Volume 2 | Number 1

HHT FRONTLINES

BRINGING SCIENCE FROM BENCH TO BEDSIDE

CLINICAL RESEARCH

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PULMONARY ARTERIOVENOUS MALFORMATIONS ARE ASSOCIATED WITH SILENT BRAIN INFARCTS IN HEREDITARY HEMORRHAGIC TELANGIECTASIA PATIENTS

W Brinjikji, DM Nasr, CP Wood, VN Iyer
Cerebrovasc Dis 2017;44:179–185

The authors of this article evaluated the prevalence of silent brain infarcts in patients with HHT.

Objective: The purpose of this study was to determine the prevalence and risk factors for silent brain infarcts (SBI) in HHT patients.

Materials and Methods: Our institutional HHT database was queried to identify HHT patients who received a baseline screening brain MRI from January 2000 to February 2017. This study group was further refined by excluding patients who had a history of clinical ischemic disease as defined by having a stroke or transient ischemic attack (TIA). Brain MRIs were reviewed for SBI. Baseline data on demographics, Curacao criteria, presence of PAVMs, and cardiovascular risk factors were collected. The primary outcome was SBI prevalence. We also examined which baseline patient characteristics were associated with SBI through univariate chi-square and Student t tests and multivariate logistic regression analyses.

Results: Three hundred fifty three consecutive HHT patients from January 2000 to February 2017 with a screening brain MRI and no prior history of stroke/TIA were included. SBI prevalence was 9.9% (35/353). SBI patients were more likely to have PAVMs than non-SBI patients (80.6 vs. 53.1%, p = 0.005). The median age was 66 in the SBI group and 52 in the non-SBI group (p = 0.006). SBI patients had higher prevalence of hyperlipidemia (34.3 vs. 9.8%, p < 0.0001), hyper- tension (48.6 vs. 22.0%, p = 0.005), and tobacco use (25.7 vs. 9.8%, p = 0.005). No patients under 30 had SBI. In the 60–69 age group, the prevalence of SBI was 18.8% with rates of 28.6% in the PAVM group and 10.5% in the non-PAVM group. For patients ≥70 years old, the prevalence of SBI was 21.4% overall and 27.6% in the PAVM group and 10.5% in the non-PAVM group. On multivariate analysis, PAVMs (OR 3.62, 95% CI 1.46–10.40) and increasing age (OR 1.04, 95% CI 1.01–1.07) were independently associated with SBI.

Implications: Although a similar 10% SBI prevalence in the HHT cohort was noted as compared to the general population, the prevalence of SBI was higher in HHT patients with PAVMs when compared to that of the general population, particularly among patients more than 60 years old. These findings highlight the need to accurately identify, and when appropriate, treat PAVMs in the HHT population especially given the multiple significant, clinical consequences of SBI.

SURGICAL TREATMENT VS NONSURGICAL TREATMENT FOR BRAIN ARTERIOVENOUS MALFORMATIONS IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA: A RETROSPECTIVE MULTICENTER CONSORTIUM STUDY

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Neurosurgery. 2017 Jul 4. doi: 10.1093/neuros/nyx168. [Epub ahead of print]

The authors of this article evaluated the outcome of surgical treatment versus nonsurgical treatment of cerebral arteriovenous malformations in HHT patients.

Cerebral arteriovenous malformations (AVMs) are common in patients with hereditary hemorrhagic telangiectasia (HHT). However, due to the rarity of HHT and little published evidence of outcomes from management of brain AVMs in this disease, current international HHT guidelines recommend an individualized approach. Specifically, the outcomes for surgical vs nonsurgical management of these lesions have not been reported to date.

Objective: Report long-term outcomes of surgical resection of brain AVMs in HHT patients compared to outcomes in non-surgically treated patients.

Methods: From the database of the Brain Vascular Malformation Consortium HHT project, 19 patients with 20 resected AVMs (group 1) and 22 patients with 33 AVMs who received nonsurgical treatment (group 2) were studied. The groups were retrospectively reviewed for changes in functional status (modified Rankin Scale score) during the follow-up period.

