Circulation Research Classics

To mark the 60th birthday of Circulation Research (1953–2013), the editors have commissioned Circulation Research Classics, a series of commentaries highlighting seminal articles published in the journal over the past 6 decades that have importantly shaped cardiovascular research. Written by leading experts, Circulation Research Classics are intended to describe the impact of these articles on the field by putting them in a historical perspective. The concept of classic is inextricably linked to time—a classic is something that maintains its value regardless of its age. Thus, an important consideration in selecting the articles to be highlighted is that they have stood the test of time, which is the most reliable indicator of the value of scientific work. By looking back at the illustrious past of Circulation Research, we hope to promote a deeper appreciation of the contributions of this journal to the advancement of knowledge.

The Discovery of the ACE2 Gene

A.J. Marian

A Novel Angiotensin-Converting Enzyme–Related Carboxypeptidase (ACE2) Converts Angiotensin I to Angiotensin 1–9

Donoghue et al

The discovery of the ACE2 gene broadened the scope of the regulatory mechanisms that govern the biological effects of the renin–angiotensin system in the cardiovascular system and beyond

The late Daniel E. Koshland Jr. (1920–2007), a former editor of Science (1985–1995), characterized scientific discoveries into 3 categories of Charge, Challenge, and Chance, which he dubbed as The Cha-Cha-Cha Theory of Scientific Discoveries.1 Citing Nobel laureate Albert Szent-Györgyi, Dr Koshland described the first Cha, as to see what everyone else has seen and think what no one else has thought before. Charge solves the obvious problems, such as the laws of heredity, which were delineated by Gregory Mendel and the theory of gravity, which was developed by Sir Isaac Newton. The second Cha refers to discovery of a new concept that pulls together the accumulated facts that had remained unexplained, such as the description of base pairing in double helix by Watson and Crick.2 The third Cha is for the serendipitous discoveries, which per Louis Pasteur favor the prepared mind.3 Sir Alexander Fleming observed clear spots on petri dish and went on to discover penicillin from the mold Penicillium notatum.

Scientific discoveries are typically incremental. The magnitude of increments follows a gradient from small to large. Major discoveries are not made in a scientific vacuum or through the Eureka moments but rather are based on a series of small discoveries that pave the path for the major breakthroughs. Thus, contributions of all steps in the ladder of scientific discoveries, whether small or large, are important. Nevertheless, certain steps epitomize the breakthroughs and apt to Dr Koshland’s Cha-Cha-Cha Theory of Scientific Discoveries. The progress in cardiovascular science is no exception. Hence, the Editors of Circulation Research have invited the audience to identify and highlight seminal discoveries that have been published in Circulation Research and have had or are expected to have major impacts in the cardiovascular sciences.

The initiative is laudable and the task is not trivial. The challenge is to recognize the big steps in the ladder of scientific discoveries. There is no predefined criterion that easily lends itself to spotting the seminal discoveries. The inability to identify the potential significance of the new findings is best illustrated in the case of the discovery of DNA, which was first isolated by Mieschner in 1869.4 For the next 75 years, it was reasoned that DNA, a large monotonous macromolecule composed only of 4 repeating units, could not have much function and that protein and not DNA was responsible for inheritance. The classic work of Avery, MacLeod, and McCarty in 1944 and later Hershey and Chase in 1952 established DNA as the molecule responsible for inheritance.5,6 Perhaps, one might reason that the Test of Time index is a reliable and the most basic element in defining a discovery as a Classic. Yet, there is no fixed Test of Time index to highlight significance of the new findings. The importance of some discoveries is self-evident, at least to the connoisseurs, as was the discovery of the double-stranded helix by Watson and Crick to John Maddox, the legendary editor of Nature (1966–1973 and 1980–1995). John Maddox apparently accepted the article without an external peer-review because the correctness and significance of the findings to him were self-evident.6

The efforts of the Editors of Circulation Research in advocating scientific discoveries that are fundamental and have stood the Test of Time is praiseworthy, particularly in a scientific publishing environment whereby journals and the articles are judged by their short-term citation index rather than the true impact on the scientific progress. Unfortunately, in judging significance of the discoveries the scientific society often gives precedence to where it is published than the actual content of the article. Yet, one might surmise that it would be

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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more challenging to have an article accepted for publication in a subspecialty journal such as Circulation Research, where top experts in the field peer-review the articles than in many of the high impact factor general scientific journals, whereby tendriness and potential breadth take precedence over robustness and depth.

The focus on the Classics also underscores the emphasis on the fundamental discoveries that fulfill Dr Koshland’s Cha-Cha Theory of Scientific Discoveries as opposed to what is chic or instigated by the excessive and often premature prominence placed on translational research. The discoveries that have had a major influence on drug development, medicine, and human health have typically originated from addressing the fundamental problems that are relevant to medicine, a point elegantly pointed out by Goldstein and Brown in a recent perspective on a golden era of Nobel laureates. The 9 Nobel Laureates who trained at the National Institutes of Health between 1964 and 1972 made their discoveries working on fundamental mechanisms. The point is not to negate a major, if not the overarching, purpose of biological sciences, which is to cure the human diseases. The point is that to cure a disease, it is essential to understand the fundamental mechanisms that govern its pathogenesis. Excessive and premature emphasis on translational research has the risk of diminishing the opportunities for fundamental discoveries, and hence, the ultimate cure of human diseases, as elegantly elaborated by physician-neuroscientist Huda Zoghbi in a recent editorial in Science. Without such fundamental understanding, translational research might simply emulate the metaphoric blind men and an elephant.

