FIGURE LEGENDS

Figure 1. Subgroup-specific changes in coagulation factor expression of human medulloblastoma. The heatmap illustrates changes in the mean mRNA expression of selected coagulation-related genes that exhibit major alterations. WNT, n= 53; SHH, n= 113; Group 3, n= 94; Group 4, n= 164; Fetal Cerebellum, n= 5; Adult cerebellum, n= 13.

Figure 2. Upregulation of F3/TF and F2R/PAR-1 mRNA expression in SHH tumors is irrespective of age group. Values represent mean ± SEM. WNT: infant, n= 0; child, n= 44; adult, n= 9. SHH: infant, n= 29; child, n= 27; adult, n= 55. Group 3: infant, n= 25; child, n= 63; adult, n= 3. Group 4: infant, n= 8; child, n= 131; adult, n= 22.

Figure 3. Parallel changes in the expression of coagulation factors and MET receptor in the SHH subtype of medulloblastoma. A-B. Coagulation factors upregulated in SHH MB include F3/TF and F2R/PAR-1, and this is correlated with MET (C), an oncogenic receptor tyrosine kinase known to influence the cellular coagulome. Abbreviation: CB, cerebellum. The black line for each subgroup is representative of the mean. WNT, n= 53; SHH, n= 113; Group 3, n= 94; Group 4, n= 164; Fetal CB, n= 5; Adult CB, n= 13. **** $p < 0.0001$, *** $p = 0.0001$ to 0.001.

Figure 4. Activation of MET and SHH pathways upregulates F3/TF and F2R/PAR-1 in cultured medulloblastoma cells. MB-derived DAOY cells were stimulated with HGF and SHH for 7 hours and assayed for mRNA levels of F3/TF and F2R/PAR-1 by real-time PCR.

Remarkably, this treatment recapitulates upregulation of F2R/PAR-1 in SHH MB samples. Values represent mean ± SEM from n=3 independent experiments. ** p= 0.001 to 0.01, * p= 0.01 to 0.05.

Figure 5. Angiogenic profile of medulloblastoma is a function of molecular subtype and changes upon exposure to oncogenic growth factors and thrombin. A. Heatmap for mRNA expression of angiogenesis-related factors in MB subgroups. B. Heatmap of normalized real-time PCR ratios of the same angiogenesis-related factors using the angiogenesis PCR array for the control (left) and HGF+SHH-treated DAOY cells with or without exposure to thrombin. Abbreviations: IIa, active thrombin.

Figure 6. Coagulation signaling activated by thrombin induces changes in the expression of angiogenesis-related genes *in vitro* in a manner modulated by oncogenic growth factors. A. mRNA expression profiling was analyzed using an Angiogenesis PCR array and data are expressed as fold change compared to untreated control DAOY cells. These data are representative of one profiling experiment. Notably, thrombin selectively induces ILB/IL1β expression in HGF+SHH pre-treated cells. B. Validation of thrombin targets identified in (A). Genes were selected from the angiogenesis profile for further validation by real-time PCR. Abbreviations: IIa, active thrombin. Values represent mean ± SEM from n=3 independent experiments. **** p< 0.0001, *** p= 0.0001 to 0.001, ** p= 0.001 to 0.01, * p= 0.01 to 0.05.

Table 1. Classes of angiogenic changes in MB cells exposed to thrombin

Class 1	Class 2	Class 3	Class 4	Class 5
EDN1, IL1B, IL6	MMP9	IL8, SERPINE1	ANG, CCL11, CDH5, CXCL10, EDN1, EGF, IGF1, IL1B, IL6, IL8, KDR, LECT1, MMP9, SERPINE1	ANG, CCL11, CDH5, CXCL10, EGF, IGF1, KDR, LECT1

Figure 1

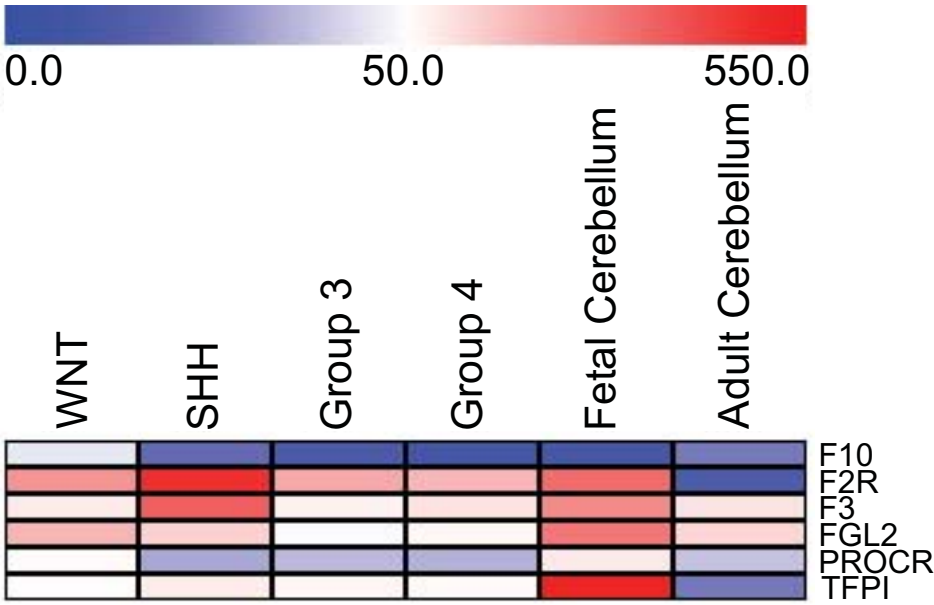
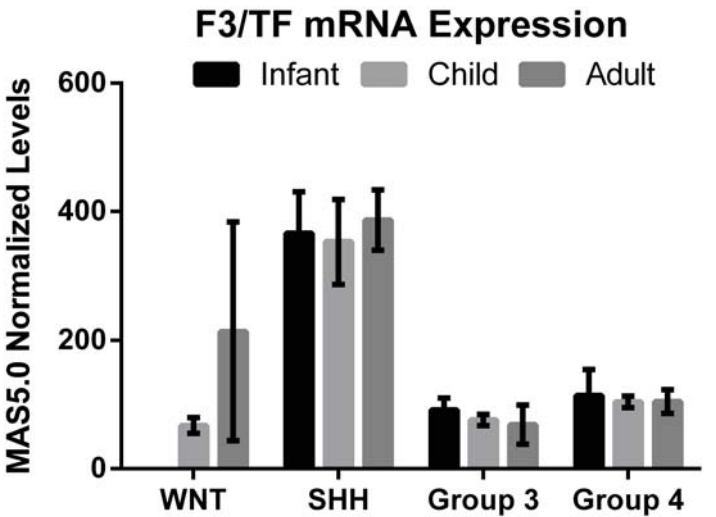

0.0
50.0
550.0
WNT
SHH
Group 3
Group 4
Fetal Cerebellum
Adult Cerebellum
F10
F2R
F3
FGL2
PROCR
TFPI

Figure 2


F3/TF mRNA Expression
Infant
Child
Adult
MAS5.0 Normalized Levels
600
400
200
0
WNT
SHH
Group 3
Group 4

