TGF-beta Receptors in Pulmonary Arterial Hypertension: The HHT Connection

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Introduction

Substantial progress in delineating the molecular and genetic basis of pulmonary arterial hypertension has placed the TGF-beta cell-signaling pathway as the centerpiece of contemporary thinking about the pathogenesis of this disorder. Although identification of deleterious mutations within the bone morphogenetic protein receptor type II (BMPR2) gene provides a compelling basis for implicating altered TGF-beta signaling in pulmonary arterial hypertension, the precise molecular mechanisms of disease development remain obscure. However, these findings already present important clinical implications, not least of which is the recognition of disorders in which pulmonary arterial hypertension should now be considered a potential and important complication. In this article, emerging features of the association of pulmonary arterial hypertension and the inherited vascular disorder known as hereditary hemorrhagic telangiectasia (HHT) are described. Recognition of this relationship not only serves to expand the clinical phenotype of HHT,[1] but the findings emphasize the importance of clinical observation in assisting the elucidation of the molecular basis of human disease, in this case the enigmatic disorder we term pulmonary arterial hypertension. Finally, the clinical association of HHT and pulmonary arterial hypertension challenges cellular biologists who now seek to provide the molecular detail of the processes that alter normal structure and function of the pulmonary vasculature.

HHT, or Rendu-Osler-Weber syndrome, describes an autosomal dominant disorder characterized by vascular malformations affecting both cutaneous and internal structures, which frequently bleed (**Figure 1**).[2] Hence, the typical symptomology of HHT includes recurrent epitaxis, fatigue, and exertional dyspnea due to chronic blood loss from the gut, together with more complex complications arising from larger arteriovenous malformations that may involve the pulmonary, cerebral, or hepatic vasculature.

By comparison to the paucity of information regarding the development of the characteristic obstructive and plexigenic lesions of pulmonary arterial hypertension, substantial detail of the emergence of the vascular lesions in HHT is available. This includes systematic electron microscopy studies of biopsied lesions during the evolution of the classic cutaneous telangiectasia, which ultimately leads to loss of the capillary bed intervening between arterioles and venules (**Figure 1B**).[3] Intriguingly, although focal, the initial

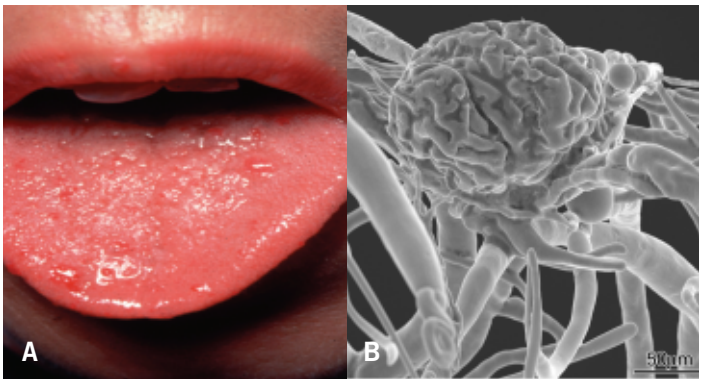


Figure 1. A. Cutaneous telangiectasia characteristic of hereditary hemorrhagic telangiectasia (HHT). B. Electron microscopic appearance of arteriovenous communication in HHT.

observation is one of venodilation, mononuclear (lymphocytic) infiltration, and vessel wall thickening, due to pericyte recruitment. Subsequently arteriole dilatation is observed with a loss of the capillary bed, replaced by up to four direct arteriovenous connections, with consequences for the local flow dynamics. Although these changes appear to reflect fundamental developmental defects, arteriovenous malformations, and more generally telangiectasias, do not typically appear clinically until early teenage life and hence may reflect more prolonged exposure to hemodynamic influences.