Figure 1. Number of the original articles, editorials, and reviews published in the Biomedical Journals on ACE2 since 2000, when the original cloning of the ACE2 gene was reported in Circulation Research.

Since its inception 60 years ago, the Editors-in-Chief of Circulation Research have been visionary leaders who have advocated publication of the fundamental scientific discoveries in the cardiovascular field. By scanning the list of published articles in the category of human molecular genetics, one identifies a number of articles that merit to be considered Classics. Among such articles, perhaps the discovery of the angiotensin-converting enzyme (ACE2) is noteworthy and might be categorized as a Classic. Since the first report of its cloning and partial characterization in 2000 in Circulation Research, 955 original, editorial, and review articles about ACE2 have been published (Figure 1), and the original article by Donoghue et al has been cited nearly 700 times as of March 2013. Although it is probably too premature to judge the full impact of this discovery on the practice of medicine, it has, nonetheless, changed our simplistic vertical concept about the renin-angiotensin-system (RAS) by pointing out the presence of dual functions of the RAS with opposing effects in cardiovascular biology.

The scientific background for cloning of the human ACE2 by Donoghue et al and Tipnis et al was the strong evidence for the presence of functionally active angiotensin (Ang) 1-7 in the brain. However, the enzyme(s) responsible for the generation of Ang 1-7, presumably through cleavage of Ang I and Ang II, were not fully known. Donoghue et al cloned the human ACE2 from a human heart failure ventricular cDNA library and showed that it was predominantly expressed in the endothelium in the heart, the kidney, and less so in the testis. Sequence analysis suggested that ACE and ACE2 exhibited 42% amino acid identity and had evolved through gene duplication. Despite sequence identity, however, ACE2 has a distinct role in generating Ang 1-7 and less so, Ang 1-9 than ACE. Although ACE generates Ang II (Ang 1–8) from Ang I, ACE2 cleaves Ang II (1–8) to generate heptapeptide Ang 1–7 (Figure 2). The latter acts as a ligand through its recently identified receptor MAS1, which is a G-protein–coupled receptor. Binding of Ang 1–7 to MAS1 receptors activates the phospholipase C signaling pathway and leads to a number of effects that are opposite to activation of the type 1 receptors by Ang II type I receptor, such as smooth muscle relaxation, hypotension, and protection against hypertrophy and fibrosis. Thus, ACE2/Ang 1–7/MAS1 pathway provides for an endogenous counter-regulatory mechanism within the RAS, which

Figure 2. Dual functions of the renin–angiotensin system with opposing effects on cardiovascular biology. Renin converts angiotensinogen to angiotensin (Ang) 1, which is then converted by the converting enzyme angiotensin-converting enzyme (ACE) to Ang II. Ang II through its type 1 receptor Ang II type I receptor (AT1R) exerts deleterious effects on the cardiovascular system. ACE2 converts Ang II to a heptapeptide Ang 1–7, which through its receptor MAS1 counters the deleterious effects of ACE/Ang II/AT1R pathway.

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balances the deleterious effects of the ACE/Ang II/Ang II type I receptor axis.

Since its cloning 13 years ago, ACE2 has emerged as an important regulator of the cardiovascular system, albeit the full spectrum of its functions has yet to be recognized. Given the role of the RAS in various physiological and pathological processes in multiple organs, the discovery and characterization of the ACE2 pathway might have implications well beyond cardiovascular medicine. Perhaps, deletion of the Mas1 gene, coding for the Ang 1-7 receptor, or the Ace2 gene provides glimpses, at least in the mouse, into the biological significance of the ACE2/Ang 1-7 MAS1 axis in the cardiovascular system (reviewed in Santos et al12). Mice deficient in MAS1 or ACE2 exhibit cardiac systolic dysfunction, increased blood pressure, myocardial interstitial fibrosis, endothelial dysfunction, susceptibility to intravascular thrombosis, metabolic abnormalities, and various other biological abnormalities that regulate the cardiovascular system.12–14 The discovery of ACE2 has also provided the impetus for investigating potential clinical use of the MAS1 receptor agonists in the prevention and treatment of various cardiovascular pathological states ranging from hypertension to atherosclerosis. Efforts are underway to harness the potential preventive and therapeutic effects of MAS receptor agonists in several cardiovascular diseases. Notwithstanding the significance of the discovery of the ACE2 gene, targeted deletion of the Ace2 gene in the mouse have led to conflicting data on the effects of ACE2 on certain cardiovascular phenotype.14–16 Although the initial report identified ACE2 as a major regulator of cardiac function,14 subsequent studies in 2 independent Ace2 knockout mouse models showed no discernible effect on cardiac function or the blood pressure at the baseline15,16 (reviewed in Gurley and Coffman17). In contrast, these studies identified ACE2 as a major regulator of Ang II metabolism in vivo and, consequently, the response of cardiac hypertrophy to pressure overload and the blood pressure to infusion of Ang II.15,16 Collectively, the studies in the Ace2 knockout mouse models showed that ACE2 contributes to cardiovascular physiology and pathology through its role in hydrolyzing Ang II and to some degree through synthesis of Ang 1-7.15 Given the broad spectrum of the biological effects of the RAS and the potential clinical significance of balancing the deleterious effects of the ACE/Ang II/Ang II type I receptor pathway by ACE2, through degradation of Ang II and generation of Ang 1-7, one might catalog the discovery of the ACE2 pathway an endogenous negative regulator of the RAS system as a Classic.

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Disclosures
None.

References
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