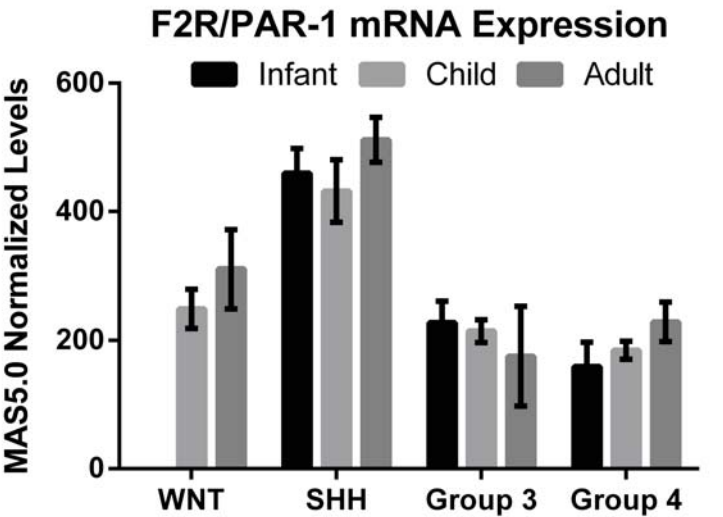

F2R/PAR-1 mRNA Expression
Infant
Child
Adult
MAS5.0 Normalized Levels
600
400
200
0
WNT
SHH
Group 3
Group 4

Figure 3

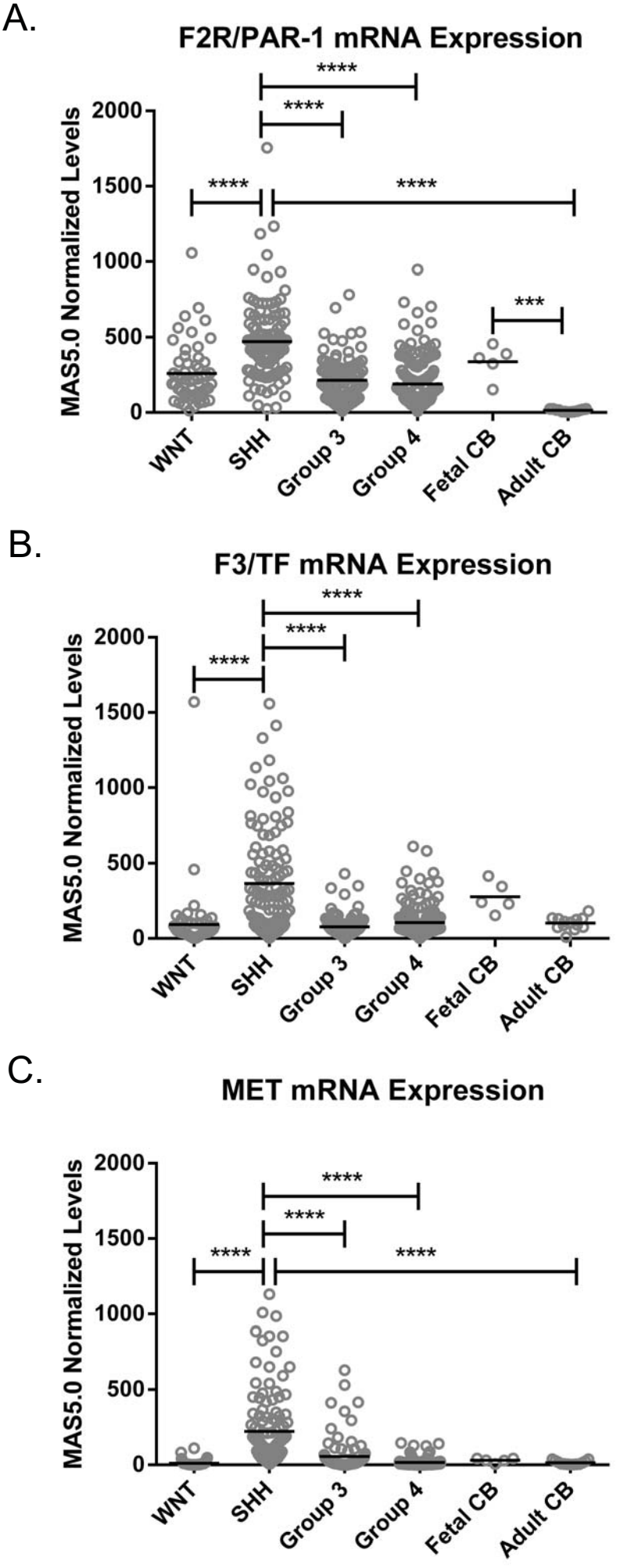
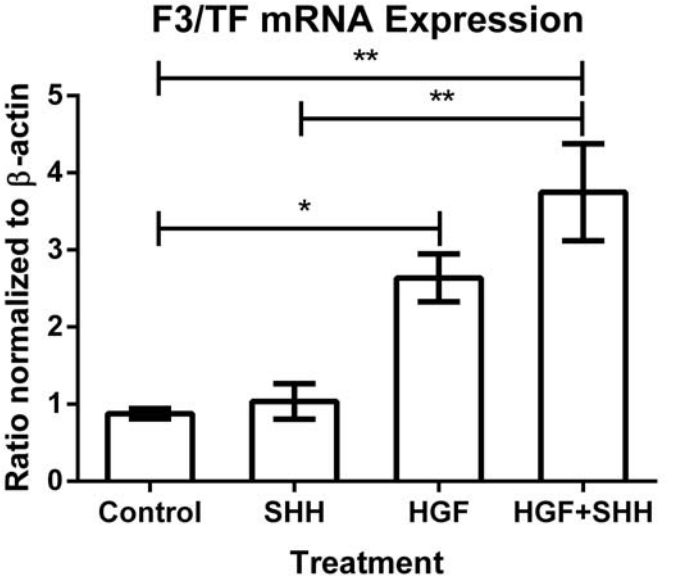

A.
F2R/PAR-1 mRNA Expression
MAS5.0 Normalized Levels
2000
1500
1000
500
0
****
****
****
****
***
WNT
SHH
Group 3
Group 4
Fetal CB
Adult CB
B.
F3/TF mRNA Expression
MAS5.0 Normalized Levels
2000
1500
1000
500
0
****
****
****
WNT
SHH
Group 3
Group 4
Fetal CB
Adult CB
C.
MET mRNA Expression
MAS5.0 Normalized Levels
2000
1500
1000
500
0
****
****
****
****
WNT
SHH
Group 3
Group 4
Fetal CB
Adult CB

Figure 4


F3/TF mRNA Expression
Ratio normalized to β-actin
0
1
2
3
4
5
**
**
*
Control
SHH
HGF
HGF+SHH
Treatment

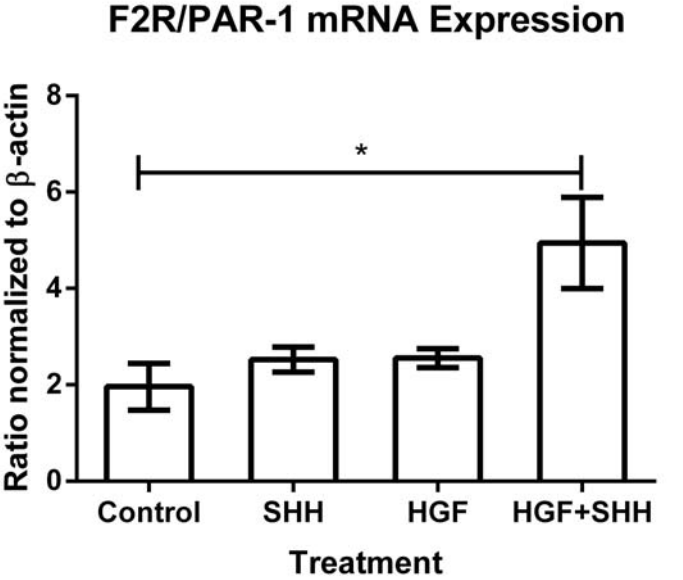

F2R/PAR-1 mRNA Expression
Ratio normalized to β-actin
0
2
4
6
8
*
Control
SHH
HGF
HGF+SHH
Treatment