HHT as a genetic syndrome and a vascular disorder has risen onto the horizon of those interested in pulmonary arterial hypertension. This has occurred because of the recognition of a group of patients who develop classical features of pulmonary arterial hypertension, including in some cases histopathological evidence of both plexiform and concentric lesions, against a background of clinical HHT or due to mutations in the genes causative of HHT. These are described in detail later in this article, but first it is appropriate to describe some of the genetic and molecular features of HHT, to enable comparison and contrast with emerging views of the pathogenesis of pulmonary arterial hypertension.

Molecular Genetic Basis of HHT

Early linkage studies of extended HHT kindreds provided compelling evidence for at least two distinct locations for

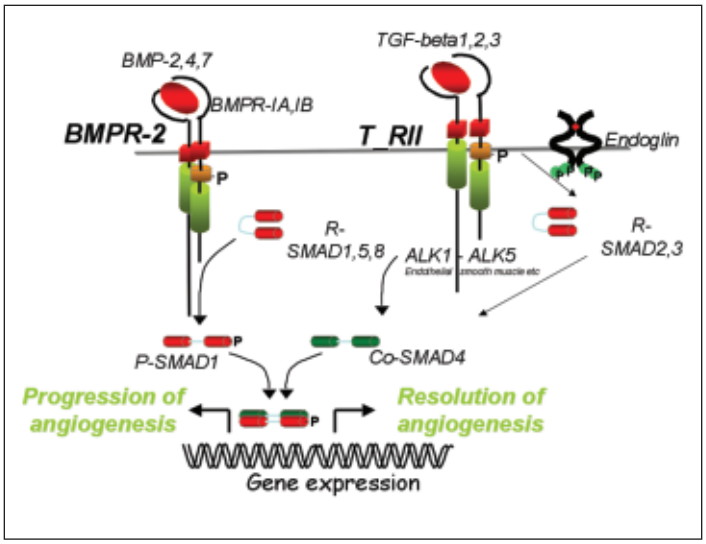
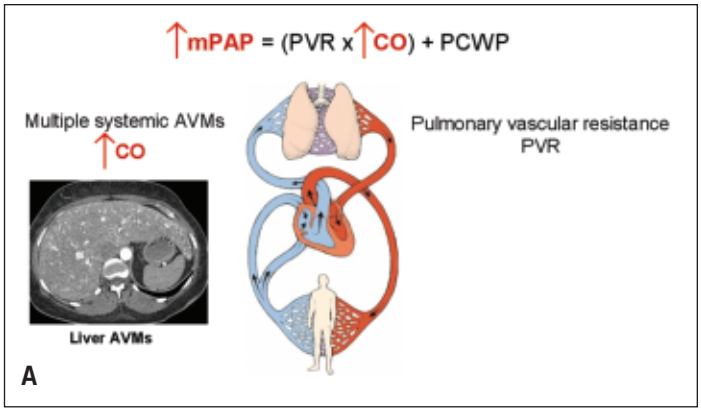


Figure 2. Line diagram of classic components of TGF-beta mediated cell signaling pathway, illustrating the potential cross-talk between the type II receptor BMPR2 and the type I receptor ALK1, modulated by the accessory receptor endoglin. The balance between activation of these pathways is considered to regulate progression or resolution of angiogenesis, which may contribute to the clinical features of vascular remodeling.

HHT genes within the human genome. Using positional and candidate gene cloning strategies, HHT1, which maps to chromosome 9q33-34, was shown to encode the type III or accessory receptor protein termed endoglin (ENG).[4] The HHT2 locus, which maps to chromosome 12q13, was shown to be a gene that encodes a further member of the TGF-beta superfamily of cytokines, known as activin A receptor, type II-like kinase 1 or activin receptor-like kinase-1 (ACVRL1 or ALK1).[5] The more general features of TGF-beta mediated signaling are described below, but of importance, transcripts from both ENG and ALK1 are expressed predominantly in endothelial cells. The majority of ENG and ALK1 mutations appear to lead to loss of function.[6] As an autosomal dominant condition, the presence of a heterozygous (single) mutant allele appears sufficient to predispose to the development of the classic vascular anomalies; however, these are site specific rather than generalized, suggesting that other events, either genetic or environmental, are necessary to switch the normal equilibrium of vascular cells from a maintenance state. To date, no evidence for a second genetic hit of either gene has been brought forward, although, and in contrast to tumor formation, the vascular process of telangiectasia formation and arteriovenous malformations is one of remodeling rather than a predominant state of cell proliferation. Molecular analysis of site-specific lesions in HHT under such conditions may therefore be less than ideal for looking, for example, for events such as loss of heterozygosity. Interestingly, early studies suggested a phenotype-genotype correlation with an increased predisposition to pulmonary arteriovenous malformations among the HHT1 (ENG) group of subjects. Beyond this, precise functions for either ENG or ALK1 in vascular processes remain unclear. However, insight will further emerge through the detailed analysis of animal models harboring defects of these genes.



