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Induced Mutant Animal Models for Studying the Genetics of Hypertension and Atherosclerosis

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ABSTRACT *: Gene targeting allows precise, predetermined changes to be made in a chosen gene in the mouse genome. To date, targeting has been used most often for generation of animals completely lacking the product of a gene of interest. Models of essential hypertension have been produced by mutated genes relating renin angiotensin system. The most significant contribution to understanding the genetic etiology of essential hypertension is probably the demonstration that discrete alterations in the expression of a variety of different genes can individually cause changes in the blood pressures of mice, even when the mice have all their compensatory mechanisms intact. These effects are readily detected in animals having moderate decreases in gene function due to heterozygosity for gene disruptions or modest increases due to gene duplication. As a species the mouse is highly resistant to atherosclerosis. However, through induced mutations it has been possible to develop lines of mice that are deficient in apolipoprotein E, a ligand important in lipoprotein clearance, develop atherosclerotic lesions resembling those observed in humans. The atherosclerotic lesions in apoE-deficient mice have been well characterized, and they resemble human lesions in their sites of predilection and progression to the fibroproliferative stage. Other promising models are mice that are deficient in the low-density lipoprotein receptor. Considerable work still remains to be done in dissecting out in a rigorous manner the effects of alterations in single genes on the induction or progression of atherosclerosis and on the control of blood pressures. Perhaps even more exciting is the opportunity now becoming available to breed animals in which the effects of precise differences in more than one gene can be studied in combination.*

Key Words *: Hypertension, Atherosclerosis, Gene disruption, Gene duplication*

I. AN *AGT* GENE TITRATION AND COMPENSATION

Smithies and Kim (1994) began to develop the idea of studying quantitative changes in blood pressure related genes was published in early 1994 under the title "Targeted gene duplication and disruption for analyzing quantitative genetic traits in mice" (Fig. 1). They argued that the experimental analysis of complex quantitative genetic traits, including essential hypertension, should be greatly facilitated by being able to manipulate the expression of a gene in living animals without altering the nucleotide sequence, chromosomal location, or regulatory elements of the gene. Jeunemaitre *et al.* (1992) reported that the T235 variant of the human Angiotensinogen (AGT) gene co-segregated with hypertension and was associated with 20% higher plasma AGT levels than its allelic form (M235).

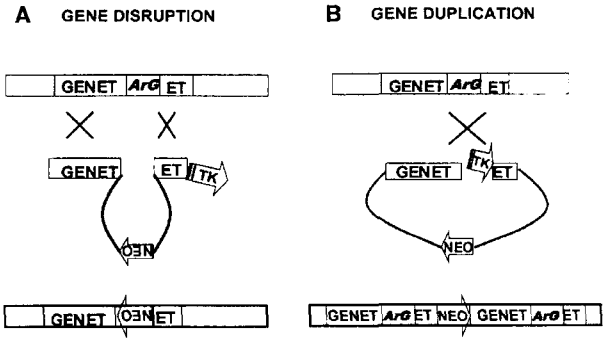

Fig. 1. The gene disruption of a locus via a generic replacement targeting event (A), and the duplication of a locus via a gap-repair targeting scheme (B).

To test whether variants of the mouse *Agt* gene that directly affect plasma AGT levels and also affect blood pressure, a two part targeting strategy was used to generate animals with plasma AGT levels raging from 0 to 145% normal levels. The strategy included conventional gene targeting to produce a disrupted *Agt*

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gene (Kim *et al.*, 1995) and a special form of gap-repair gene targeting to duplicate a wild-type *Agt* gene at its normal chromosomal location together with known or suspected controlling elements (Smithies and Kim, 1994). Suitable breeding of chimeras and F1 progeny derived from the targeted embryonic stem (ES) cells allowed generation of animals having 0, 1, 2, 3, or 4 copies of the wild-type. These animals had a highly satisfactory gradation of the steady-state plasma AGT levels, which spanned the relative levels observed in the human patients. This method of gene titration is applicable in principle to any gene and should provide a valuable tool for analyzing quantitative genetic traits by experiments in mice. Blood pressure measurements were made on F2 mice having the genetically controlled gradations in plasma AGT levels. A highly significant increase in blood pressures with increasing numbers of functional *Agt* genes was observed (Smithies and Maeda, 1995).