Figure 5

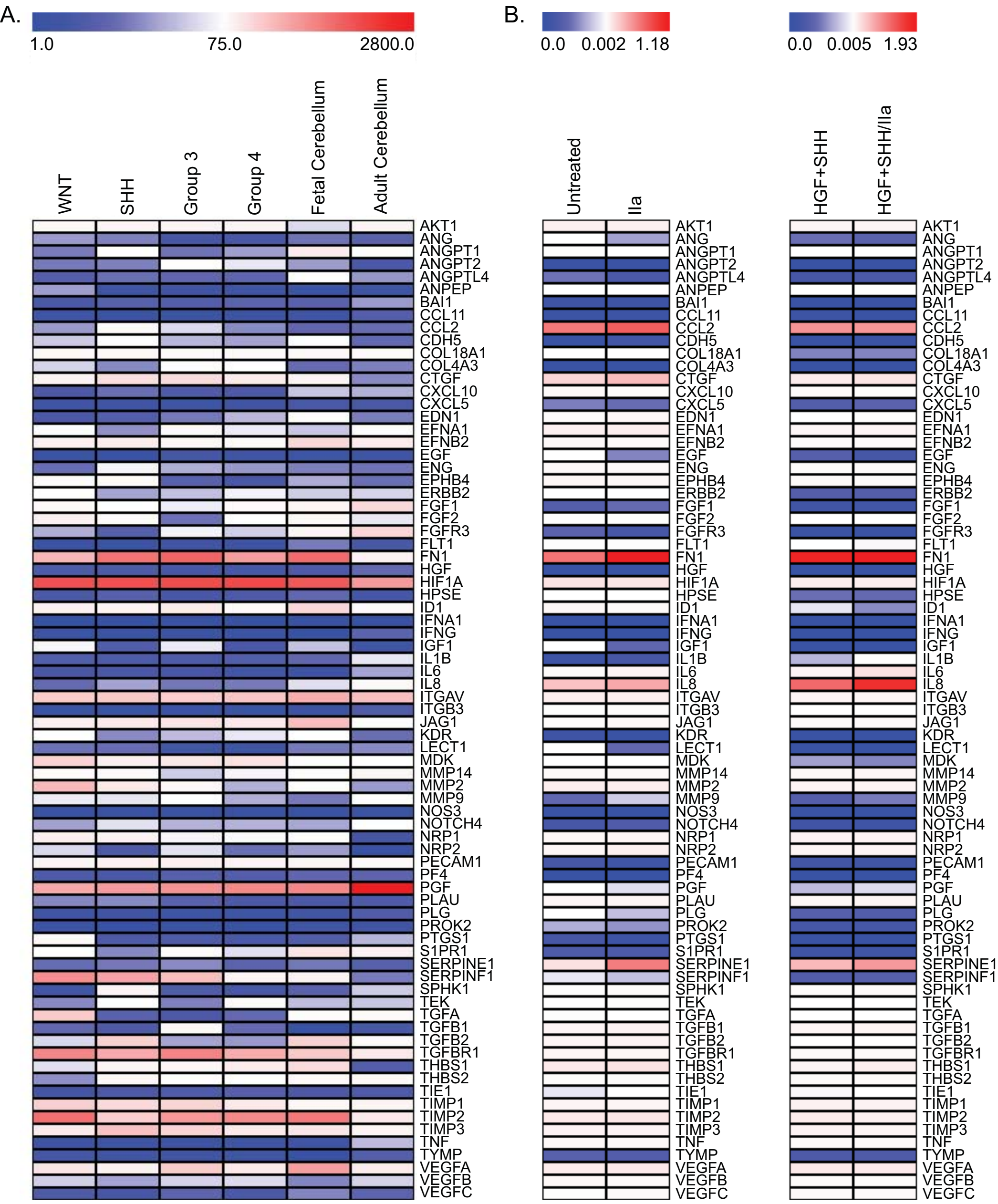
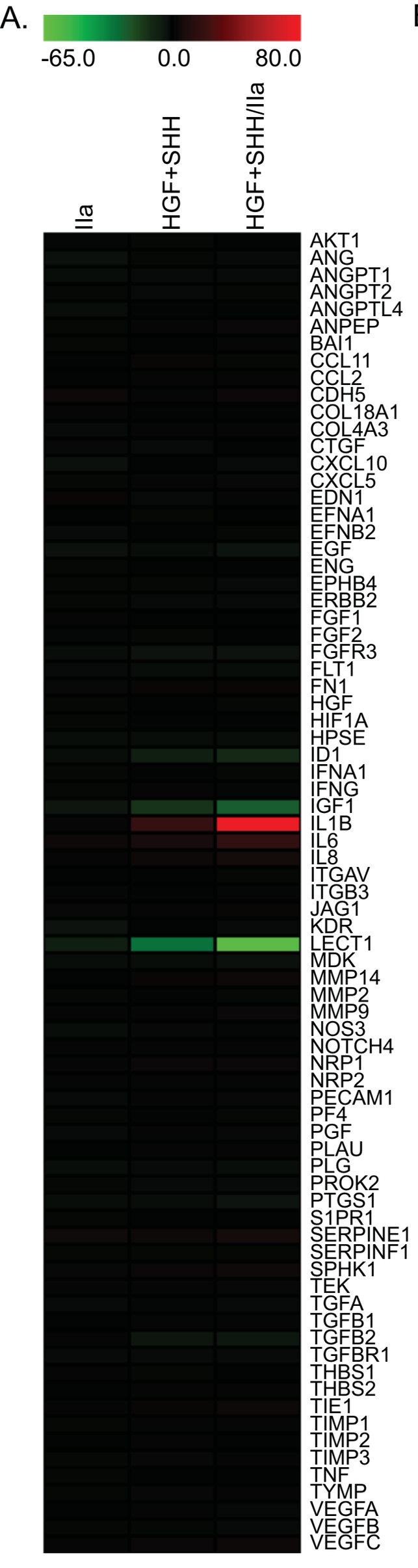

A.
1.0
75.0
2800.0
WNT
SHH
Group 3
Group 4
Fetal Cerebellum
Adult Cerebellum
B.
0.0
0.002
1.18
Untreated
IIa
0.0
0.005
1.93
HGF+SHH
HGF+SHH/IIa
AKT1
ANG
ANGPT1
ANGPT2
ANGPTL4
ANPEP
BAI1
CCL11
CCL2
CDH5
COL18A1
COL4A3
CTGF
CXCL10
CXCL5
EDN1
EFNA1
EFNB2
EGF
ENG
EPHB4
ERBB2
FGF1
FGF2
FGFR3
FLT1
FN1
HGF
HIF1A
HPSE
ID1
IFNA1
IFNG
IGF1
IL1B
IL6
IL8
ITGAV
ITGB3
JAG1
KDR
LECT1
MDK
MMP14
MMP2
MMP9
NOS3
NOTCH4
NRP1
NRP2
PECAM1
PF4
PGF
PLAU
PLG
PROK2
PTGS1
S1PR1
SERPINE1
SERPINF1
SPHK1
TEK
TGFA
TGFB1
TGFB2
TGFBR1
THBS1
THBS2
TIE1
TIMP1
TIMP2
TIMP3
TNF
TYMP
VEGFA
VEGFB
VEGFC