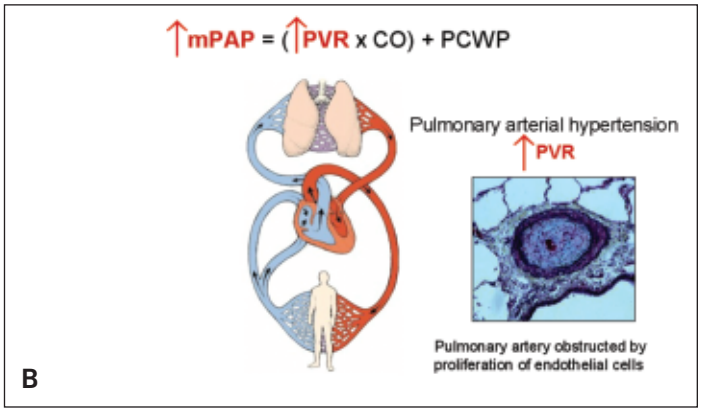


Figure 3. Hemodynamic determinants of mean pulmonary arterial pressure (mPAP). A. Raised mPAP due to high flow through large arteriovenous malformations (AVMs) leading to raised cardiac output (CO) but typically normal pulmonary vascular resistance (PVR) and pulmonary capillary wedge pressure (PCWP). B. Contrast to raised mPAP due to raised PVR from obstructive pulmonary vascular lesions in HHT-related pulmonary arterial hypertension.

TGF-beta signaling in vascular disease

BMPR2, ENG, and ALK1 are membrane-bound receptor members of the TGF-beta superfamily that form structurally related polypeptides that regulate a number of biological processes that include cell cycle control, embryogenesis, growth, development, and differentiation of cell types. The general and most studied model of TGF-beta signaling requires ligand binding with a constitutively phosphylated type II receptor. This process enables recruitment of the type I receptor into the activated complex, with the intracellular kinase domain of the type II receptor subsequently phosphorylating the type II receptor at serine and threonine residues, located in the cytoplasmic GS-domain. Once activated, the type I receptor phosphylates members of the SMAD family of intracellular mediators, which act as cell type-specific transcription factors. Specificity for response is at least in part determined by the components of the cascade that are activated. For example, interaction of the type I receptor ALK1 leads to phosphorylation of receptor SMAD 1, 5, and 8. Less well characterized SMAD independent pathways, together with SMAD dependent processes, may be further regulated by the expression of type III receptors such as endoglin.[7] Despite the lack of evidence for a direct interaction between BMPR2 (the familial pulmonary arterial hypertension gene) and ALK1/endoglin complexes, there is extensive cross-talk between intracellular signalling path-