Results: During the follow-up period, 9% of patients in group 1 suffered from worsening of functional status, whereas this figure was 16% for group 2 (P > .05). Functional outcomes were not statistically different between the 2 groups at the latest follow-up (P > .05).

Implications: HHT patients treated surgically for brain AVMs appear to have long-term functional outcomes comparable to nonsurgical (including observational) therapy with fewer unfavorable outcomes. It is therefore reasonable to consider surgical resection as a management option in the multidisciplinary team's individualized treatment strategy for HHT patients with brain AVMs.

CEREBRAL ABSCESS ASSOCIATED WITH ODONTOGENIC BACTEREMIAS, HYPOXEMIA, AND IRON LOADING IN IMMUNOCOMPETENT PATIENTS WITH RIGHT-TO-LE SHUNTING ROUGH PULMONARY ARTERIOVENOUS MALFORMATIONS

EJ Boother, S Brownlow, HC. Tighe, KB Bamford, JE Jackson, CL Shovlin
Clinical Infectious Diseases 2017; 65:595-603

The authors of this article investigated the relation between cerebral abscess and odontogenic bacteremias, hypoxemia, and iron loading.

Cerebral abscess is a recognized complication of pulmonary arteriovenous malformations (PAVMs) that allow systemic venous blood to bypass the pulmonary capillary bed through anatomic right-to-le shunts. Broader implications and mechanisms remain poorly explored.

Methods: Between June 2005 and December 2016, at a single institution, 445 consecutive adult patients with computed tomography–confirmed PAVMs (including 403 [90.5%] with hereditary hemorrhagic telangiectasia) were recruited to a prospective series. Multivariate logistic regression was performed and detailed periabscess histories were evaluated to identify potential associations with cerebral abscess. Rates were compared to an earlier non-overlapping series.

Results: Thirty-seven of the 445 (8.3%) patients experienced a cerebral abscess at a median age of 50 years (range, 19–76 years). e rate adjusted for ascertainment bias was 27 of 435 (6.2%). Twenty-nine of 37 (78.4%) patients with abscess had no PAVM diagnosis prior to their abscess, a rate unchanged from earlier UK series. Twenty-one of 37 (56.7%) suffered residual neurological deficits (most commonly memory/cognition impairment), hemiparesis, and visual defects. Isolation of periodontal microbes, and precipitating dental and other interventional events, emphasized potential sources of endovascular inoculations. In multivariate logistic regression, cerebral abscess was associated with low oxygen saturation (indicating greater right-to-le shunting); higher transferrin iron saturation index; intravenous iron use for anemia (adjusted odds ratio, 5.4 [95% confidence interval, 1.4–21.1]); male sex; and venous thromboemboli. there were no relationships with anatomic attributes of PAVMs, or red cell indices often increased due to secondary polycythemia..

Implications: Greater appreciation of the risk of cerebral abscess in undiagnosed PAVMs is required. Lower oxygen saturation and intravenous iron may be modifiable risk factors (by treating PAVMs under iron supplementation).

SCREENING CHILDREN FOR PULMONARY ARTERIOVENOUS MALFORMATIONS: EVALUATION OF 18 YEARS OF EXPERIENCE

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Pediatr Pulmonol. 2017 Sep;52(9):1206-1211

The authors of this article evaluated 18 years of experience screening children for pulmonary arteriovenous malformations (PAVMs) with a conservative screening method.

Background: Hereditary Hemorrhagic Telangiectasia (HHT) is an autosomal dominant disease with multi-systemic vascular dysplasia. Early diagnosis through screening is important to prevent serious complications. How best to screen children of affected parents for pulmonary arteriovenous malformations (PAVMs) is often subject to debate. Transthoracic contrast echocardiogram (TTCE) is considered optimal in screening for PAVMs in adults. Guidelines for the screening of children are not specific, reflecting the lack of scientific evidence on the best method to use.

Objectives: Aims of this study are (i) to evaluate our current screening method (in use since 1998), consisting of history, physical examination, pulse oximetry, and chest radiography and (ii) to assess whether postponing more invasive screening for PAVMs until adulthood is safe.