Figure 6


A.
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IIa
HGF+SHH
HGF+SHH/IIa
AKT1
ANG
ANGPT1
ANGPT2
ANGPTL4
ANPEP
BAI1
CCL11
CCL2
CDH5
COL18A1
COL4A3
CTGF
CXCL10
CXCL5
EDN1
EFNA1
EFNB2
EGF
ENG
EPHB4
ERBB2
FGF1
FGF2
FGFR3
FLT1
FN1
HGF
HIF1A
HPSE
ID1
IFNA1
IFNG
IGF1
IL1B
IL6
IL8
ITGAV
ITGB3
JAG1
KDR
LECT1
MDK
MMP14
MMP2
MMP9
NOS3
NOTCH4
NRP1
NRP2
PECAM1
PF4
PGF
PLAU
PLG
PROK2
PTGS1
S1PR1
SERPINE1
SERPINF1
SPHK1
TEK
TGFA
TGFB1
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TNF
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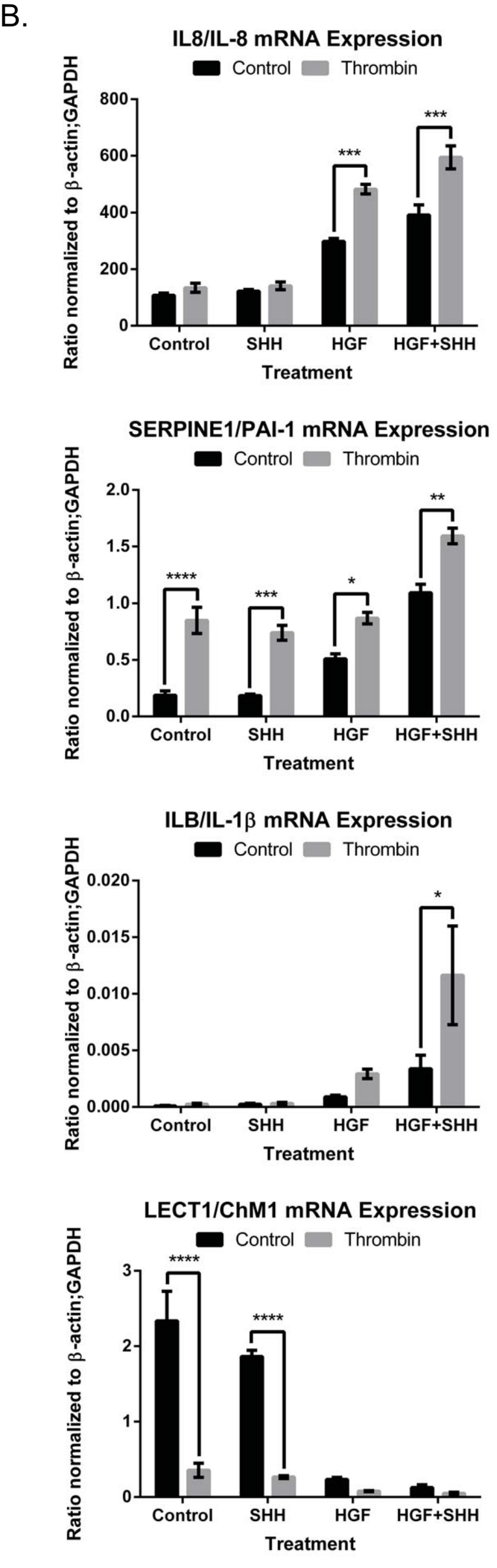
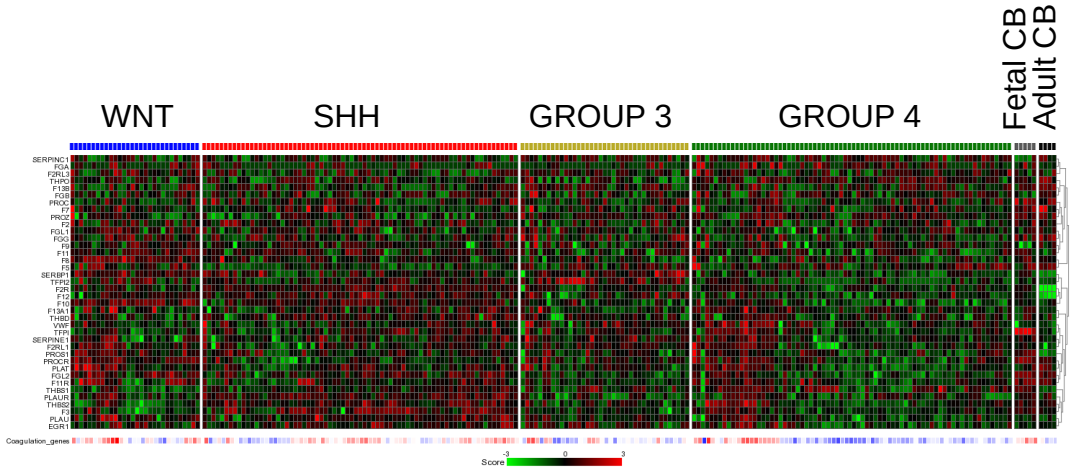
VEGFA
VEGFB
VEGFC

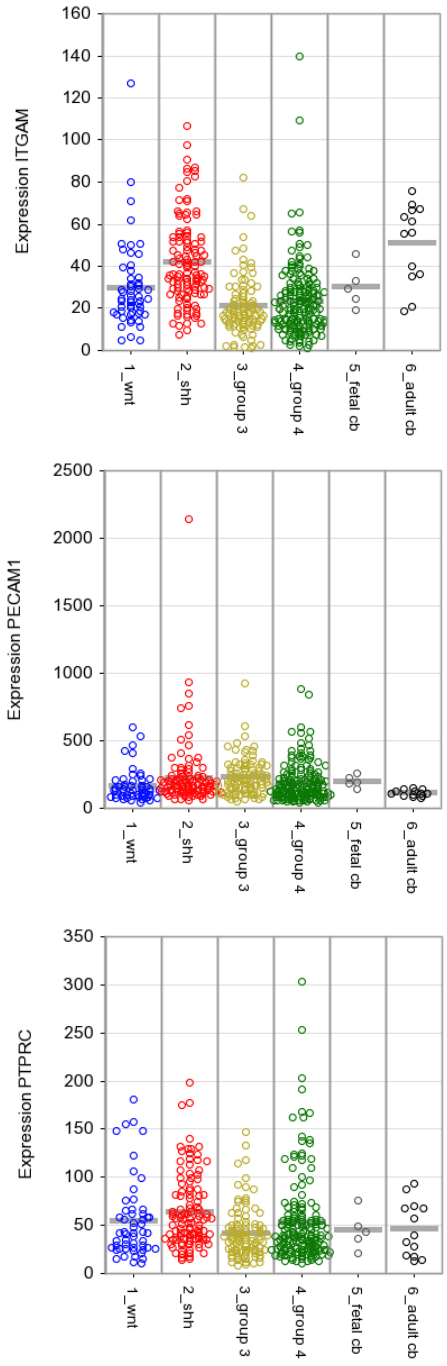

B.
IL8/IL-8 mRNA Expression
Control
Thrombin
Ratio normalized to β-actin;GAPDH
800
600
400
200
0
***
***
Control
SHH
HGF
HGF+SHH
Treatment
SERPINE1/PAI-1 mRNA Expression
Control
Thrombin
Ratio normalized to β-actin;GAPDH
2.0
1.5
1.0
0.5
0.0
****
***
*
**
Control
SHH
HGF
HGF+SHH
Treatment
ILB/IL-1β mRNA Expression
Control
Thrombin
Ratio normalized to β-actin;GAPDH
0.020
0.015
0.010
0.005
0.000
*
Control
SHH
HGF
HGF+SHH
Treatment
LECT1/ChM1 mRNA Expression
Control
Thrombin
Ratio normalized to β-actin;GAPDH
3
2
1
0
****
****
Control
SHH
HGF
HGF+SHH
Treatment