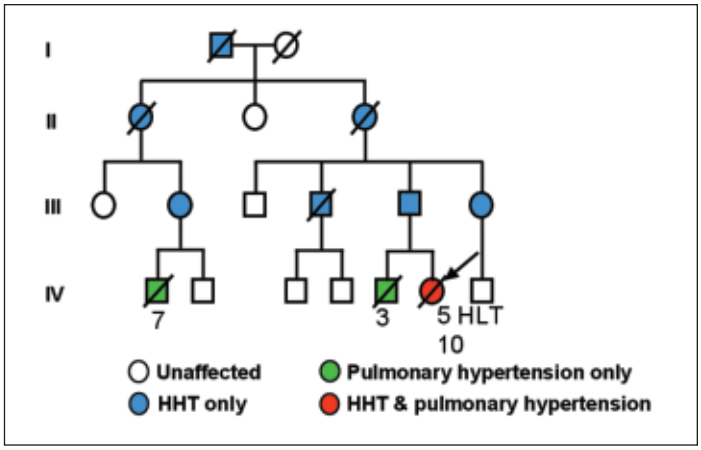


Figure 4. Family A reported in detail in Trembath et al.[9] Pedigree shows diversity of vascular phenotypes consequent upon mutation of ALK1 segregating within the kindred.

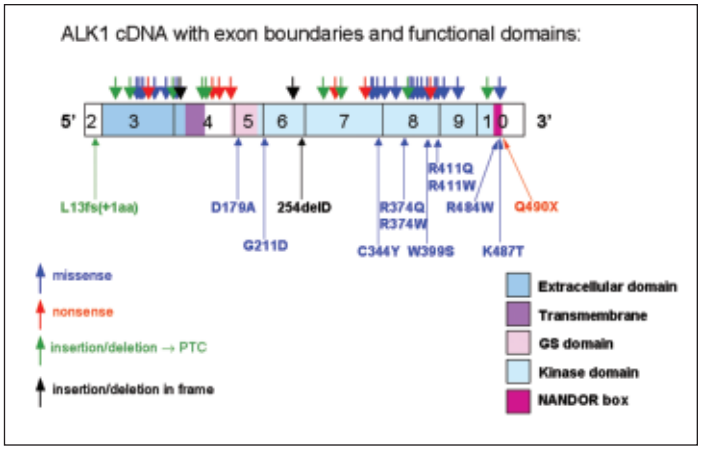


Figure 5. Diagram of ALK1 cDNA with exons encoding protein functional domains. Location of previously reported mutations in classic HHT (above) and all ALK1 mutations reported to date in HHT-related pulmonary arterial hypertension (below). Note potential clustering within the so-called NANDOR box, a region from codons 479 to 489 apparently necessary for TGF-beta signaling regulation.[10]

ways activated by these receptor complexes.[8] For example, signalling via ALK1 and BMP receptors occurs through the same set of receptor-regulated SMAD proteins that complex with the comediator SMAD 4. This allows transolaction into the nucleus and transcriptional regulation of a restricted range of genes in both a cell type and context-specific manner (**Figure 2**). Hence, we might anticipate that elucidation of the impact of mutations in either BMPR2 or ALK1 on these downstream pathways will point to molecular mechanisms critical to the development of pulmonary arterial hypertension.

Pulmonary Arterial Hypertension in HHT

Abnormalities of vascular pressure-flow dynamics have long been recognised in HHT. The development of large arteriovenous malformations has the potential to significantly impact on cardiac output and peripheral resistance as well as to generate significant shunting of blood flow, particularly within the pulmonary vasculature. The hemodynamic consequences of these clinical scenarios are illustrated in **Figure 3**.

The association of HHT with familial pulmonary arterial hypertension was first reported in detail in 2001.[9] Six mutations and 10 cases of pulmonary arterial hypertension were identified in five families with HHT and one in one individual with no available family history. In one of the families with an extended HHT kindred, three young relatives (<7 years age) each developed rapidly progressive and fatal pulmonary arterial hypertension, the proband surviving a few years following heart-lung transplantation (**Figure 4**). Histology of the explanted lung revealed obliterature and plexiform lesion, and investigations of this child and the affected siblings revealed evidence of significantly raised pulmonary vascular resistance (**Figure 4**). Intervening adult relatives exhibited typical features of HHT, which was present in only one of the three children with pulmonary arterial hypertension. Hence, these subjects have HHT-related pulmonary arterial hypertension, characterised by a significantly raised pulmonary artery pressure, high pulmonary vascular resistance, normal pulmonary arterial wedge pressure, and a normal or low cardiac output. Molecular studies of this and additional kindreds revealed pathogenic heterozygous mutations of the ALK1 gene (**Figure 5**).[10]

