Translational medicine in hereditary hemorrhagic telangiectasia

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ABSTRACT

Scientific community have gained lots of new insights in the genetic and biochemical background of different conditions, rare diseases included, settling the basis for preclinical models that are helping to identify new biomarkers and therapeutic targets. Translational Medicine (TM) is an interdisciplinary area of biomedicine with an essential role in bench-to-bedside transition enhancement, generating a circular flow of knowledge transfer between research environment and clinical setting, always centered in patient needs. Here, we present different tools used in TM and an overview of what is being done related to hereditary hemorrhagic telangiectasia (HHT), as a disease’s model. This work is focused on how this combination of basic and clinical research impacts in HHT patient’s daily clinical management and also looking into the future. Further randomized clinical trials with HHT patients should assess the findings of this bench-to-bedside transition. The benefits of this basic and clinical research combination, may not only be important for HHT patients but for patients with other vascular diseases sharing angiogenic disturbances.

1. HHT as a model for Translational Medicine

Hereditary hemorrhagic telangiectasia (HHT) or Rendu–Osler–Weber syndrome (ORPHA:774) is a rare autosomal dominant vascular disease characterized by systemic telangiectases and larger vascular malformations (VMs) [1]. The hallmark of HHT is telangiectasis, which are dilated postcapillary venules directly connected with dilated arteries losing the capillary bed [2]. HHT can be diagnosed either using the Curaçao clinical criteria (recurrent epistaxis, cutaneous/mucosal telangiectasia, visceral VMs, and a first-degree family member with HHT) or through molecular genetic test [2,3]. Mutations in the endoglin (ENG) and activin A receptor type II-like 1 (ACVRL1) genes are detected in approximately 90% of cases submitted for molecular diagnosis and cause HHT1 and HHT2, respectively [4,5]. Endoglin (encoded by ENG) is an auxiliary co-receptor at the endothelial cell (EC) surface that promotes BMP9 (Bone morphogenetic protein 9) signaling through the activin receptor-like kinase 1 (ALK1; encoded by ACVRL1). Thus, HHT is now considered a disease of the hub formed by BMP9–Endoglin–ALK1–Smad, rather than a disease of the TGFB pathway [6].

Although many HHT features have been elucidated, there are still several questions that remain unsolved. It is unknown why telangiectases are typically found in characteristics sites, as in fingertips of the hands or nasal mucosa (causing recurrent epistaxis, which are the most
frequent clinical manifestation) or the reason why pulmonary arteriovenous malformations (AVMs) are more common in patients with HHT1 and hepatic VMs in HHT2. Also, the genetic landscape of HHT is unclear, as there are 10-15% of patients with HHT phenotype who are genetically orphan. Moreover, despite showing equal pathogenic variants, there is high inter- and intra-familial variability in vascular involvement and clinical manifestations [2,3]. Another unsolved question is that, in spite of HHT being a congenital condition, it exhibits an age-related penetrance as epistaxis is the earliest clinical manifestation and most patients have developed larger VMs before 40 years [2] but surprisingly, gastrointestinal bleeding symptoms usually develop at fifth or sixth decades [7]. Moreover, the low prevalence and high clinical variability make understanding HHT a challenge, especially in uncommon situations [8]. Together, these unresolved questions have hampered the development of a curative therapy for HHT patients. Thus, increasing understanding of HHT is vital for providing insights into molecular regulation of vascular development and improving care of patients.

The European Society for Translational Medicine (EUSTM) defined Translational Medicine (TM) in 2015 as "an interdisciplinary branch of the biomedical field supported by three main pillars: benchside, bedside, and community. The goal of TM is to combine disciplines, resources, expertise, and techniques within these pillars to promote enhancements in prevention, diagnosis, and therapies" [9]. Accordingly, a multidisciplinary approach is essential in TM and correlates indeed, with the importance of a multidisciplinary medical team when dealing with patients affected by rare diseases, such as HHT. TM must depart from medical attention to patients, so this multidisciplinary team should be the starting point. Because of clinical research difficulties in rare diseases, including basic research is crucial to improve management of these patients. However, there is usually a wide gap in the transition between preclinical ("basic") and clinical stages because basic experiments may not reflect the real patients' demands. Thus, TM seems a good strategy to bring the two types of research closer as it implies both, clinical and basic researchers, working together. In fact, the adjective "translational" refers to the transference of the basic scientific findings from a laboratory setting into a clinical one. This is not only important as a way to get new discoveries, but also to enhance bench-to-bedside transition. The aim of this review is to exhibit some of the benefits that TM provide to HHT patients management, as a disease’s model.