SUPPL FIGURE 1



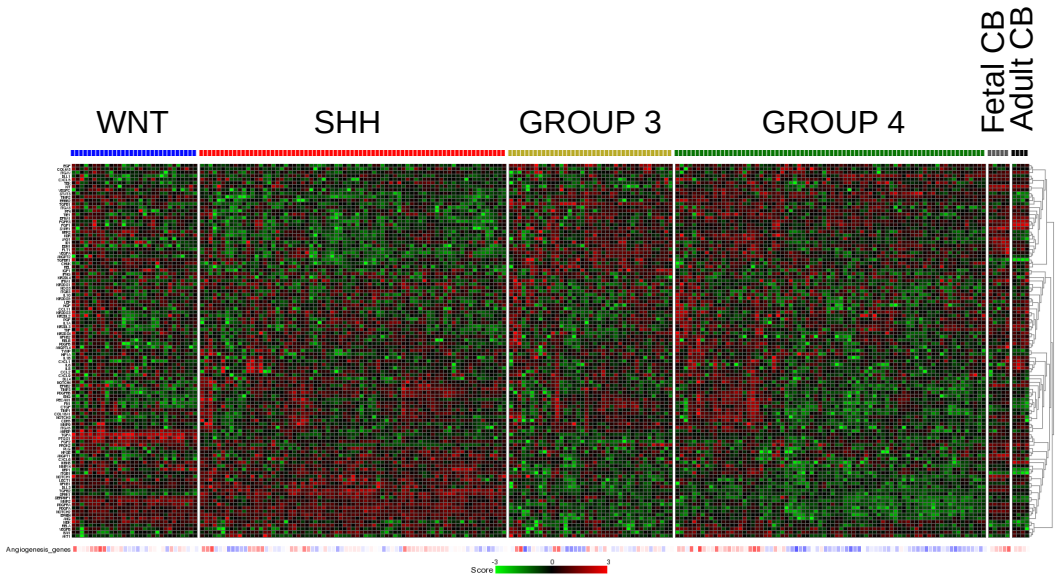
Supplementary Figure 1. Subgroup-specific changes in coagulation factor expression of human medulloblastoma. The heatmap illustrates mRNA expression levels of 38 effectors of the coagulation and fibrinolytic systems in 424 patient specimens and an additional 31 samples of fetal and adult cerebellum. Abbreviation: CB, cerebellum. WNT, n= 53; SHH, n= 113; Group 3, n= 94; Group 4, n= 164; Fetal cerebellum, n= 5; Adult cerebellum, n= 13.

SUPPL FIGURE 2



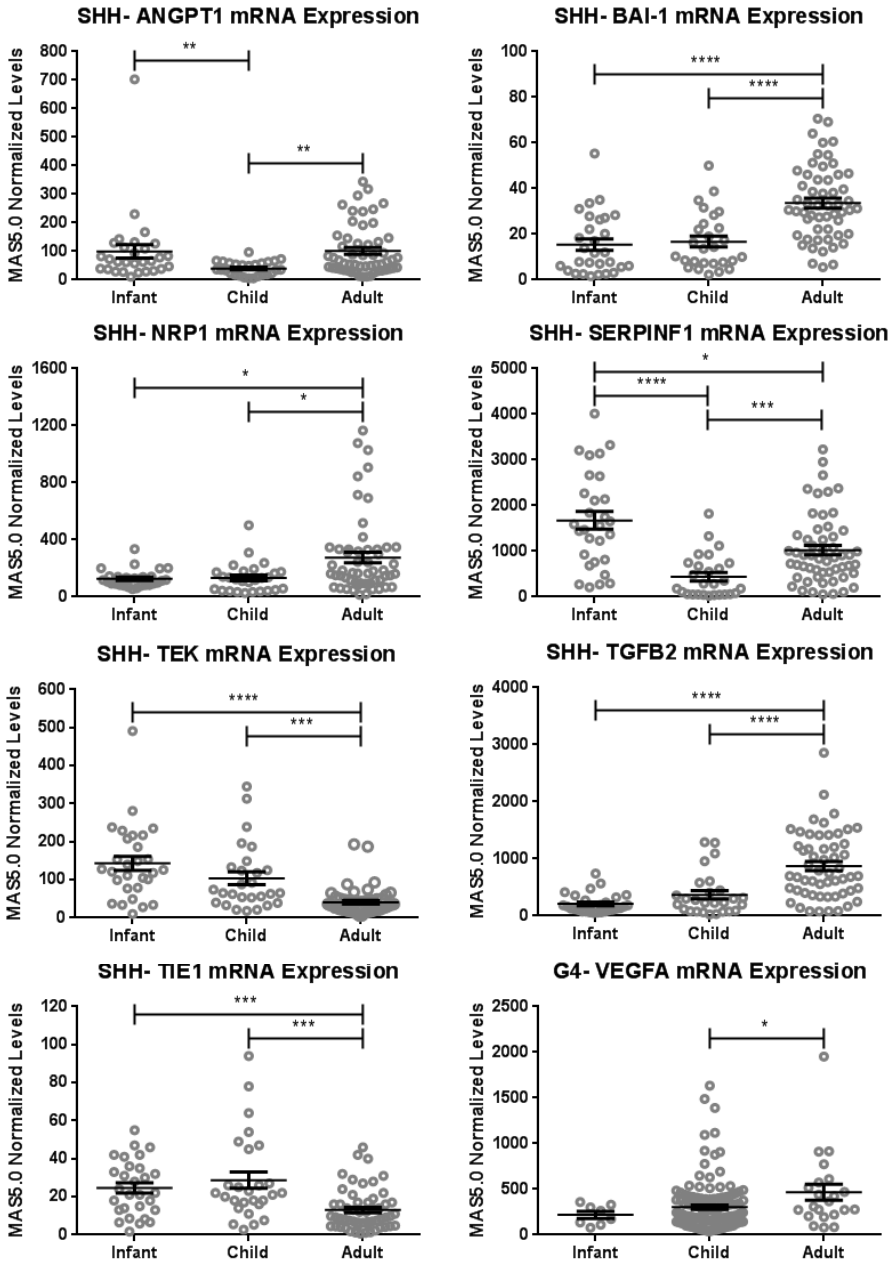
Supplementary Figure 2. Minimal overall differences in the mRNA expression of stromal cell markers ITGAM, PECAM1, and PTPRC. Abbreviation: CB, cerebellum. The black line for each subgroup is representative of the mean. WNT, n= 53; SHH, n= 113; Group 3, n= 94; Group 4, n= 164; Fetal CB, n= 5; Adult CB, n= 13.

SUPPL FIGURE 3



Supplementary Figure 3. Subgroup-specific changes in the expression of angiogenesis-related factors of human medulloblastoma. The heatmap illustrates mRNA expression levels of 77 angiogenesis-related factors in 424 patient specimens and an additional 31 samples of fetal and adult cerebellum. Abbreviation: CB, cerebellum. WNT, n= 53; SHH, n= 113; Group 3, n= 94; Group 4, n= 164; Fetal cerebellum, n= 5; Adult cerebellum, n= 13.

SUPPL FIGURE 4



Supplementary Figure 4. SHH tumours frequently demonstrate age-dependent alterations in angiogenesis-related factors. Abbreviations: G4, Group 4. The black line for each subgroup is representative of the mean. SHH: infant, n= 29; child, n= 27; adult, n= 55. Group 4: infant, n= 8; child, n= 131; adult, n= 22. **** p< 0.0001, *** p= 0.0001 to 0.001, ** p= 0.001 to 0.01, * p= 0.01 to 0.05.

Supplementary Table 1. Significant changes in the mRNA expression of coagulation-related factors (p= <0.0001)

Gene	WNT	SHH	Group 3	Group 4	Fetal Cerebellum	Adult Cerebellum
F10	45.66ABC**	21.23BC	12.42	8.98^^	9.46	24.79
F2R	259.74AD	479.38BCD	218.06D	189.25^^^	337.12^^^	13.54
F3	92.22Ac**	367.03BC	78.85*	104.74	277.66	101.98
FGL2	193.16BC	140.13BC	49.46***^^	62.81***^^	315.16	131.83
PROCR	57.42ABC	33.15**	36.57**	35.01**	93.28	38.32
TFPI	52.22a***	85.47CD	68.87**^^	59.39***^	519.76D	24.52

Data are representative of the mean. A-D, p<0.0001 compared to SHH (A), Group 3 (B), Group 4 (C), and adult cerebellum (D); a, p= 0.0001 to 0.001 compared to SHH; c, p= 0.01 to 0.05 compared to Group 4; *** p= 0.0001 to 0.001 compared to fetal cerebellum; ** p= 0.001 to 0.01 compared to fetal cerebellum; * p= 0.01 to 0.05 compared to fetal cerebellum; ^^^ p= 0.0001 to 0.001 compared to adult cerebellum; ^^ p= 0.001 to 0.01 compared to adult cerebellum; ^ p= 0.01 to 0.05 compared to adult cerebellum.