Methods: Prospective observational cohort study using a patient database.

Results: Over a period of 18 years (mean follow-up 9.21 years, SD 4.72 years), 436 children from HHT families were screened consecutively. A total of 175/436 (40%) children had a diagnosis of HHT. PAVMs were detected in 39/175 (22%) children, 33/39 requiring treatment by embolotherapy. None of the screened children suffered any PAVM-associated complications with this screening method.

Implications: This study provides a good indication of the safety of a conservative method of screening for PAVMs, performed in a large cohort, over a long period of time. Smaller PAVMs will be missed in children, but in 18 years of experience, with a mean follow-up of more than 9 years and more than 1600 patient years, none of the patients have suffered complications from these undetected small PAVMs. The authors support continuing the screening of all children of HHT parents for relevant PAVMs but suggest that using less invasive and less stressful screening of children for pulmonary involvement, based on physical examination, pulse oximetry and a chest radiograph, instead of TTCE or chest CT, is sufficient to prevent serious complications. By doing so, screening with TTCE and chest CT can be postponed until adult age.

TOPICAL PROPRANOLOL IMPROVES EPISTAXIS IN PATIENTS WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA - A PRELIMINARY REPORT

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Journal of Otolaryngology - Head and Neck Surgery (2017) 46: 58-62

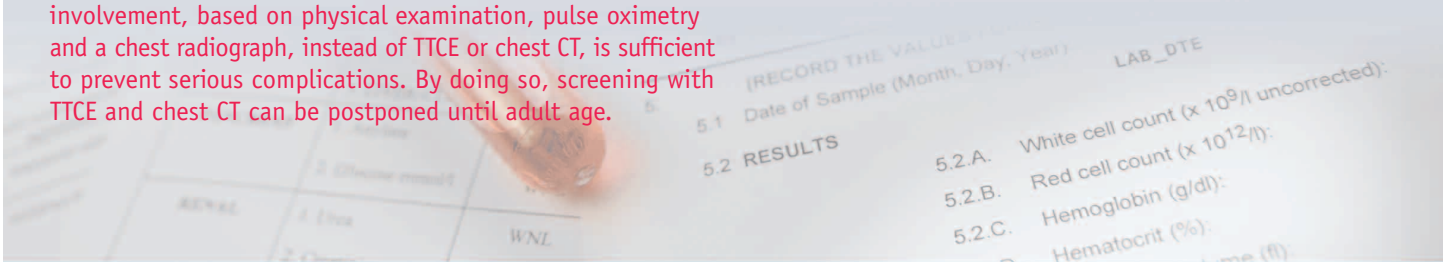
The authors of this article conducted a study to evaluate the effect of topical use of propranolol on epistaxis in HHT patients.

Severe epistaxis is often difficult to control in patients with hereditary hemorrhagic telangiectasia (HHT). Propranolol has been shown to have antiangiogenic properties in vitro and in vivo and is commonly used to treat hemangiomas. We present our experience with topical nasal propranolol for the treatment of moderate to severe epistaxis in patients with HHT.

Methods: Retrospective case series. Six patients with HHT were treated with 0.5 cm3 of 1.5% propranolol gel, applied to each nostril twice daily for at least 12 weeks. Outcome measures were epistaxis severity score (ESS), hemoglobin level, and number of blood transfusions prior to and while on treatment. Local and systemic side effects were recorded.

Results: The mean duration of treatment was 30 ± 5.6 weeks. A significant improvement in the ESS was found in all patients, with a mean decrease from 6.4 ± 2.1 at treatment onset to 3.5 ± 1.7 at 12 weeks (p = 0.028). Hemoglobin level increased significantly from 8.4 ± 3.1 to 11.0 ± 1.8 g/dL at 12 weeks (p = 0.043). The mean number of blood transfusions decreased from 4.5 ± 4.9 before treatment to 2.5 ± 2.9 at 12 weeks and 0.3 ± 0.8 at 24 weeks, but the difference did not reach statistical significance (p = 0.109 for both). No significant side effects of treatment were recorded.

