Molecular Advances in Cardiovascular Biology

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Cardiovascular disease is the leading cause of mortality in the United States and consumes a large portion of the health care budgets of many developed nations. It is, therefore, one of the prime targets for biotechnology and drug development. Current surgical and interventional therapies, while efficacious in certain clinical settings, are costly and primarily palliative, and do not attack the basic cause of the disease. However, recent advances in gene transfer, mouse and human genetics, and molecular biology are initiating a revolution in both cardiovascular biology and medicine as the field enters the molecular era.

High-tech cardiovascular medicine is unexpectedly intersecting with molecular biology. When the arteries of the heart are occluded by atherosclerotic plaques, they can be opened up by mechanical dilation with catheters. However, a few months after successful coronary dilation, the vessels often become reoccluded, largely due to the migration and proliferation of smooth muscle cells into the vascular lumen of the fractured arteriosclerotic plaque. In the rat carotid vessel, this proliferation can be inhibited by antibodies to platelet-derived growth factor (PDGF) (*1*) or antisense oligonucleotides to a proto-oncogene (c-*myb*) (*2*), pointing the way for molecular therapy to improve long-term vessel patency. Specialized catheters have been designed to deliver the molecules to the exact site of the coronary lesion (*3*); the efficiency of delivery can be determined by monitoring the direct transfer and expression of reporter genes in the arterial wall. This technological advance may eventually allow percutaneous, site-specific gene therapy for vascular lesions, if we can also achieve long-term, highly efficient, and targeted expression to specific cell types in the vascular wall. Recombinant viruses with tissue-specific tropism and cell-type specific promoters will be valuable in achieving this cell-specific targeting.

Perhaps the most fascinating examples of the power of genetics models for cardiovascular medicine are transgenic mice harboring human genes that have been implicated in either the progression or inhibition of coronary artery disease. Although rabbit and pig models of atherosclerosis have proven valuable, transgenic and embryonic stem cell biology is less well developed in these species. By mating into different mouse genetic backgrounds with varying degrees of inherent resistance or susceptibility to atherogenesis, mouse models allow the identification of factors that can inhibit the onset or progression of vascular lesions, independent of effects on absolute concentrations of cholesterol or cholesterol metabolism (*4*). These models may also be useful for the identification of interacting genes that accelerate the atherogenic phenotype in other disease states (for example, diabetes). However, due to species-specific differences in lipoprotein metabolism, the value of mice as models of human atherogenesis has been questioned, and the challenge has been to "humanize" the mouse by transgenic techniques. Two laboratories have established lines of "knockout" mice that lack the gene for apolipoprotein E (*5*). Both lines have cholesterol levels approaching 1800 mg per deciliter when they eat a high-fat diet like that of humans in the United States. They also have widespread intimal foam cells (an early sign of atherosclerosis) and more complicated atherosclerotic lesions (see figure, left). These mice may ultimately prove valuable for optimizing conditions for newly developed gene therapies for hypercholesterolemia (*6*) or for investigating restenosis after catheter dilation.

The polygenic nature of clinical hypertension has posed a formidable experimental obstacle to the identification of "hypertension genes" in humans. Nevertheless, genetic analyses of rodent models (*7*) and human populations (*8*) support an important role for the renin-angiotensin system in the hypertensive phenotype. Renin is an aspartyl protease that cleaves angiotensinogen into the decapeptide, angiotensin I, which is the rate-limiting step in the renin-angiotensin system (see figure, center). Subsequent cleavage by angiotensin converting enzyme (ACE) results in the production of the octapeptide angiotensin II, which regulates blood pressure and salt retention. Molecular variants of the renin substrate angiotensinogen have been found to cause an inherited predisposition to essential hypertension (*8*). Although previous linkage studies have suggested that the ACE gene is not linked to clinical hypertension (*9*), a deletion polymorphism in the ACE gene is a significant risk factor for myocardial infarction in humans (*10*). These results, coupled with the well-documented efficacy of ACE inhibitors as antihypertensive agents, imply that the targeted inhibition of other molecules in this cascade (for example, with renin inhibitors or angiotensin II receptor antagonists) may be clinically useful with potentially fewer side effects than the currently available ACE inhibitors. The availability of cloned cDNAs for each of the components of the renin-angiotensin system has offered a prime opportunity for the rational design of peptide inhibitors, such as the peptide-based renin inhibitors (*11*). With these therapeutic agents, it may be possible to determine if the secondary disease that accompanies long-standing hypertension (in kidney, heart, retina, and so forth) arises from elevated blood pressure per se or rather reflects other, perhaps nonvasopressor, actions of angiotensin II on vascular smooth muscle cell growth and proliferation.

In response to long-standing hypertension, myocardial injury, or other demands for increased cardiac work, the myocardium adapts through the activation of a "hypertrophic" response. The hypertrophic heart is enlarged and characterized by the accumulation of contractile proteins in individual myocardial cells, but with no concomitant increase in muscle cell proliferation (*12*). The hypertrophic response largely depends on the transcriptional activation of genes that encode myosin and other components of the muscle cell architecture, which mediate contraction of the heart. One of these activated components, tubulin, is of particular importance, because agents that inhibit polymerization of microtubules prevent the contractile abnormality associated with hypertrophy (*13*). Although this process is initially compensatory, there can be a pathological transition in which the myocardium becomes irreversibly enlarged and dilated, with the accompanying onset of overt cardiac muscle failure. Discrete, and perhaps novel, growth factors might mediate various stages of ventricular muscle growth, hypertrophy, and failure, thereby allowing the design of specific agents to promote the compensatory phenotype or to repress the onset of pathologic forms of hypertrophy. Now that well-characterized in vitro cardiac muscle cell model systems are available that display morphological and genetic markers of the hypertrophic phenotype (*14*), it appears feasible to characterize potential factors (such as angiotensin II, endothelin, and adrenergic agonists), as well as to identify downstream intracellular signaling molecules (such as protein kinase C, G_q, and H-Ras) that orchestrate this adaptive response (*13,14*). By fusing constitutively active mutants of these signaling proteins to cardiac muscle promoters that can target expression to specific cardiac chambers in transgenic mice (*15*), the