Supplementary Table 2. Significant changes in the mRNA expression of angiogenesis-related factors (p= <0.0001)

Gene	WNT	SHH	Group 3	Group 4	Fetal Cerebellum	Adult Cerebellum
ANG	46.76BC	40.26BC	15.49*	16.83*	35.98	27.77
ANGPT2	38.21BC	39.65BC	76.75D	68.33^^^	47.42	18.97
ANPEP	47.92ABC*^^^	11.17	13.12	10.33	8.58	12.19
EPHB4	97.13BC^^	114.24BCD	27.38	18.09	50.98	34.59
FGF2	215.32ABC^^^	110.61Bc	35.06C**^	77.32	122.36	68.95
FN1	840.06AB	1580.13CD	1731.51CD	1107.63^^^	1638.90^^	209.08
MDK	549.92Ab**D	241.34c^^	325.99^^^	369.26*D	93.78	78.98
MMP14	108.42BC	105.75BC	60.72D	72.80D	87.2	155.22
MMP2	817.83aBC*D	299.97BCD	128.81C	53.18	90.14	46.58
NRP1	249.512D	199.55BCD	72.90^^	105.23D	96.18^	14.96
PTGS1	127.66ABC	21.45D	21.15D	18.54D	23.12	53.98
S1PR1	80.82A*^^^	42.14BCDE	102.50^^	66.53**D	347.46	209.45
SERPINF1	1261.907CD	1041.47CD	758.68CD	182.01	207.96	38.55
SPHK1	13.73A7D	170.89BC	22.23^	17.63^^^	27.74	60.48
TEK	42.29aC	82.48B	40.12C	105.37	56.56	58.82
TGFA	642.23ABC	27.303*D	19.71C***D	32.22^^^	91.48	111.25
TGFB1	31.14B	21.72Bc	118.11C***D	32.51	4.18	16.45
TGFB2	64.04A*	574.65BC	49.40***^^	46.49***^^^	536.5	102.82
TGFBR1	1356.62AC**D	978.76BD	1378.09C**D	923.19D	659	308.52
TIMP2	1600.28AbD	590.24BC***	1179.28D	1324.37D	1496.50D	311.56
TIMP3	289.66A7	728.07bCD	531.42c^	294.43	337.82	171.5
VEGFA	357.70A2	218.24BC***^	617.44C	318.75*	1082.12	297.44

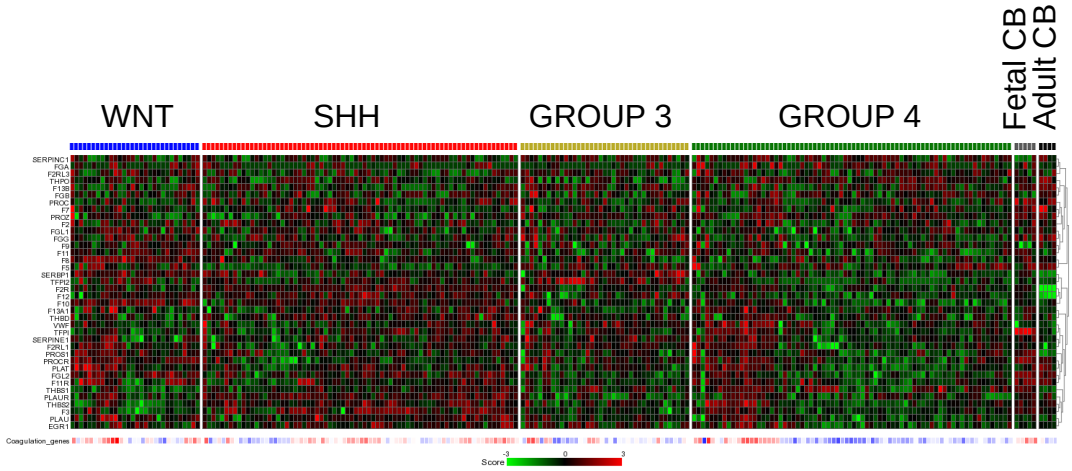
Data are representative of the mean. A-D, p<0.0001 compared to SHH (A), Group 3 (B), Group 4 (C), and adult cerebellum (D); a, p= 0.0001 to 0.001 compared to SHH; b, p= 0.0001 to 0.001 compared to Group 3; c, p= 0.0001 to 0.001 compared to Group 4; 2, p= 0.001 to 0.01 compared to Group 3; 3, p= 0.001 to 0.01 compared to Group 4; 7, p= 0.01 to 0.05 compared to Group 3; *** p= 0.0001 to 0.001 compared to fetal cerebellum; ** p= 0.001 to 0.01 compared to fetal cerebellum; * p= 0.01 to 0.05 compared to fetal cerebellum; ^^^ p= 0.0001 to 0.001 compared to adult cerebellum; ^^ p= 0.001 to 0.01 compared to adult cerebellum; ^ p= 0.01 to 0.05 compared to adult cerebellum.

Supplementary Table 3. Gene names

Gene	Official Full Name
AKT1	v-akt murine thymoma viral oncogene homolog 1
ANG	angiogenin
ANGPT1	angiopoietin 1
ANGPT2	angiopoietin 2
ANGPTL4	angiopoietin-like 4
ANPEP	alanyl (membrane) aminopeptidase
BAI1	brain-specific angiogenesis inhibitor 1
CCL11	chemokine (C-C motif) ligand 11
CCL2	chemokine (C-C motif) ligand 2
CDH5	cadherin 5, type 2
COL18A1	collagen, type XVIII, alpha 1
COL4A3	collagen, type IV, alpha 3
CTGF	connective tissue growth factor
CXCL10	chemokine (C-X-C motif) ligand 10
CXCL5	chemokine (C-X-C motif) ligand 5
EDN1	endothelin 1
EFNA1	ephrin-A1
EFNB2	ephrin-B2
EGF	epidermal growth factor
ENG	endoglin
EPHB4	EPH receptor B4
ERBB2	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2
F10	coagulation factor X
F2R	coagulation factor II receptor
F3	coagulation factor III
FGF1	fibroblast growth factor 1 (acidic)
FGF2	fibroblast growth factor 2 (basic)
FGFR3	fibroblast growth factor receptor 3
FGL2	fibrinogen-like 2
FLT1	fms-related tyrosine kinase 1
FN1	fibronectin 1
HGF	hepatocyte growth factor
HIF1A	hypoxia inducible factor 1, alpha subunit
HPSE	Heparanase
ID1	inhibitor of DNA binding 1
IFNA1	interferon, alpha 1
IFNG	interferon gamma
IGF1	insulin-like growth factor 1
IL1B	interleukin 1, beta
IL6	interleukin 6
IL8	interleukin 8

