HHT International Scientific Conference Summary
Basic Science Summary

Talks 1-3,12, 14, 16-18, 21,22, 28-31, 37,38, 43, 45, 54-56

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Genetics of HHT

What is the evidence for other HHT causing genes?

Talk 12. **Liz Cirulli Rogers** (Duke) - Used existing whole exome DNA Sequencing data for a large **normal** cohort to simulate the use of this technology in gene discovery in a disease cohort.
Take home lesson. LOTS of variants are found and by chance, many will be found in the same gene across multiple samples. Rigorous statistical methods are required to separate the signal from the overwhelming noise of sequence variation in the genome.

Talk 19. Jamie McDonald (Utah) - When Curacao diagnostic criteria are used, 96% of DNA samples submitted to Utah Center show a mutation in the known HHT genes

Talk 31. Pernille Torring (Odense, Denmark) - Danish HHT Center - 89% of patients have a known gene mutation.

Talks 17 (Klaus Brusgaard) and 21 (Whitney Wooderchak-Donahue). Described their pipeline for HHT mutation analyses. Improved systems and pipelines will improve the success rate for DNA diagnostics.

What is the role of somatic mutation in AVM formation? (Workshop)

Nearly all highly pentrant animal models of AVM formation in HHT require loss of both alleles. Usually then another event too

No data exists for human AVMs in HHT.
Advances in Deep resequencing can help resolve this if tissue is available

Therapeutic implications. If the normal copy is lost in the AVM, then strategies to up regulate the normal copy of the gene are not useful. BUT
Tal**k 18 Paul Oh** Upregulation of ALK1 rescued AVM formation in endoglin mouse model.

New(-ish) Tools and Approaches to Study HHT

Two cell based tools were discussed.

Talk 1. **Stephanie Bowers.** (Missouri) Endothelial cell + Pericyte Capillary Tube 3 dimensional co assembly assay

More physiologically relevant than ECs grown in monolayers.

Reanalyze the role of TGF-beta in this system: TGF beta enables better tube formation but poor pericyte coverage

Talk 16. Valeria Orlova (Leiden) Induced Pluripotent Stem Cell. Generate any cell type (Endothelial, Pericyte, Smooth muscle) with a specific HHT associated mutation. Study cellular phenotypes such as EC / pericyte interaction. Two different ENG mutations showed different EC phenotypes

These systems (and others) will help validate ideas (and generate new ones) about signaling and cellular behavior in more relevant contexts.

Ideas for HHT Therapy

Since HHT is an autosomal dominant disease, one normal copy of the mutated gene is present in every cell. All cells expressing the relevant protein are reduced (theoretically) by 50%

Can we treat HHT by upregulating the remaining normal copy to restore normal protein levels?

Talk 18. Paul Oh (Florida) Mouse Model of AVM formation in the skin.
ALK1 overexpression could inhibit AVM formation in his HHT2 (ALK1) AND HHT1 (Endoglin) models
Significance: It is theoretically possible to inhibit AVM formation by increasing the amount of ALK1 protein. Pharmacologic up-regulation of ALK1 expression might then be valuable *for both* of the major forms of HHT.

Talk 3. Petr Nachtigal (Prague and Madrid). Atovastatin induced eNOS expression in ECs. Side point Atorvastatin modestly upregulated endoglin levels. (approximately 2 fold). Maybe this is all one needs.

A CAUTION. Session 4. Workshop on the Role of "second hits" in the AVM development.

What if an AVM forms only after the remaining normal copy of the endoglin (or ALK1) gene is also mutated? *Then it will not be available for pharmacological manipulation.*

Evidence for second hit comes from mouse models of HHT. Nearly all require both copies of the gene to be mutated for AVM formation. Human data is lacking.

Mutation Specific Therapy

Approximately 20% of HHT associated mutations are due to a mutation that leads to an early stop codon.

This mutation mechanism is present in many other inherited diseases. Thus, there is much work underway to try to correct this class of mutations in general.

Talk 43 Micheala Aldred (Cleveland) The separation of the canonical (signaling pathway) and non canonical (miRNA processing) pathways in PAH due to BMPR2 mutations. Some of these are also early stop codon mutations.

Ataluren is a new drug that causes the cellular machinery to read through the stop codon. In various phases of clinical trials/approval for this class of mutations in Cystic Fibrosis and for Duchene Muscular Dystrophy.

Ataluren works in vitro (cellular assays) to read through some nonsense mutations in BMPR2.

Will this work with HHT associated stop codon mutations? These will be tested soon.

Caution. Will not help for premature stop codons caused by frameshift mutations or splice site mutations that cause a reading frame change.

Some New Data on BMP 9 signaling

Talk 28 Bob Friesel (Maine) BMP9 signaling in ECs involves the Hippo/YAP/TAZ pathway. "hot" topic in biology these days. *If this pathway proves relevant to HHT pathogenesis, new ideas will come rapidly due to the explosion of this area of research.*

Talk 29 Sabine Bailly (Grenoble) BMP9 mutant (null, -/-) mice. Slightly elevated pulmonary arterial pressure under normoxic conditions, but paradoxically reduced pressure under hypoxia. (the classic models of PH include moving the animals to a hypoxic chamber).
Do these mice also have an "HHT like" phenotype?