2. Genetics and big data in Translational Medicine

Following the improvement of sequencing technology during the second decade of the 21st century, a lot of new genetic data has been served to researchers and clinicians, allowing to a better understanding of the molecular basis of lots of diseases. Although not always easy to interpret, Whole Exome Sequencing (WES) and Next Generation Sequencing (NGS) results are decisive to continue learning and elucidating the foundations of many diseases.

A prompt understanding of HHT genetic landscape was performed early in the 1990s [10,11]. Although HHT is a germline disease, the multiple VMs in HHT patients occur focally, rather than manifesting as a systemic vascular defect. The observation that VM only appear upon biallelic loss of HHT genes specifically in endothelial cells (EC) pointed towards the existence of a second-hit genetic event as a trigger for the induction of VMs. This Knudsonian second-hit mechanism is known to contribute to Capillary Malformation-Arteriovenous Malformation Syndrome type 1 (ORPHA:137667; MIM: 608354), a disease caused by autosomal-dominant loss-of-function mutations in RASA1 gene [1]. Indeed, by sequencing cutaneous telangiectases biopsies from 5 HHT patients it has been recently demonstrated that 9 out of 19 telangiectases contain a low-frequency somatic mutation in trans configuration [12]. Key questions that emanate from that study is how the second-hit appear and how the remaining half telangiectases are induced. In another study, despite local loss of both ENG and ACVR1I genes using Cre-Lox technology in mouse, brain VMs only develop after local vascular endothelial growth factor (VEGF) injection [13]. Thus, the necessity for an angiogenic stimulus suggests that loss of both copies of the relevant HHT gene is necessary, but not sufficient, for development of VM. In fact, some studies suggest that both second-hit combined with environmental pro-angiogenic trigger are necessary for VM development, establishing the so-called “three event hypothesis” [13-15]. This could explain why HHT patients debut during puberty when a wave of hormones and growth factors are constantly produced and released into the bloodstream [5].

Another issue is why mutations in the same gene do not recapitulate a similar phenotype. In this sense, resequencing patients and applying new informative tools to reclassify variants as pathogenic or possible pathogenic and crossing these data with clinical information, would allow to a better risk profiling and a most personalized follow up for HHT patients [16]. Another approach has been looking for RNA signatures differences when comparing HHT nasal telangiectasia and non-telangiectasia HHT tissues [17]. More recently, other authors have suggested detecting polymorphisms in other genes related to HHT even when a driver mutation has been identified, to feed the hypothesis that modifier genes may influence the phenotype and might explain heterogeneity [18]. Other pathways related to vascular development may be responsible for HHT-like phenotypes in those 10-15% of patients who full-fill the Curaçao Clinical Criteria but are genetically orphan [19].

Under the umbrella of bioinformatics, TM is growing massively, and rare diseases must take advantage of its improvement. There are emerging bioinformatic tools that allow correlating mutations with protein-to-protein interface and, after comparing it with clinical data, explain how diseases apparently driven by the same gene have different behaviors in terms of treatment response [20]. Others have used artificial intelligence to reposition technologies created for other purposes to suggest new ways to diagnose HHT [21] or have based a treatment proposal in network analysis of metabolomic profiles [22]. In artificial intelligence and in silico predictions is probably where TM has the bigger potential of growth as a way to scout new research niches and provide rational to built new models and paradigms.