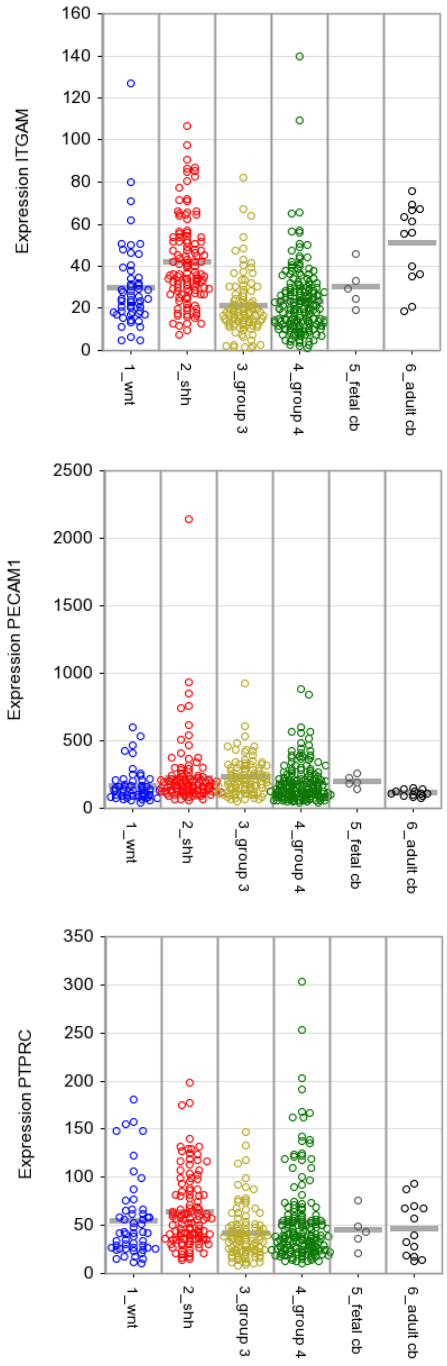
ITGAV	integrin, alpha V
ITGB3	integrin, beta 3
JAG1	jagged 1
KDR	kinase insert domain receptor
LECT1	leukocyte cell derived chemotaxin 1
MDK	midkine
MMP14	matrix metallopeptidase 14
MMP2	matrix metallopeptidase 2
MMP9	matrix metallopeptidase 9
NOS3	nitric oxide synthase 3
NOTCH4	notch 4
NRP1	neuropilin 1
NRP2	neuropilin 2
PECAM1	platelet/endothelial cell adhesion molecule 1
PF4	platelet factor 4
PGF	placental growth factor
PLAU	plasminogen activator, urokinase
PLG	plasminogen
PROCR	protein C receptor
PROK2	prokineticin 2
PTGS1	prostaglandin-endoperoxide synthase 1
S1PR1	sphingosine-1-phosphate receptor 1
SERPINE1	serpin peptidase inhibitor, clade E
SERPINF1	serpin peptidase inhibitor, clade F
SPHK1	sphingosine kinase 1
TEK	TEK tyrosine kinase
TFPI	tissue factor pathway inhibitor
TGFA	transforming growth factor, alpha
TGFB1	transforming growth factor, beta 1
TGFB2	transforming growth factor, beta 2
TGFBR1	transforming growth factor, beta receptor 1
THBS1	thrombospondin 1
THBS2	thrombospondin 2
TIE1	tyrosine kinase with immunoglobulin-like and EGF-like domains 1
TIMP1	TIMP metallopeptidase inhibitor 1
TIMP2	TIMP metallopeptidase inhibitor 2
TIMP3	TIMP metallopeptidase inhibitor 3
TNF	tumor necrosis factor
TYMP	thymidine phosphorylase
VEGFA	vascular endothelial growth factor A
VEGFB	vascular endothelial growth factor B
VEGFC	vascular endothelial growth factor C

SUPPL FIGURE 1



Supplementary Figure 1. Subgroup-specific changes in coagulation factor expression of human medulloblastoma. The heatmap illustrates mRNA expression levels of 38 effectors of the coagulation and fibrinolytic systems in 424 patient specimens and an additional 31 samples of fetal and adult cerebellum. Abbreviation: CB, cerebellum. WNT, n= 53; SHH, n= 113; Group 3, n= 94; Group 4, n= 164; Fetal cerebellum, n= 5; Adult cerebellum, n= 13.

SUPPL FIGURE 2



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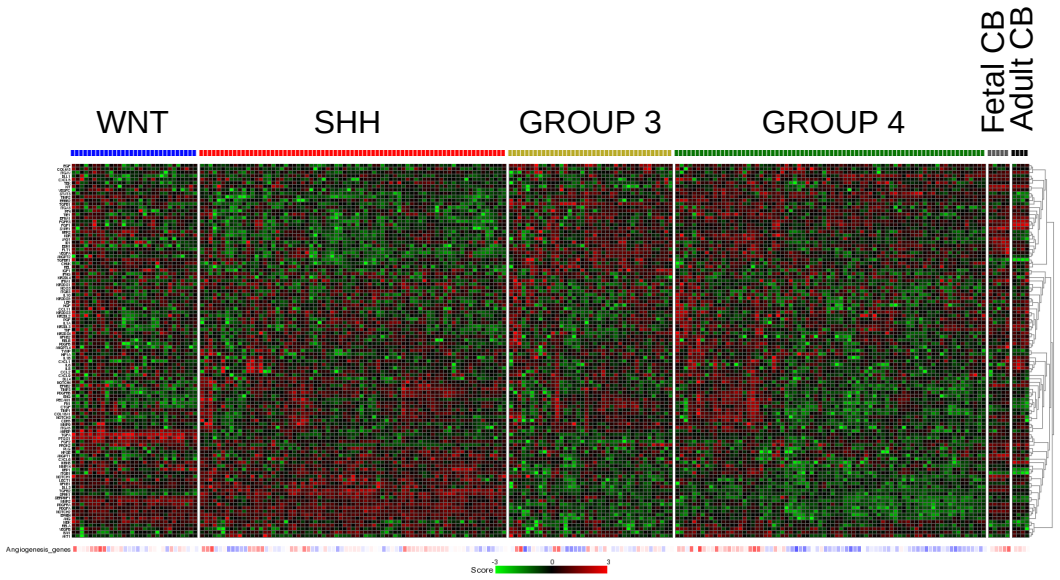
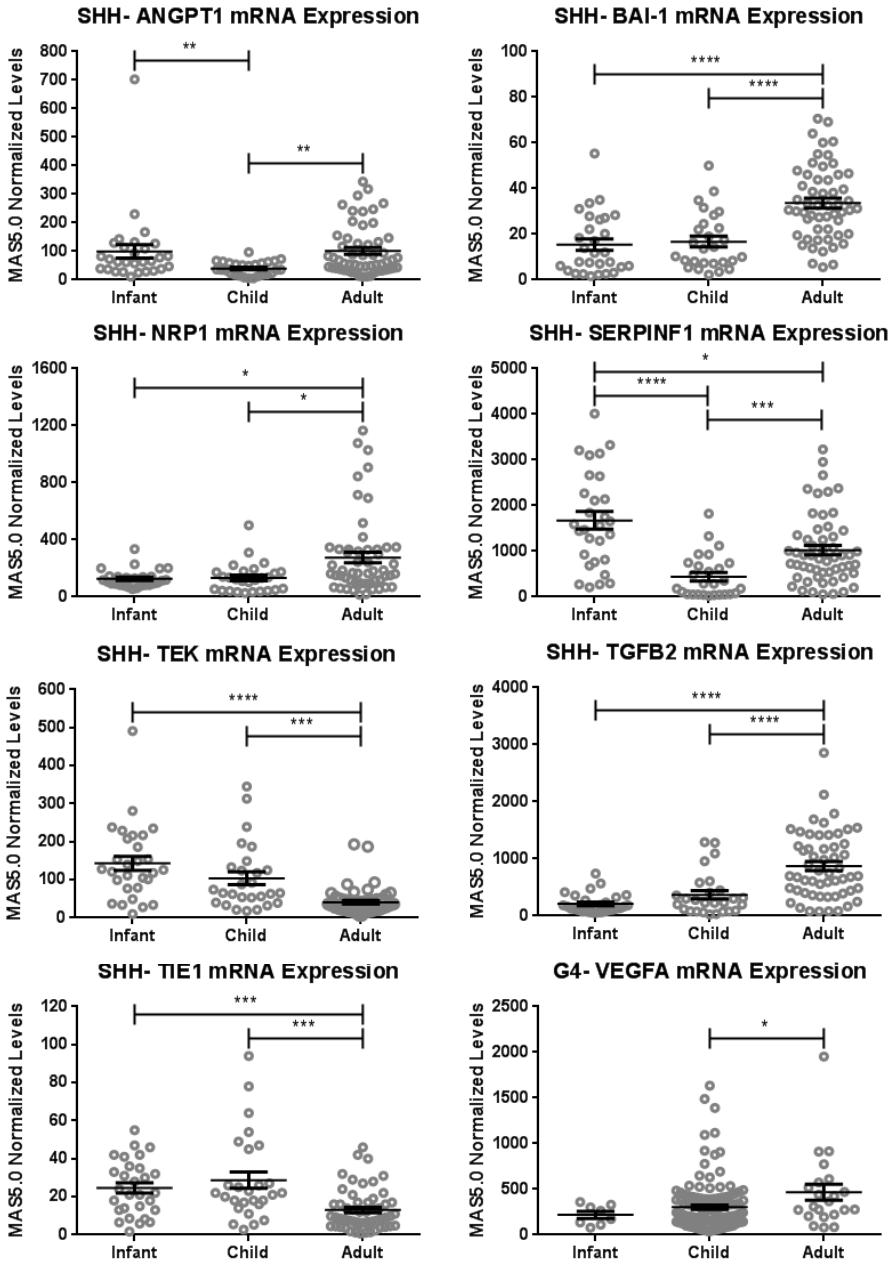
SUPPL FIGURE 3