Implications: These preliminary results suggest that topical propranolol may be effective for the treatment of epistaxis in patients with HHT. A prospective controlled trial is required to confirm the findings.



PULMONARY HYPERTENSION SUBTYPES ASSOCIATED WITH HEREDITARY HAEMORRHAGIC TELANGIECTASIA: HAEMODYNAMIC PROFILES AND SURVIVAL PROBABILITY

S Revuz, E Decullier, I Ginon, N Lamblin, PY Hatron, P Kaminsky, MF Carette, P Lacombe, AC Simon, S Rivière, JR Harle´, A Fraisse, C Lavigne, V Leguy-Seguin, A Chaouat, C Khouatra, S Dupuis-Girod, E Hachulla
PLoS One. 2017 Oct 5;12(10):e0184227. doi: 10.1371/journal.pone.0184227. eCollection 2017

The authors of this article investigated the prevalence of pulmonary hypertension in HHT patients and the mechanisms of pulmonary hypertension in these patients.

Methods and results: A retrospective study was conducted of all suspected cases of PH (echocardiographically estimated systolic pulmonary artery pressure [sPAP] 40 mmHg) in patients with definite HHT recorded in the French National Reference Centre for HHT database. When right heart catheterization (RHC) was performed, PH cases were confirmed and classified among the PH groups according to the European guidelines. Among 2,598 patients in the database, 110 (4.2%) had suspected PH. Forty-seven of these 110 patients had RHC: 38/47 (81%) had a confirmed diagnosis of PH. The majority of these had isolated post-capillary PH (n = 20). For the first time other haemodynamic profiles were identified: pre-capillary pulmonary arterial hypertension (PAH) cases (n = 3) with slightly raised pulmonary vascular resistances (PVR), and combined post- and pre-capillary PH cases (n = 4). Compared to controls, survival probability was lower in patients with PAH.

Implications: This study revealed the diversity of PH mechanisms in HHT. The description of combined post- and pre-capillary PH with/or without high cardiac output (CO) suggests either a continuum between the pre- and post-capillary haemodynamic profiles or a different course in response to high cardiac output.

PULMONARY HYPERTENSION IN A LARGE COHORT WITH HEREDITARY HEMORRHAGIC TELANGIECTASIA.

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Respiration 2017;94(3): 242-250

The authors of this article investigated the prevalence and mechanisms of pulmonary hypertension in patients with HHT and compared the findings with findings in HHT-negative controls.

Hereditary hemorrhagic telangiectasia (HHT) is a vascular disorder characterized by arteriovenous malformations in the brain, liver, and lungs. Pulmonary hypertension (PH) is increasingly recognized as a severe complication of HHT. However, there are no studies describing the prevalence of PH in HHT compared to HHT-negative controls.

Objective: To assess the estimated prevalence of PH in patients with HHT compared to HHT-negative controls.

Methods: All consecutive subjects screened for HHT with available genetic testing and echocardiography-based peak tricuspid regurgitation velocity (TRV) measurement were included. Increased-probability PH was defined as a TRV >2.8 m/s

Results: In 578 subjects, both echocardiography and genetic testing were available. A reliable TRV was measured in 383 (66.3%), of whom 127 had HHT type 1 (HHT1), 150 had HHT type 2 (HHT2), and 106 were HHT-negative controls, with a mean TRV of 2.3 ± 0.4, 2.4 ± 0.5, and 2.2 ± 0.3 m/s, respectively ($p = 0.008$ and $p < 0.001$ vs. controls). Increased-probability PH was found in 42 subjects (8.7% in HHT1, 18.0% in HHT2, and 3.8% in HHT-negative controls). HHT2 and hepatic arteriovenous malformations (HAVMs) were the most important predictors for increased-probability PH (odds ratio 5.6, $p = 0.002$, and odds ratio 11.3, $p < 0.001$, respectively). Heritable pulmonary arterial hypertension (HPAH) was diagnosed in 2 patients (0.7%) and only found in HHT2 (1.3%).

