Wave mice: a new tool in the quest to characterize aortic valvular disease etiologies

Ana M. Porras, Kristyn S. Masters

Department of Biomedical Engineering, University of Wisconsin-Madison, Madison, WI 53706, USA
Correspondence to: Kristyn S. Masters, PhD. Department of Biomedical Engineering, University of Wisconsin-Madison, 1550 Engineering Drive, #2152, Madison, WI 53706, USA. Email: kmasters@wisc.edu.

Introduction

Although many patients with non-rheumatic aortic regurgitation (AR) are asymptomatic and do not require surgical intervention, chronic AR can lead to left ventricular (LV) hypertrophy and, eventually, heart failure (1). The progression from mild to chronic AR is poorly understood and relatively few animal models of chronic AR have been described. In a recent study published in *Arteriosclerosis, Thrombosis*, and *Vascular Biology*, Hajj *et al*. report the characterization of a "Wave" mouse model whose predominant valvular function abnormality is AR, despite the valves exhibiting many of the features traditionally associated with aortic stenosis (AS) (2). Unlike previously described models of AS (3,4), the valvular dysfunction exhibited by Wave mice is independent of the fibrocalcific changes found in the aortic valves of these mice. Thus, the results of this study challenge some of the current paradigms of aortic valve disease and are likely to impact pathophysiological conclusions related to both AR and AS.

Challenging the AR *vs.* AS paradigm

Wave mice possess a single nucleotide mutation in the gene encoding the epidermal growth factor receptor (EGFR), resulting in over 90% global reduction of EGFR tyrosine kinase activity (5). This mutation affects the valvulogenesis of the semilunar valves, yielding valvular dysfunction, LV hypertrophy, and eventual heart failure (5,6). However, previous studies of Wave mice have produced conflicting reports regarding whether AS or AR was the predominant valvular pathology (5,6). The aortic valves of Wave mice exhibit numerous AS hallmarks—such as thickened leaflets,

proteoglycan enrichment, lipid deposition, osteoblastic differentiation of the valvular interstitial cells (VICs), calcification, and increased aortic valve pressure gradient—which would appear to make AS a logical diagnosis. But the elegant work by Hajj *et al*. provides multiple lines of evidence indicating that AR is the principal valvular disorder in Wave mice.

At 6 months of age, Wave mice possessed aortic valves that showed signs of thickening, lipid deposition, and calcification that were consistent with prior mouse models of AS (4,7). Valvular pressure gradient and LV volume and mass were also significantly elevated. However, in 52 of 55 mice, measurement of aortic cusp separation revealed no difference between Wave and control mice, indicating the absence of AS. Meanwhile, moderate to severe AR was detected in 81% of Wave mice at this age. The authors went on to further prove that the valvular dysfunction was not due to calcific events by treating the mice with pioglitazone, a compound previously found to attenuate valve calcification (4). While pioglitazone treatment successfully reduced both calcification and osteogenic differentiation in Wave mouse aortic valves, it did not have any effect on the prevalence or severity of AR in these valves. Thus, despite displaying several hallmarks of AS, the aortic valves of Wave mice appear to serve almost exclusively as a model for myxomatous AR. This dissociation of fibrocalcific changes from valve function is an important finding that is likely to impact future investigations of both AR and AS.

A critical role for the extracellular matrix (ECM)

In addition to challenging the current paradigms of

evaluating valvular disease, the findings by Hajj *et al.* suggest that changes in the valve ECM potentially play a major role in driving valvular pathogenesis—an observation that could influence not only our understanding of aortic valve pathologies, but also our treatment of them. By as early as 1.5 months of age, the aortic valves of Wave mice exhibit increased collagen and proteoglycan deposition relative to controls. Proteoglycan enrichment is a hallmark of both myxomatous valvular disease and AS, and is hypothesized to be an initiating event in the progression of both pathologies (8). Although only correlative in nature, the findings by Hajj *et al.* support this hypothesis, as alterations in ECM composition preceded all other histopathological changes. Moreover, the authors specifically identified increased levels of intact versican (but not biglycan) in Wave mice. An earlier study found that mice developed myxomatous valvular disease due to a decrease in versican cleavage (9). It remains unclear why versican, and not other proteoglycans, contributed to the pathology observed in both papers—a mechanism which warrants further study. Overall, it is becoming increasingly apparent that the valve ECM is a delicately balanced structure that can exert a powerful influence on the development of valvular pathologies (10). The ability of the ECM to potentially drive subsequent events in the development of both AR and AS also raises the possibility of targeting molecules involved in ECM remodeling to stop disease progression.

Elucidating the sequence of pathological events in aortic valvular diseases

The investigation by Hajj *et al.* also serves to further highlight our gaps in knowledge with respect to understanding the sequence of pathobiological events that lead to either AR or AS, as well as the challenges in obtaining causative evidence linking these events. One intriguing observation is that the Wave mice exhibit ECM disruption and valve dysfunction prior to upregulation of fibrotic markers and fibrosis. While these results imply that myofibroblast activity is not necessary to induce ECM disarray and that fibrosis is not necessary for development of AR and LV dysfunction, the causative factor for initiation of fibrotic activity cannot be determined from this study. Transdifferentiation of VICs can be driven by ECM composition (10) as well as the mechanical environment (11), so both ECM changes and hemodynamic alterations are possible contenders for guiding the fibrotic and osteogenic events described in Wave mice. The finding that AR and

AS can share so many histopathological similarities also raises the question of which cellular- and ECM-level characteristics are responsible for progression towards each of these pathologies. The Wave mouse model, in combination with existing models of AS (3), provides an important tool in addressing such questions. Tissue-engineered models of valvular disease are also poised to serve a critical role in such investigations (12); although they lack the full complexity of native valves, they enable a controlled manipulation of causative connections that is often not possible with *in vivo* models.

In the analysis of data obtained from mouse models, it is also important to consider their limited potential in recapitulating human valve anatomy and physiology. For example, mouse aortic leaflets do not possess the same trilayered ECM structure characteristic of human aortic valves (13). Myxomatous valve disease as described in this particular model of AR is not considered common in the aortic valves of humans in the absence of congenital abnormalities or rheumatic heart disease (14). Moreover, the extent of calcification and lipid deposition reported for both Wave mice and mouse models of AS is substantially lower than what is found in humans. For instance, the amount of calcification in mouse AS models (4,7) (and Wave mice) has been <10% of the total leaflet area, while mild to moderate AS in humans can be accompanied by 40-80% mineralization (15).

Conclusions

In summary, the development of a much-needed mouse model of AR is a significant scientific contribution on its own, but the work by Hajj *et al.* has implications that reach beyond this accomplishment. Their report not only has consequences for the pathophysiological evaluation of valve tissues, but also highlights the current gaps in our knowledge of the mechanistic pathways of both AR and AS, and what features may be common *vs.* divergent across these two pathologies. The continued development of novel animal models, combined with tissue engineering-based approaches, is likely to be needed to fully elucidate these etiologies and causative relationships.

Acknowledgements

of Health (R01 HL093281) for research into calcific aortic valve disease mechanisms.

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