3. Biomarkers in Translational Medicine

The term “biomarker”, a portmanteau of “biological marker”, refers to a measurable and quantifiable indicator of some biological state or condition. In 2001, the National Institutes of Health (NIH) working group standardized the definition of a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [23]. Biomarkers are a relatively new clinical toolset used in the new “Precision medicine”. This is a medical model that proposes the customization of healthcare, with medical decisions, treatments or practices being tailored to a subgroup of patients, instead of a one-drug-fits-all model. In “Precision medicine”, individual biomarkers are often employed for selecting appropriate and optimal therapies and guiding treatment decisions. Current knowledge of the affected genes and signalling pathways involved in HHT has allowed the identification of potentially biomarkers. Studies in HHT patients and animal models have reported altered levels of VEGF, transforming growth factor-beta 1 (TGF-b1), angiopoietin 2 (ANGPT2) or adrenomedullin (AM).

VEGF is a potent pro-angiogenic factor that acts as a critical modulation of angiogenesis and has been extensively studied in HHT patients. Plasma levels and tissue expression in the nasal mucosa of VEGF have been found to be significantly higher in HHT patients compared to healthy controls [24,25]. Interestingly, HHT patients without pulmonary AVM had significantly lower VEGF levels than pulmonary AVM HHT patients [26]. Some data have allowed the benefits of using anti-angiogenic therapy against VEGF (bevacizumab) in HHT patients [27]. Whether determination of plasma VEGF may be a useful marker of severity or may correlate to anti-VEGF treatment response is not well
established.

Several groups have evaluated TGF-b1 levels in patients with HHT showing controversial results [24,26]. In a mouse model of HHT (Smad4+/-ECKO), ANGPT2 levels were strongly increased and administration of ANGPT2 monoclonal antibodies resulted in the prevention and resolution of retinal AVMs [28]. AM is a vasoactive peptide mostly secreted by EC with vasodilatory properties that helps to regulate vascular stability and permeability by modulating the endothelial barrier [29]. Some studies have shown that AM-induced angiogenesis in EC is mediated by activation of PI3K/akt [30]. Moreover, given the vasodilatory properties of AM, its enhanced local expression in endothelial and smooth muscle cells may cause variations in blood flow and contribute to development of VMs [31]. Higher AM serum levels have been recently found in 45 HHT patients compared to 50 healthy volunteers and telangiectases’ biopsies from eight HHT patients expressed higher AM immunoreactivity involving the endothelium than normal skin from five healthy donors. [29].

4. In vitro models in Translational Medicine

In spite its limitations of mimicking biological processes, in vitro models are an essential tool for the study and comprehension of the mechanistic clues underneath clinical queries. It deserves a particular mention the role of gene delivery and gene editing as cornerstones of the in vitro model. The already established CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats - associated protein 9) technique allows researchers generating cell lines with genetic landscapes similar to those exhibited by patients, widening the possibilities of molecular characterization and interactor knowledge.

BMP9 is a high affinity ligand for endoglin and ALK1 co-receptors in EC context and induce either stimulation or repression of target gene expression mainly in a phospho-Smad-dependent manner [4,32]. The main biological output of BMP9/10 signaling is the induction of vascular quiescence [32]. However, ALK1 activation can also cross-talk with VEGF, ANGPT2, or Notch pathways [33]. Moreover, through down-regulation of PTEN (Phosphatase and TENsin homolog) phosphorylation, BMP9 increases PTEN activity and in turn decreases PI3K (phosphatidylinositol-4,5 bisphosphate 3-kinase) and Akt, two key components of the VEGF and ANGPT2 signaling [34-36]. In fact, it has been stated by immunohistochemistry of cutaneous telangiectasia from HHT1 and HHT2 patients that loss of endoglin and ALK1 proteins’ function provoke anomalous vascular overgrowth due to the over-activation of PI3K signaling [36,37]. This is not surprising given that EC are exclusively regulated by the PI3K pathway, and overactivation of this pathway is a hallmark of several types of VMs [38]. However, it is important to note that the degree of PI3K overactivation in ENG and ACVR1L mutant cells is quite likely moderate compared to the impact of activating mutations in PIK3CA gene in other vascular-related diseases, such as venous and lymphatic malformations as well as PROS (PIK3CA-related overgrowth spectrum) [38,39].

