Surviving Infarction One Gene at a Time
Decreased Remodeling and Mortality in Engineered Mice Lacking the Angiotensin II Type 1A Receptor

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Angiotensin II (Ang II), named for its first-discovered physiological role as a potent vasoconstrictor, is perhaps the most widely studied paradigm of a pleiotropic agent in cardiovascular biology and medicine, with diverse targets and effects far beyond those first envisioned.1 Notably, Ang II has received scrutiny as an intramyocardial growth factor (one, among others) that is produced locally within the myocardium, is released from cardiac cells after mechanical load, and is subsequently expressed at higher levels as part of the “hypertrophic phenotype.”2 Both cardiac myocytes and fibroblasts are responsive to Ang II,3 which suffices in tissue culture experiments not only to trigger most of the molecular features of hypertrophy but also to drive fibroblast proliferation. In clinical heart failure, this gives credence to the beneficial effects of treatments that decrease Ang II production (ACE inhibitors) or that decrease binding to its type I receptors, thereby preserving ventricular function at doses that do not lower blood pressure and consequently are thought to be mediated by blocking of local actions of this peptide.4 A second clinical setting, related but distinct, in which blockers of Ang II production or function in the heart have proven to be especially valuable is the more acute context of adverse ventricular remodeling after myocardial infarction.5,6 In the instance of Ang II, the universal caveat that a pharmacological intervention might actually work through unanticipated mechanisms has given rise to perennial controversy, because ACE inhibitors, apart from their nominal role, also impair the degradation of bradykinin.4

Given the foreseeable, recurrent, and vexing limitations of reliance on drug studies alone to decipher protein function in vivo, in recent years it has become a conventional wisdom that the “gold standard” for such insights is to be found in animal models that have been genetically engineered to lack the protein in question (so-called knockout mutations). As one relevant example, mice lacking the bradykinin B2 receptor recently were used to prove that kinins do not mediate the antihypertrophic action of ACE inhibitors, discussed above.7 Most typically, knockout mutations have been created by the swapping of a defective copy of the gene for the endogenous, wild-type gene (“homologous recombination”) with primitive, totipotent cells (embryonic stem cells) that are reintroduced into early embryos and give rise to all cell types in the resulting organism. If the mutation is successfully transmitted to the offspring, and heterozygous mutants are mated to one another, the desired “null” genotype results, ie, animals with 2 defective copies of the gene and no normal copies. The attractions of this experimental strategy are many: precision, by comparison with random insertions or chemical mutagenesis; certainty, by comparison with techniques that might inhibit gene expression or function incompletely; and specificity, by comparison with methods that might factitiously affect the expression or function of another gene.

With the alluring promise of a genetic resolution to these issues in mind, investigators have tested the presumptive role of Ang II in pressure-overload hypertrophy, as had been predicted from a number of whole-animal drug studies. Perhaps surprisingly, the outcome, found independently by 2 research teams,8–10 turned out to be that the “critical” Ang II receptor, AT1A, was wholly dispensable for the development of hypertrophy when models of either pressure or volume overload were used. (Functional compensation by alternative Ang II receptors, AT1B or AT2, cannot be disregarded as a potential explanation for this, which could be addressed, in principle, with double-knockout mice, triple-knockout mice, or, more simply, mice lacking the angiotensinogen gene.11) Both groups’ conclusion that cardiac hypertrophy can be induced in the absence of AT1A-mediated Ang II signaling also was supported by the persistence of normal responses to mechanical stretch in vitro, demonstrated by use of cardiac myocytes isolated from AT1A-null versus wild-type mice.12 Together, these results might be consistent with an obligatory role for some alternative secreted factor, such as endothelin (another vasoconstrictor turned hypertrophic agonist),13 or with the operation of an exclusively intracellular pathway coupling mechanical signals to cardiac growth, perhaps via integrins as a physical bridge to the cells’ environment.14

In contrast to the failure of this seemingly straightforward test to substantiate an essential role for Ang II signaling through AT1A in load-induced hypertrophy, Harada et al15 report in this issue of Circulation that survival, heart failure, remodeling, and fibrosis after myocardial infarction were markedly improved in mice lacking the AT1A receptor. No significant difference was seen in infarct size between control and AT1A receptor-deficient

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mice, in agreement with an earlier investigation by this group using a murine model of ischemia-reperfusion injury; a decreased prevalence of reperfusion arrhythmias was the one positive finding in that report. In the present study, after permanent occlusion, the cumulative 4-week mortality rate was reduced from 22.7% to 5.9% in AT1A knockout mice; left ventricular (LV) wall thickness, fractional shortening, and end-diastolic pressure were preserved; LV dilatation was minimized; and fibrosis was prevented. Although several early responses 1 week after infarction were equivalent in wild-type and receptor-deficient mice (with regard to mRNA levels for atrial natriuretic peptide, β-myosin heavy chains, and collagen III), others were attenuated (transforming growth factor β-1 and collagen I). Even more striking effects on the normalization of gene expression were seen at 4 weeks, including return of both collagen genes to sham-operated control levels.

In short, the lack of AT1A receptors was associated with improved survival after infarction, despite an infarct size similar to controls, and with improvements in myocardial structure, myocardial function, and myocardial gene expression. These findings are remarkably congruent with the clear-cut protective effect of ACE inhibitors after infarction in humans, and this genetic evidence thus attests to Ang II as the responsible agent in the clinical setting. The present study, deleting the gene for only 1 of 3 Ang II receptor genes, also demonstrates a complex range of cardiac responses contingent on AT1A, although some of these responses may reflect the favorable effects of this knock-out on LV geometry and, hence, wall stress.

Arguably, the single largest effect of the deletion of AT1A was on the extent of interstitial fibrosis, which in turn may account for some, much, or all of the other improvements observed. Recent evidence demonstrates the existence of complex cross talk between cardiac myocytes and cardiac fibroblasts that could underlie the cardiac fibrosis induced by Ang II. 17 One cannot conclude from the present work the extent to which these improved outcomes might be due to absence of AT1A in cardiac fibroblasts versus its absence from cardiac myocytes themselves. Refinements in gene-targeting technology in mice (“conditional” knockouts that use a DNA recombinase to excise critical segments of suitably tagged genes) now permit gene deletion to be confined to any lineage or region for which an appropriate, specific promoter or gene is available. 18 The feasibility and utility of cardiac-specific knockouts have been established, and an approach that clearly is suited to resolving the relative importance of myocytes versus other cell types as Ang II targets in the myocardium. Another, subtle issue raised by previous work on AT1A receptor-deficient mice is the basis for abnormal phe- notypes of angiotensin nullizygotes. 19, 20

References

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