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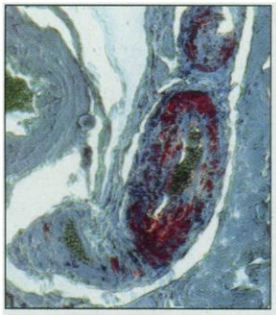

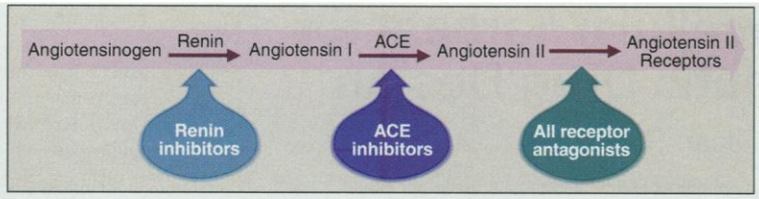

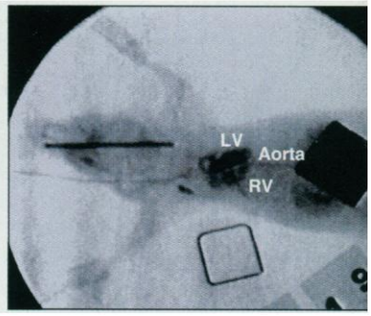

Steps toward molecular cardiovascular therapy. (**Left**) Coronary atherosclerotic lesion in apolipoprotein E–deficient mice. (**Center**) Therapeutic targets in the renin-angiotensin system, which controls blood pressure and salt retention. (**Right**) Digitized ventricular angiography for in vivo assessment of cardiac function in a mouse.

possibility of generating genetic-based mouse models of cardiac muscle hypertrophy and failure is on the horizon. The difficulties in assessing hemodynamic and contractile function in a mouse with a 1-mm aortic diameter has been obviated by the development of microsurgical and miniaturized technology to quantitatively assess hemodynamics and ventricular angiography in normal and transgenic mice (*16*) (see figure, right). These mouse genetic models could serve as valuable screening systems for therapeutic agents and may allow an examination of other factors that repress the pathologic phenotype, through mating into other genetic backgrounds.

Studies of an autosomal dominant form of familial hypertrophic cardiomyopathy are beginning to shed additional light into this adaptive physiologic response of the myocardium. Patients with this disorder display marked enlargement of the heart in the absence of hypertension, pathologic myocardial injury, or other external stimuli. The disease appears to be a primary disorder of cardiac muscle. Some of these families harbor missense mutations in conserved amino acid residues of the b-myosin heavy chain gene (*17*), which have been identified as being responsible for the disease. Although the mechanism by which a defective myosin produces the hypertrophic phenotype is not clear, it may represent an adaptive response to the expression of dysfunctional myosin. Because this disorder is genetically heterogeneous, the elucidation of other disease loci might identify new control points in the signaling pathway for cardiac hypertrophy and cardiomyopathy. Alternatively, other loci may represent mutations in different sarcomeric proteins.

Although our current understanding of cardiovascular developmental defects is itself at an embryonic stage, this area is likely to be one of the major beneficiaries of advances in transgenic model systems. Congenital heart disease currently affects 1 in 200 births in the United States each year, and there is a constellation of well-defined, distinct phenotypes (defects in septation, valvular formation, anomalous vascular development, cardiac chamber growth, right-to-left positional orientation of the heart and great vessels, and so forth) for which there are few currently recognized candidate genes or molecular insights. Recent advances in our understanding of the developmental regulation of genetic markers of cardiac chamber formation and specification, combined with insights into the molecular switches that regulate the expression of these markers, are beginning to provide a foundation from which to analyze the complex process of cardiogenesis (*18*). The in vitro differentiation of totipotent mouse embryonic stem cells into cardiac muscle cells (*19*) with ventricular specific properties (*20*) may ultimately allow very early studies of chamber specification in genetically engineered cardiac muscle cells. As the factors that play a role in cardiac expression become identified (*21*), gene knockouts of these candidate loci may uncover connections with defined congenital defects in humans, a long awaited event in the field. Toward this end, *splotch* mice, which harbor mutations in a homeotic gene (*Pax-3*), display neural crest defects (*22*), as well as persistent truncus arteriosus, an anomalous communication between the great vessels of the heart. A knockout of the *Hox1.5* gene results in abnormal cardiovascular development (*23*), although the observed cardiovascular phenotype does not coincide with a previously characterized clinical entity. A number of groups are also exploring the potential of zebrafish as a model to study heart tube development (*24*), because this species is amenable to transgenic technology and allows the external in vivo visualization of the linear heart tube during embryogenesis.

The past two decades of cardiovascular biology and medicine have been based largely upon the consideration of the heart and vasculature as an integrated physiological system, a view that has resulted in major therapeutic advances. The field is now on the threshold of a molecular therapeutic era. By allowing the molecular analysis of in vivo cardiovascular physiology, recent advances in mouse and human genetics may lead to the generation of a host of novel, biologically targeted therapeutic options. Given the multifactorial and polygenic nature of cardiovascular diseases, the prime targets for drug development may remain to be discovered. Nevertheless, a word of caution is in order. History can be a painful reminder of what can become of the best laid plans of mice and men.

References and Notes

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