Apart from phospho-Smad-dependent manner, another pro-mitogenic pathway inhibited by BMP9/ALK1/Endoglin is the ERK (extracellular signal-regulated kinases) /MAPK (mitogen-activated protein kinase) pathway. The ERK/MAPK cascade is one of the most characterized signaling pathways that connects different types of membrane receptors to the nucleus after mitogenic stimulation or differentiation [40,41]. The activity of ERK/MAPK downstream of proangiogenic factors, as VEGF-A, is essential for physiological sprouting, proliferation and migration of EC [40]. Beyond the formation of new vessels, ERK-signaling is also essential to maintain vascular integrity in quiescent EC, altogether demonstrating a central role for ERK in normal vascular biology [41].

Albeit these well established mechanisms, there are other that remain unknown and that may be relevant for HHT patients management. The means by which the ERK/MAPK pathway is altered remains to be clarified, although some data imply a different kinase, SGK1 (serum/glucocorticoid-regulated kinase 1). SGK1 is an early gene induced by the BMP9/ALK1/Endoglin cascade [42,43], and its deletion is embryonic lethal due to problems in cardiovascular development [44]. In all, available data suggests that SGK1 plays a key role as a signaling hub for the BMP9/ALK1/Endoglin pathway including ERK/MAPK repression and centralizing different actions played by this pathway during angiogenesis: cell proliferation blockade while maintaining an active cell metabolism. Alterations in this protein could also be involved in the pathological phenotype of EC in different vascular diseases as HHT.

All these data mean that EC are permanently receiving pro- and anti-angiogenic inputs from different pathways and that disturbances in this homeostasis could be responsible for abnormal vascular overgrowth and HHT phenotype.

5. Mouse models in Translational Medicine

Preclinical mouse models have become a powerful tool to understand the pathological mechanisms underlying HHT and to test novel therapies. However, modelling the HHT-related AVMs in mouse models is not an easy task. This is partially due to the lack of fundamental principles on how these mutations induce vascular malformations. Whereas homozygous ENG and ACVR1L gene inactivation in mouse result is embryonic lethal because of severe VMs, mice heterozygous for constitutional deletion of either gene recapitulates only some features of these diseases and show extremely mild phenotypes with relatively few, if any, detectable AVMs [45,46,37]. Yet, embryonic phenotypes are not an optimal system for preclinical studies.

To overcome embryonic lethality induced by homozygous global ENG and ACVR1L gene inactivation, postnatal tamoxifen (Tx)-inducible approaches have been pursued [45,46]. Based on the germline nature of these disease, ENG and ACVR1L were first ubiquitously deleted using the Rosa26CreERT2 mouse strain which resulted in rapid signs of illness and lethality, quite likely due to the formation of aberrant arteriovenous shunts [14]. The use of cell-specific Tx-induced models allowed to identify that HHT-like VM in mice occur upon biallelic knockout (KO) of the ENG and ACVR1L in EC [35,47]. Of note, blocking antibodies against BMP9 and 10 also efficiently induce AVMs in mice [35].

Given that the abluminal surface of EC are in proximity with mural cells (i.e., pericytes, vascular smooth muscle cells), it was also questioned whether these cells would contribute to the pathogenesis of HHT. However, homozygous deletion of HHT genes upon Tx specifically in mural cells does not result in AVMs [14]. Instead, deletion of ACRL1 in these cells rewires the phenotype of vascular smooth muscle cells from contractile to synthetic, resulting in pulmonary and cardiac hypertension implications [48]. Moreover, blood flow is a required process for vascular remodeling. Thus, using sophisticated CreERT2 lines which allow to delete genes in specific subsets of EC (capillaries, veins or arteries) shows that only biallelic loss of ACVR1L in capillary-venous EC is sufficient to induce AVM formation [49]. This suggests that AVM spatially originate from capillary and venous cells and then aberrantly connect to arteries. Indeed, a close-up analysis of these mutant EC shows that loss of ACVR1L impairs capillary and venous EC to migrate against the flow. This fits with the data that expression of HHT genes is enriched in venous and capillaries but not in arteries [47,31].

Taken together, modelling HHT genes in cultured cells and in animals have provided some important molecular and cellular insights into the onset and development of VM: (1) modelling VMs by genetic approaches becomes a powerful strategy to study the onset of HHT phenotypes; (2) HHT-like AVMs occur by an EC autonomous defect; (3) VMs only appear during growth phases supporting the mentioned “three event hypothesis”; (4) activation of PI3K signaling is a hallmark of VM.