Implications: The estimated prevalence of PH is higher in HHT patients compared to HHT-negative controls. This increase is especially present in HHT2 and mainly associated with the presence of hepatic arteriovenous malformations (HAVMs). HPAH appears to be rare in HHT patients and was only diagnosed in HHT2.

ENDOGLIN PREVENTS VASCULAR MALFORMATION BY REGULATING FLOW-INDUCED CELL MIGRATION AND SPECIFICATION THROUGH VEGFR2 SIGNALLING

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Nat Cell Biol. (2017) 19: 639–652.

Loss-of-function (LOF) mutations in the endothelial cell (EC) enriched gene endoglin (ENG) causes the human disease hereditary haemorrhagic telangiectasia-1, characterized by vascular malformations promoted by vascular endothelial growth factor A (VEGFA). How ENG deficiency alters EC behavior to trigger these anomalies is not understood. Mosaic ENG deletion in the postnatal mouse rendered Eng LOF ECs insensitive to flow-mediated venous to arterial migration. Eng LOF ECs retained within arterioles acquired venous characteristics and secondary ENG-independent proliferation resulting in arterio-venous malformation (AVM). Analysis following simultaneous Eng LOF and overexpression (OE) revealed that ENG OE ECs dominate tip cell positions and home preferentially to arteries. ENG knock-down altered VEGFA-mediated VEGFR2 kinetics and promoted AKT signalling. Blockage of PI3K/AKT partly normalized flow-directed migration of ENG LOF ECs in vitro and reduced the severity of AVM in vivo. This demonstrates the requirement of ENG in flow-mediated migration and modulation of VEGFR2 signaling in vascular patterning.

Implications: In previous years, it has been shown that ALK1-deficiency results in impaired migration of arterial endothelial cells against flow in a zebrafish model (by Beth Roman's group at Pittsburgh), and also that ALK1-deficiency increased PI3 kinase/AKT pathway signaling (by Anne Eichmann's group at Yale). In this paper, the authors have shown that ENG-deficiency also leads to impairment of EC migration and increased AKT signaling, similar to what is seen in ALK1-deficiency. The authors also provide evidence suggesting that ENG may directly interact with a VEGFA receptor (VEGFR2) and modulate its kinetics. It was shown that AKT/PI3 kinase inhibitors reduced severity of AVMs by normalizing flow-directed EC migration and also by inhibiting the proliferating ECs in established AVMs.

ENDOGLIN CONTROLS BLOOD VESSEL DIAMETER THROUGH ENDOTHELIAL CELL SHAPE CHANGES IN RESPONSE TO HAEMODYNAMIC CUES

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Nat Cell Biol. (2017) 19: 653–665

The hierarchical organization of properly-sized blood vessels ensures the correct distribution of blood to all organs of the body, and is controlled via haemodynamic cues. In current concepts, an endothelium-dependent shear stress set-point causes blood vessel enlargement in response to higher flow rates, while lower flow would lead to blood vessel narrowing, thereby establishing homeostasis. We show that during zebrafish embryonic development increases in flow, after an initial expansion of blood vessel diameters, eventually lead to vessel contraction. This is mediated via endothelial cell shape changes. We identify the transforming growth factor beta co-receptor endoglin as an important player in this process. Endoglin mutant cells and blood vessels continue to enlarge in response to flow increases, thus exacerbating pre-existing embryonic arterial-venous shunts. Together, our data suggest that cell shape changes in response to biophysical cues act as an underlying principle allowing for the ordered patterning of tubular organs.

Implications: HHT patients treated at high volume centers (those seeing large numbers of HHT patients) experienced better outcomes with lower in-hospital mortality and higher rates of discharge to home (rather than short term rehabilitation facilities). These improved outcomes occurred despite the fact that HHT patients treated at high volume centers were sicker with higher rates of co-morbidities. Data from this study strongly supports the expansion of HHT centers of excellence worldwide.