Talk 30 Pierre Guihard (UCLA) He found oscillatory (mRNA) expression patterns for BMP9, CV2 (aka BMPER), MGP (matrix Gla protein; mutation of the gene causes lung AVM formation) in pulmonary arterial ECs. MGP and CV2 are negative regulators of BMP signaling. Presented a mathematical model of how these proteins might regulate levels of each other. The model predicts the mRNA expression data. (no Protein data yet). If ALK1 levels are reduced, these oscillations are perturbed.

Does this oscillation control the balance of the EC proliferative and quiescent phenotypes?
Does perturbation of this oscillation have any bearing on HHT pathogenesis?

Role of Macrophages in HHT pathogenesis

Talk 37 Paul Oh (Florida) Using his wound model of AVM development in HHT2 mouse model

Gene expression array analyses ALK1 -/- cells revealed up regulation of chemokines that regulate macrophage recruitment. (CCL2, CCL5, CXCL10).

Historic (Braverman et al) and new data from Paul and Hua (see below) reveal movement of macrophages to perivascular area of the developing AVM in the mouse model

Blocking macrophage recruitment (clodronate liposomes - causing macrophage apoptosis) inhibits AVM formation. Ditto when using an antagonist to CCL5.
Macrophages are cellular chaperons for the formation of vascular anastomoses. *Is this process acting during AVM formation?*

Talk 38 Hua Su (USCF) Brain AVM model in Endoglin and ALK1 mutant mice (inducible KO). Label bone marrow derived macrophages (genetic lineage marker) with a fluorescent dye to track their location. Increased numbers of these macrophages in the area of the brain AVM.

Is macrophage recruitment a cause or an effect (response) of the AVM? Paul's data suggest some level of causality.

How does Anti-VEGF Therapy work?

During the Session on Epistaxis Therapies. Resultings using Bevacizumab in HHT are promising ! (antibody to VEGF)

At least in some cases mucocutaneous (lips, tongue) telangiectasia seem to regress during treatment for epistaxis (**Talk 47 Vivek Iyer,** Mayo)

Talk 37 Paul Oh He had already shown that blocking antibody to VEGF could inhibit and even regress AVMs in his mouse model. VEGF has two receptors on ECs. VEGFR2 (R2) is thought to be the authentic "signaling" receptor that regulates angiogenesis, with VEGFR1 (R1) being anti-angiogenic, possibly by acting as a "decoy"
BUT, when Paul blocks signaling through R2, he does not inhibit AVM formation in adult skin. Instead blocking R1 shows the inhibitory effect.

Talk 45 Roxana Ola (Yale). Studied AVM shunt formation in retinal vasculature during early postnatal life. Mosaic analysis using lineage tracing of the mutant cells shows that the mutant cells are found in the shunt. These mutant cells have a high(er) proliferation profile than other cells in the developing retinal vasculature and are depleted of pericytes. Lineage markers suggest an identity crisis for Arterial venous identity.

Caveats. The Yale study was AVM formation during retinal development. The Florida study was AVM formation in response to wounding in the adult skin. Do AVMs formed during development (some internal organs) differ in mechanism than others (telangiectases) formed later in life?

Talks from this morning

Plenary Talk by Rong Wang (UCSF) created a mouse model of brain AVM by precisely timed (and controllable) expression of an activated Notch4 receptor. Gave us 10 take home lessons

1 AVMs occur via the enlargement of capillaries
2 Notch4 reprograms the venous segment towards an arterial identify
3 Notch4 permits flow-selected growth - increased flow in lower resistance vessels leads to enlargement and shunt formation, decreased flow in higher resistance vessels leads to thinning (and loss?) of these vessels (the importance of flow has been emphasized by others, especially Beth Roman)

Rong showed two pathways that could be perturbed to inhibit and even regress brain AVMs in this model Soluble Eph4B (Notch signaling) and

Talk 54 Lawrence Huang - Nitric Oxide Synthase (NOS) inhibitors also inhibit brain AVMs in the Notch mouse

Talk 55 Beth Roman - Beth showed that the ALK1 and Notch signaling pathways interact to regulate expression of certain Notch target genes, but that the pattern was target (gene) and context (Organ/tissue) specific. Bonus, AVMs formed via dysregulation of Notch in zebrafish resemble those from her ALK1 Fish

However, AVMs formed by these two mutants are different in origin AND, Notch inhibition by two methods fails to rescue the AVMs in the ALK1 mutants

Take home: Notch signaling may not be critical for AVM formation in HHT. More work needs to be done here to determine if Rong's work has applicability to HHT.

Final Thoughts: Where do we go from here?

1. Does AVM formation in HHT require loss of expression of both copies of the relevant gene? Animal data suggests this, no human data – at least with modern technology) is available to address this question. This is not merely academic as it has profound implications for any therapeutic strategy that involves up regulation of the remaining normal copy of the gene.
2. What is the role of new angiogenesis via VEGF signaling in AVM formation? What are the pathways activated? What are the downstream effects?
3. Is BMP9 the authentic ligand for AVM pathogenesis in HHT? If not, then who is the culprit?
4. Does Notch signaling play a central role in HHT AVM pathogenesis? (central as defined by therapeutic potential)
5. What is the role of macrophages in AVM formation? Can they be a therapeutic target?
6. Are there any other targets for therapy based on new findings?