These studies have also highlighted that current genetic models can only be used for a short period of time given the reduced life span of these mouse models. Hence, an effort to overcome this is urgently needed as we still do not know whether targeted therapies would prevent the genesis or they would also regress AVMs. This becomes extremely
important in HHT as it is difficult to predict what triggers the formation of an AVM and most patients already have an AVM at the time of diagnosis.

Mouse models of HHT1 and HHT2 should be addressed to examine many outstanding questions in the field; for example, whether AVMs always form in the same manner and at what point in their development they are reversible; whether EC proliferation always contributes to enlargement of AVMs and to what extent the shunts are maintained, or exacerbated, by blood flow. These and many more questions will be answered as the mouse models are further refined.

6. Translational Medicine impact in HHT management

By the use of all these different tools, TM has helped elucidating the basis of HHT pathogenesis and therefore, provided new potential therapeutic targets as a perfect example of the adding value of the clinical an basic research association. We will now describe the recent most interesting targets and associated therapies identified by this translational strategy (Fig. 1). Importantly, these new therapies are not only useful for HHT but for diseases that share angiogenesis disturbances.

The loss of BMP9/ALK1/Endoglin pathway causes overstimulation of the PI3K and ERK/MAPK signaling hubs and in consequence, endothelial overgrowth [34,36]. So there are two interesting pathways to block. On the one hand, the overstimulation of the PI3K/AKT/mTOR pathway, could be reversed using mTOR (mammalian Target of Rapamycin) inhibitors such as sirolimus or everolimus [50]. In fact, it has been published an important improvement after a liver transplant in HHT patients receiving mTOR inhibitors [51]. Interestingly, Ruiz et al. reported that the combination of sirolimus and a tyrosine kinase receptor inhibitor (nintedanib) could synergistically reverse retinal AVMs in a BMP9/10-immunoblocked mouse model of HHT [52]. The authors found that sirolimus acted as a dual modulator, stimulating Smad1/5/8 while inhibiting mTOR, thus correcting two key signalling defects in HHT. Moreover, astonishing results after using sirolimus in patients with other different types of VMs have been published [53]. By using mouse models for HHT, it has been shown that pharmacological directly inhibition of PI3K prevents ALK1-induced vascular hyperplasia in retinas [35,37]. Test a new more specific inhibitor against PI3K-alfa, alpelisib in animal models could be the next step for treating HHT patients.

Genetic or pharmacological inhibition of ERK-signalling results in impaired blood vessel development in different models [54]. In consequence, a failure in ERK activation control could also be involved in the overgrowth of VMs in HHT. Thus, blocking ERK/MAPK activity by repositioning Ulixertinib, that has been studied in advanced solid tumors with ERK/MAPK cascade alterations, could become a new therapeutic approach. Another considered strategy has been trying to overcome the haploinsufficiency caused by ENG and ACVRL1 mutations by boosting the BMP9/10 pathway with the use of tacrolimus [55]. Tacrolimus is a potent activator of the BMP9/ALK1/Smad signaling hub and it has been tested in HHT animal models showing a significant decrease in retinal AVMs, previously induced by BMP9/10 immunodepleting [56]. Indeed, a recent case study reported the dramatic reduction of epistaxis in HHT2 patient who was treated with oral low dose of tacrolimus due to progressive PAH [57]. Finally, several pharmacological modulators of AM, recently involved in HHT, have been described and could be used to intervene in these patients [29]. These modulators include monoclonal antibodies, such as adrecizumab, a non-neutralizing
AM binding antibody that has been shown to restore the impaired vascular barrier function that causes hemodynamic instability in sepsis [58].

Preclinical studies with cellular and animal models and the resulting randomized clinical trials with HHT patients, should assess the efficacy and safety of these drugs, that already have a solid physiopathological basis. The benefits of this basic and clinical research community, may not only be important for HHT patients but for patients with other vascular diseases sharing angiogenic disturbances. In fact, this is the objective of TM: transfer the insight from a laboratory environment into a clinical one, avoiding any gap in the transition between both settings.

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Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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