Supplementary Figure 3. Subgroup-specific changes in the expression of angiogenesis-related factors of human medulloblastoma. The heatmap illustrates mRNA expression levels of 77 angiogenesis-related factors in 424 patient specimens and an additional 31 samples of fetal and adult cerebellum. Abbreviation: CB, cerebellum. WNT, n= 53; SHH, n= 113; Group 3, n= 94; Group 4, n= 164; Fetal cerebellum, n= 5; Adult cerebellum, n= 13.

SUPPL FIGURE 4



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Supplementary Table 1. Significant changes in the mRNA expression of coagulation-related factors (p= <0.0001)

Gene	WNT	SHH	Group 3	Group 4	Fetal Cerebellum	Adult Cerebellum
F10	45.66ABC**	21.23BC	12.42	8.98^^	9.46	24.79
F2R	259.74AD	479.38BCD	218.06D	189.25^^^	337.12^^^	13.54
F3	92.22Ac**	367.03BC	78.85*	104.74	277.66	101.98
FGL2	193.16BC	140.13BC	49.46***^^	62.81***^^	315.16	131.83
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Supplementary Table 2. Significant changes in the mRNA expression of angiogenesis-related factors (p= <0.0001)

Gene	WNT	SHH	Group 3	Group 4	Fetal Cerebellum	Adult Cerebellum
ANG	46.76BC	40.26BC	15.49*	16.83*	35.98	27.77
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ANPEP	47.92ABC*^^^	11.17	13.12	10.33	8.58	12.19
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PTGS1	127.66ABC	21.45D	21.15D	18.54D	23.12	53.98
S1PR1	80.82A*^^^	42.14BCDE	102.50^^	66.53**D	347.46	209.45
SERPINF1	1261.907CD	1041.47CD	758.68CD	182.01	207.96	38.55
SPHK1	13.73A7D	170.89BC	22.23^	17.63^^^	27.74	60.48
TEK	42.29aC	82.48B	40.12C	105.37	56.56	58.82
TGFA	642.23ABC	27.303*D	19.71C***D	32.22^^^	91.48	111.25
TGFB1	31.14B	21.72Bc	118.11C***D	32.51	4.18	16.45
TGFB2	64.04A*	574.65BC	49.40***^^	46.49***^^^	536.5	102.82
TGFBR1	1356.62AC**D	978.76BD	1378.09C**D	923.19D	659	308.52
TIMP2	1600.28AbD	590.24BC***	1179.28D	1324.37D	1496.50D	311.56
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ANG	angiogenin
ANGPT1	angiopoietin 1
ANGPT2	angiopoietin 2
ANGPTL4	angiopoietin-like 4
ANPEP	alanyl (membrane) aminopeptidase
BAI1	brain-specific angiogenesis inhibitor 1
CCL11	chemokine (C-C motif) ligand 11
CCL2	chemokine (C-C motif) ligand 2
CDH5	cadherin 5, type 2
COL18A1	collagen, type XVIII, alpha 1
COL4A3	collagen, type IV, alpha 3
CTGF	connective tissue growth factor
CXCL10	chemokine (C-X-C motif) ligand 10
CXCL5	chemokine (C-X-C motif) ligand 5
EDN1	endothelin 1
EFNA1	ephrin-A1
EFNB2	ephrin-B2
EGF	epidermal growth factor
ENG	endoglin
EPHB4	EPH receptor B4
ERBB2	v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2
F10	coagulation factor X
F2R	coagulation factor II receptor
F3	coagulation factor III
FGF1	fibroblast growth factor 1 (acidic)
FGF2	fibroblast growth factor 2 (basic)
FGFR3	fibroblast growth factor receptor 3
FGL2	fibrinogen-like 2
FLT1	fms-related tyrosine kinase 1
FN1	fibronectin 1
HGF	hepatocyte growth factor
HIF1A	hypoxia inducible factor 1, alpha subunit
HPSE	Heparanase
ID1	inhibitor of DNA binding 1
IFNA1	interferon, alpha 1
IFNG	interferon gamma
IGF1	insulin-like growth factor 1
IL1B	interleukin 1, beta
IL6	interleukin 6
IL8	interleukin 8

ITGAV	integrin, alpha V
ITGB3	integrin, beta 3
JAG1	jagged 1
KDR	kinase insert domain receptor
LECT1	leukocyte cell derived chemotaxin 1
MDK	midkine
MMP14	matrix metallopeptidase 14
MMP2	matrix metallopeptidase 2
MMP9	matrix metallopeptidase 9
NOS3	nitric oxide synthase 3
NOTCH4	notch 4
NRP1	neuropilin 1
NRP2	neuropilin 2
PECAM1	platelet/endothelial cell adhesion molecule 1
PF4	platelet factor 4
PGF	placental growth factor
PLAU	plasminogen activator, urokinase
PLG	plasminogen
PROCR	protein C receptor
PROK2	prokineticin 2
PTGS1	prostaglandin-endoperoxide synthase 1
S1PR1	sphingosine-1-phosphate receptor 1
SERPINE1	serpin peptidase inhibitor, clade E
SERPINF1	serpin peptidase inhibitor, clade F
SPHK1	sphingosine kinase 1
TEK	TEK tyrosine kinase
TFPI	tissue factor pathway inhibitor
TGFA	transforming growth factor, alpha
TGFB1	transforming growth factor, beta 1
TGFB2	transforming growth factor, beta 2
TGFBR1	transforming growth factor, beta receptor 1
THBS1	thrombospondin 1
THBS2	thrombospondin 2
TIE1	tyrosine kinase with immunoglobulin-like and EGF-like domains 1
TIMP1	TIMP metallopeptidase inhibitor 1
TIMP2	TIMP metallopeptidase inhibitor 2
TIMP3	TIMP metallopeptidase inhibitor 3
TNF	tumor necrosis factor
TYMP	thymidine phosphorylase
VEGFA	vascular endothelial growth factor A
VEGFB	vascular endothelial growth factor B
VEGFC	vascular endothelial growth factor C