TACROLIMUS RESCUES THE SIGNALING AND GENE EXPRESSION SIGNATURE OF ENDOTHELIAL ALK1 LOSS-OF-FUNCTION AND IMPROVES HHT VASCULAR PATHOLOGY

Ruiz S, Chandakkar P, Zhao H, Papoin J, Chatterjee PK, Christen E, Metz CN, Blanc L, Campagne F, Marambaud P
Human Molecular Genetics (2017) Sep.

Hereditary Hemorrhagic Telangiectasia (HHT) is a highly debilitating and life-threatening genetic vascular disorder arising from endothelial cell (EC) proliferation and hypervascularization, for which no cure exists. Because HHT is caused by loss-of-function mutations in bone morphogenetic protein 9 (BMP9)-ALK1-Smad1/5/8 signaling, interventions aimed at activating this pathway are of therapeutic value. We interrogated the whole-transcriptome in human umbilical vein ECs (HUVECs) and found that ALK1 signaling inhibition was associated with a specific pro-angiogenic gene expression signature, which included a significant elevation of DLL4 expression. By screening the NIH clinical collections of FDA-approved drugs, we identified tacrolimus (FK-506) as the most potent activator of ALK1 signaling in BMP9-challenged C2C12 reporter cells. In HUVECs, tacrolimus activated SMAD1/5/8 and opposed the pro-angiogenic gene expression signature associated with ALK1 loss-of-function, by notably reducing Dll4 expression. In these cells, tacrolimus also inhibited AKT and P38 stimulation by vascular endothelial growth factor, a major driver of angiogenesis. In the BMP9/10-immunodepleted postnatal retina—a mouse model of HHT vascular pathology—tacrolimus activated endothelial Smad1/5/8 and prevented the Dll4 overexpression and hypervascularization associated with this model. Finally, tacrolimus stimulated SMAD1/5/8 signaling in C2C12 cells expressing BMP9-unresponsive ALK1 HHT mutants and in HHT patient blood outgrowth ECs. Tacrolimus repurposing has therefore therapeutic potential in HHT.

Implications: The authors screened the NIH clinical collections of FDA-approved drugs for molecules that can enhance ALK1 signaling, and found tacrolimus (FK506) as a top candidate. They found that tacrolimus treatment could normalize the expression of key ALK1 downstream genes and inhibit the formation of retinal AVMs in a mouse model. Repurposing Tacrolimus for use in HHT, has therefore, great potential for normalizing expression of genes downstream of ALK1 in ALK1-deficient conditions (HHT2).

SELECTIVE EFFECTS OF ORAL ANTIANGIOGENIC TYROSINE KINASE INHIBITORS ON AN ANIMAL MODEL OF HEREDITARY HEMORRHAGIC TELANGIECTASIA.

Kim YH, Kim MJ, Choe SW, Sprecher D, Lee YJ, Oh SP
J Thromb Haemost. (2017) 15:1095-1102

Objectives: The goal of this study is to evaluate potential therapeutic effects of oral delivery of four anti-angiogenic tyrosine-kinase inhibitors (TKIs) in the development of adult onset AVMs in a murine model of HHT.

Methods: Adult activin receptor-like kinase 1 (Alk1)-inducible knockout (iKO) model was utilized to evaluate the effect of oral administration of sorafenib, sunitinib, erlotinib, and a pazopanib analog (GW771806), on hemoglobin level, GI hemorrhages, and formation of wound-induced skin AVMs.

Results and Conclusions: Sorafenib (trade name Nexavar) and GW771806 significantly improved, yet Erlotinib (trade name Tarceva) worsened anemia and GI-bleeding in the Alk1-iKO model. However, none of these TKIs appeared to be effective for inhibiting the development of wound-induced skin AVMs. Taken together, these results suggest that oral delivery of anti-angiogenic TKIs are selectively more effective on GI bleeding than mucocutaneous AVMs, and it may provide an experimental basis for selective therapeutic options depending on the symptoms of HHT.

Implications: Four oral anti-angiogenic drugs that have been used for various cancers were evaluated in an Alk1-mouse model, in which adult-onset skin AVMs and GI bleeding can be assessed. While sorafenib, sunitinib, and pazopanib were effective in preventing GI bleeding and anemia, erlotinib (EGFR inhibitor) exacerbated the symptoms. Oral delivery was not effective for wound-induced skin AVMs, but topical applications of pazopanib were shown to be effective in some cases. Taken together, these results suggest an experimental basis for selective therapeutic options depending on the symptoms of HHT.