Additional reports of patients with HHT-related pulmonary arterial hypertension have subsequently been published. Eight missense mutations of ALK1 were reported in 11 probands, one of which was observed in two families.[11] Four of the eight mutations were novel. In addition, heterozygous mutations of ENG were identified in two subjects, each representing a novel frameshift mutation and predicted to lead to premature truncation of the transcript. None of the mutations was identified among appropriate matched control groups. More recently, ALK1 mutations were identified in four additional families with HHT.[12] Three novel mutations in exon 10 leading to truncated proteins were found. In the fourth family, a missense mutation previously reported, was identified.

The potential for the development of lesions characterised by either vessel dilation or pulmonary hypertension caused by concentric obstruction and abnormal remodeling, seems at first sight counterintuitive. The recognition that the genes involved in familial pulmonary arterial hypertension and HHT are so closely related and may function in common cell signaling pathways, likely holds the clues to the clinical overlap. This report now implicates mutations of ENG in the pulmonary arterial hypertension process; however, the clinical consequences may be a little more complex. A germline mutation of ENG was identified in a woman with HHT and pulmonary arterial hypertension, who has also been exposed to anorexic drugs, which are also known to predispose to the development of pulmonary arterial hypertension. We have observed that patients with pulmonary hypertension and ENG mutations underlying HHT typically have substantially reduced pulmonary vascular resistance due to arteriovenous malformations and formation of high flow shunts. We have recently reported a child with an ENG mutation who presented at a very young age (<3 months old) with evidence of substantially increased pulmonary vascular resistance. As this child was followed over time, it was of considerable

interest to note that the pulmonary vascular resistance gradually decreased, a change likely to be associated with the subsequent development of microvascular pulmonary arteriovenous malformations. In essence, this likely represents the clinical transition from the hemodynamic response seen in **Figure 3B** to the pressure flow response depicted in **Figure 3A**.

Conclusion

Overall, the findings of germline mutations in the ALK1 and rarely the ENG gene, in pulmonary arterial hypertension raise key points. First, of clinical relevance, these recent findings show that pulmonary arterial hypertension is an uncommon yet serious presentation of the inherited disorder HHT even in childhood and can be the initial presentation of HHT within a kindred. Hence, detailed family history and critical clinical examination of apparently healthy parents is warranted, looking for the subtle manifestation of HHT. For example, we have observed at least two subjects with no personal or family history of HHT, who have presented with pulmonary arterial hypertension due to de novo ALK1-mediated disease.

In comparison, clinical features at presentation, particularly in young children, failed to distinguish between patients with mutations in the different genes or those without identified mutations. Hence, clinicians need to consider the possibility of germline mutation of TGF-beta receptor genes in patients presenting with pulmonary arterial hypertension across all age groups. In terms of molecular mechanisms, it is already apparent that substantial overlap exists between those mutations of ALK1 associated with HHT and those that have been identified in patients with HHT-related pulmonary arterial hypertension (**Figure 5**). We interpret these findings as providing further evidence of a role for additional genetic and/or environmental factors that influence both the age of onset or presentation with pulmonary arterial hypertension in subjects with ALK1 mutations. Taken together, these recent genetic studies have demonstrated that pulmonary arterial hypertension is an important and not infrequent complication of ALK1-mediated disease. HHT should also be considered, and if appropriate screened for, in patients presenting with apparent idiopathic pulmonary arterial hypertension.

Acknowledgements

Work described in this article has been undertaken with financial support from the British Heart Foundation (RCT Programme Grant with Dr Nick Morrell, Cambridge, England, and Clinical Fellowship to Dr Rachel Harrison). I wish to recognize the support and collaboration of many clinical colleagues, who have generously introduced patients to these research studies and provided clinical details following informed consent.

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