Letter To The Editor

Nobel Prize in Physiology or Medicine for the Year 2007: Breakthrough in Pathophysiology and Experimental Therapy of Cardiovascular and Other Diseases

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In the year 2007, Professor of Human Genetics and Biology Mario Capecchi from University of Utah, USA, Professor of Mammalian Genetics Martin J. Evans from School of Biosciences, Cardiff University, Wales, UK, and Professor of Pathology and Laboratory Medicine Oliver Smithies from University of North Carolina, USA shared the Nobel Prize in Physiology or Medicine for developing a way to eliminate or "knockout" specific genes in mice.[1,2] Joint efforts of these distinguished scientists allowed the creation of a variety of animal models to study pathophysiology and experimental therapy of mono- and polygenic human diseases, including cardiovascular diseases.

The scientists employed specific gene modifications in embryonic stem cells in mice. Exchange of DNA sequences within chromosome pairs increases genetic variations in the population. It takes place by a process called homologous recombination. Mario Capecchi and Oliver Smithies proposed that homologous recombination could be used to modify genes in mammalian cells. Capecchi demonstrated that homologous recombination could occur between introduced DNA and chromosomes. He showed that defective genes might be repaired by homologous recombination with the incoming DNA. Smithies tried to repair mutated genes in human cells. He postulated that certain inherited blood diseases could be cured by treating mutations in bone marrow stem cells. He discovered that endogenous genes could be targeted. This suggested that genes could be accessible for modification by homologous recombination.

On the other hand, the cell types initially studied by Capecchi and Smithies could not be used to create gene-targeted animals. For this purpose, another cell type which could give rise to germ cells was required. In other words, inherited DNA modifications were necessitated.

Martin Evans worked with mouse embryonal carcinoma cells. Although these cells originate from tumors, they have the capacity to differentiate into a variety of cell types. He suggested using embryonal carcinoma cells as vehicles to introduce genetic material into the mouse germ lines. His early attempts had been unsuccessful because embryonal carcinoma cells carried abnormal chromosomes which prevented their contribution to germ cell formation. On the other hand, Evans searched for alternatives and discovered that cells with normal chromosomes obtained from mouse embryos could be cultivated. These cells were embryonic stem cells.

The next step was to investigate the contribution of embryonic stem cells to the germ line. Embryos of one mouse strain were injected with embryonic stem cells from another mouse strain. Then, these mosaic embryos were carried by surrogate mothers and delivered. The mosaic offspring was grown and subsequently mated. The presence of embryonic stem cell-derived genes was investigated in the 2nd generation and they were present in the newborns. It gave rise to the understanding of gene inheritance according to Mendel's laws. In his further experiments, Evans modified the embryonic stem cells, using retroviruses capable of integrating their genes into chromosomes. Evans used embryonic stem cells to generate porter mice carrying new genetic material.

In 1986, the first gene-targeted embryonic stem cells were created. Capecchi and Smithies demonstrated that genes can be targeted by homologous recombination in cultured cells. The first reports on homologous recombination in embryonic stem cells used to generate gene-targeted mice were published in 1989. Since then, the number of knockout mouse strains has increased drastically. Gene targeting became a highly challenging issue, and it is now possible to introduce mutations that can be activated at specific time

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points or in specific cells or organs.

Importantly, gene targeting is a basis for understanding the role of genes in mammalian fetal development. Capecchi clarified the role of genes involved in mammalian organ development and indicated possible causes of several congenital malformations. Evans applied gene targeting in several experimental models of human diseases, particularly cystic fibrosis, and tested effects of gene therapy. Smithies used gene targeting to develop mouse models for cystic fibrosis, thalassemia, diabetes, cardiac fibrosis and hypertrophy, hypertension, and atherosclerosis.[3-5] He provided a genetic basis for the so-called inflammatory theory of atherosclerosis by targeting genes encoding apolipoprotein E. It was shown that apoE-knockout homozygous mice could still spontaneously develop atherosclerosis in the absence of high fat and cholesterol diet. In a series of experiments, Smithies targeted candidate genes of hypertension coding angiotensinogen, aldosterone synthase, and atrial natriuretic peptide and clarified many hemodynamic and metabolic mechanisms of essential hypertension. In genetically modified mice lacking natriuretic peptide receptor A, the scientist developed a model of hypertension that expressed cardiac hypertrophy and sudden cardiac death. Thus, he proved that multifactorial cardiovascular diseases can be explored by studying knockout mice.

In conclusion, gene targeting in mice has been a major focus in most fields of medicine over the past decades. It is obvious that a better understanding of genes function throughout the whole life cycle, starting from embryonic stage, will provide clues for the treatment of a variety of human diseases with a complex pathophysiology and gradually progressive course (e.g. essential hypertension). Specific gene modification in mice has great implications for studying the effects of numerous biomarkers, particularly those involved in atherogenesis, endothelial dysfunction, and heart failure (e.g. C reactive protein, natriuretic peptide, angiotensin II, etc.). Although most experimental achievements of the past decades are not immediately applicable to clinical practice, one should appreciate beginning of an era of studies aimed at the clarification of genotype-phenotype associations in humans based on our understanding of experimental models of diseases.

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