STRUCTURAL BASIS OF THE HUMAN ENDOGLIN-BMP9 INTERACTION: INSIGHTS INTO BMP SIGNALING AND HHT1

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Sara Zamora-Caballero, Ling Han, Michelle Letarte,
Daniele de Sanctis, and Luca Jovine
Cell Reports (2017) 19, 1917–1928

Endoglin (ENG)/CD105 is an essential endothelial cell co-receptor of the transforming growth factor β (TGF-β) superfamily, mutated in hereditary hemorrhagic telangiectasia type 1 (HHT1) and involved in tumor angiogenesis and preeclampsia. Here, we present crystal structures of the ectodomain of human ENG and its complex with the ligand bone morphogenetic protein 9 (BMP9). BMP9 interacts with a hydrophobic surface of the N-terminal orphan domain of ENG, which adopts a new duplicated fold generated by circular permutation. The interface involves residues mutated in HHT1 and overlaps with the epitope of tumor-suppressing anti-ENG monoclonal TRC105. The structure of the C-terminal zona pellucida module suggests how two copies of ENG embrace homodimeric BMP9, whose binding is compatible with ligand recognition by type I but not type II receptors. These findings shed light on the molecular basis of the BMP signaling cascade, with implications for future therapeutic interventions in this fundamental pathway.

Implications: Crystallographic and biochemical data in this paper provides fundamental understandings about several functional domains of ENG and on how ENG functions in BMP9 signaling. With the data in this paper as well as previously reported data, the authors present clear models: ENG captures BMP9 by hydrophobic interactions and ALK1 forms a ternary complex by binding to BMP9. The regions of BMP9 interacting with ENG and ALK1 are non-overlapping while the area of ENG-BMP9 interaction overlaps with type II receptor-BMP9. For functional signaling of BMP9, ENG is replaced with type II receptors in the ENG-BMP9-ALK1 complex. Dissection of functional domains of ENG provides tremendous information for understanding the function of ENG, mutations of ENG, and development of drugs to inhibit specific function of ENG.

SOMATIC ACTIVATING KRAS MUTATIONS IN ARTERIOVENOUS MALFORMATIONS OF THE BRAIN

Nikolaev SI, Vetiska S, Bonilla X, Boudreau E, Jauhiainen S, Rezai Jahromi B, Khyzha N, DiStefano PV, Suutarinen S, Kiehl T-R, Mendes Pereira V, Herman AM, Krings T, Andrade-Barazarte H, Tung T, Valiante T, Zadeh G, Tymianski M, Rauramaa T, Yla-Herttuala S, Wythe JD, Antonarakis SE, Frosen J, Fish JE, Radovanovic I
N Engl J Med (2018) 378: 250-61

Background: Sporadic arteriovenous malformations of the brain, which are morphologicallyabnormal connections between arteries and veins in the brain vasculature, are a leading cause of hemorrhagic stroke in young adults and children. The genetic cause of this rare focal disorder is unknown.

Methods: We analyzed tissue and blood samples from patients with arteriovenous malformations of the brain to detect somatic mutations. We performed exome DNA sequencing of tissue samples of arteriovenous malformations of the brain from 26 patients in the main study group and of paired blood samples from 17 of those patients. To confirm our findings, we performed droplet digital polymerase-chain-reaction (PCR) analysis of tissue samples from 39 patients in the main study group (21 with matching blood samples) and from 33 patients in an independent validation group. We interrogated the downstream signaling pathways, changes in gene expression, and cellular phenotype that were induced by activating KRAS mutations, which we had discovered in tissue samples.

Results: We detected somatic activating KRAS mutations in tissue samples from 45 of the 72 patients and in none of the 21 paired blood samples. In endothelial cell–enriched cultures derived from arteriovenous malformations of the brain, we detected KRAS mutations and observed that expression of mutant KRAS (KRASG12V) in endothelial cells in vitro induced increased ERK (extracellular signal-regulated kinase) activity, increased expression of genes related to angiogenesis and Notch signaling, and enhanced migratory behavior. These processes were reversed by inhibition of MAPK (mitogen-activated protein kinase)–ERK signaling.

Conclusions: We identified activating KRAS mutations in the majority of tissue samples of arteriovenous malformations of the brain that we analyzed. We propose that these malformations develop as a result of KRAS-induced activation of the MAPK–ERK signaling pathway in brain endothelial cells.

Implications: High mortality and morbidity in HHT are often caused by hemorrhages from Brain Arteriovenous malformations (BAVMs). Most of BAVMs found in the general population are in a non-familial form and not associated with HHT. In this paper, the authors searched for the causative genes in these sporadic BAVMs by exome sequencing. Surprisingly they found that the majority of BAVM samples contained mutations in the KRAS gene. These mutations keeping KRAS in an active status were found in a subset of endothelial cells in BAVM lesions. It was shown that the endothelial cells expressing the mutant KRAS had no changes in cell death or proliferation, but decreased cell-cell adhesions. While KRAS is an important regulator of many intracellular signal transductions, the KRAS mutants specifically increased ERK pathway (not PI3 kinase pathway which has shown to be increased in ALK1-deficient cells). Future studies about BAVM pathogenesis by KRAS mutations would broaden our knowledge about AVM development and the knowledge could be applied to cure HHT.

INACTIVATING MUTATIONS IN DROSHA MEDIATE VASCULAR ABNORMALITIES SIMILAR TO HEREDITARY HEMORRHAGIC TELANGIECTASIA

Jiang X, Wooderchak-Donahue WL, McDonald J, Ghatpande P, Baalbaki M, Sandoval M, Hart D, Clay H, Coughlin S, Lagna G, Bayrak-Toydemir P, Hata A.
Sci. Signal. 11, eaan6831 (2018)

The transforming growth factor–β (TGF-β) and bone morphogenetic protein (BMP) family of cytokines critically regulates vascular morphogenesis and homeostasis. Impairment of TGF-β or BMP signaling leads to heritable vascular disorders, including hereditary hemorrhagic telangiectasia (HHT). Drosha, a key enzyme for microRNA (miRNA) biogenesis, also regulates the TGF-β and BMP pathway through interaction with Smads and their joint control of gene expression through miRNAs. We report that mice lacking Drosha in the vascular endothelium developed a vascular phenotype resembling HHT that included dilated and disorganized vasculature, arteriovenous fistulae, and hemorrhages. Exome sequencing of HHT patients who lacked known pathogenic mutations revealed an overrepresentation of rare nonsynonymous variants of Drosha. Two of these Drosha variants (P100L and R279L) did not interact with Smads and were partially catalytically active. In zebrafish, expression of these mutants or morpholino-directed knockdown of Drosha resulted in angiogenesis defects and abnormal vascular permeability. Together, our studies point to an essential role of Drosha in vascular development and the maintenance of vascular integrity, and reveal a previously unappreciated link between Drosha dysfunction and HHT.

Implications: Micro RNAs (miRNAs) are important for regulating gene expression. Drosha is an enzyme that is essential for generating miRNAs by cleaving the primary transcripts of miRNAs to make precursor miRNAs. In this paper, the authors have shown that Drosha-deficient zebrafish and mice exhibited vascular phenotypes. There is no clear demonstration of arteriovenous shunts or malformations in these mutant animals, but the authors suspected that HHT-like vascular lesions were present in some vessels based on vascular casting experiments. This led to exome sequencing of 98 individuals who are diagnosed as HHT without mutations in ENG, ALK1, SMAD4, and BMP9 genes. The authors found that 7 out of 98 had sequence variations in the Drosha gene. This is thousands fold higher frequency to be found in healthy individuals. It warrants further studies to investigate whether miRNAs are directly involved in HHT pathogenesis, and if so, additional studies would be needed to determine the identities of specific miRNAs and their target genes involved in HHT.