This study also provided clear evidence that the single-copy (*Agt* 1/0) animals had chronic homeostatic changes directed towards normalizing blood pressures, although this compensation failed to bring their blood pressures completely back to normal. Specifically, they had approximately twice normal plasma renin level, and their plasma AGT levels (35% normal) were less than the expected levels of around 50%. The animals had a chronic homeostatic increase in plasma renin leading to a higher than normal rate of conversion of AGT to AngiotensinI (ATI) and thence to AngiotensinII (ATII) by the action of angiotensin converting enzyme (ACE). No evidence was found for any compensatory up-regulation of the functional copy of the *Agt* gene in the 1/0, single copy animals. Plasma ACE levels were unaffected.

II. OTHER GENES IN THE RENIN/ ANGIOTENSIN SYSTEM

Ito *et al.* (1995) generated mice heterozygous (+/-) and homozygous (-/-) for inactivation form of the gene (*Agtr1A*) coding for the type 1A angiotensin II (ATII) receptor. There was no type 1 angiotensin receptor-specific binding of ATII in the kidneys of the -/- mutants, and approximately half normal levels in the heterozygotes. No evidence was found for any compensatory up-regulation of the single functional *Agtr1A* gene in the heterozygotes (+/-) animals. Pressor responses to infused ATII were virtually absent in the homozygous (-/-) mutants, and were altered in the heterozygotes (+/-). The homozygotes had a resting blood pressure 24 mmHg below normal, and the heterozygotes had pressure 12 mmHg below normal. It may conclude that altered expression of the *Agta1A* gene has marked effects on blood pressures. Thus a genetically determined decrease in expression of the type 1A receptor has comparable effects to those of Dup 753, a specific antagonist of type 1 receptors that is now under clinical trial as an antihypertensive drug.

The *Ace* gene is unusual although not unique in having an internal duplication that gives the enzyme two active centers. It is more unusual in having a testis-specific promoter that causes the gene to produce only the second half of the protein in large amounts in post-meiotic spermatogenetic cells. The gene targeting event used in this study disrupted the *Ace* gene on the 3' side of the testis promoter. Consequently, neither somatic nor testis *Ace* can be produced in the -/- animals. Krege's study (1995 an and b, 1996) showed that the -/- males had greatly reduced fertility; the -/- females were normally fertile. Further analysis of this fertility reduction is currently in progress. Blood pressures were about 33 mmHg lower in the -/- animals. Pressures in the heterozygous females did not differ from pressures in their +/+ sisters, but the pressures in male heterozygotes were significantly lower by 15~20 mmHg than pressures in their brothers.

III. STUDIES ON ENVIRONMENTAL FACTORS

John *et al.* (1995) carried out studies of the effects of dietary salt on animals heterozygous or homozygous for the *proANP* genes disruption. The gene *proANP* codes for the precursor of ANP, the arterial natriuretic peptide. Animals homozygous for the disrupted *proANP* gene had no circulating or arterial ANP and their blood pressures were elevated by 8 to 23 mmHg when fed standard (0.5% NaCl) and intermediate (2% NaCl) salt diets. The heterozygotes had normal amounts of circulating ANP and their blood pressures were normal when fed standard salt diets. However on high (8% NaCl) salt diets the heterozygotes were hypertensive, with blood pressures elevated by 27 mmHg. It

may conclude that genetically reduced production of ANP can lead to salt-sensitive hypertension. This finding that ANP modulates the blood pressure response to dietary salt should encourage the search for human genetic variants affecting the function of the ANP system. Detecting such variants might identify hypertensive patients likely to have benefit from a reduced salt intake.

IV. APOE DEFICIENCY-INDUCED ATHEROSCLEROSIS

In 1992, two laboratories used gene knockout technology to generate mice deficiency in apolipoprotein E (*ApoE*) (Zhang *et al.*, 1992; Plump *et al.*, 1992). *ApoE* which is a surface constituent of lipoprotein particles and a ligand for lipoprotein recognition and clearance by lipoprotein receptors. *ApoE* deficiency mice have delayed clearance of lipoproteins, and on a low cholesterol, low fat diet, their cholesterol levels reach 400 to 600 mg/dl as a result of accumulation of chylomyclon and very low density lipoprotein (VLDL) remnants enriched in esterified and free cholesterol. Notably, these mice develop not only fatty streaks but also widespread fibrous plaque lesions at vascular site typically affected human artherosclerosis. Lesions form at the base of aorta and the lesser curvature of the thoracic aorta; at the branch points of the carotid, intercostal, mesenteric, renal, and iliac arteries; and in the proximal colonary, carotid, femoral, subclavian, and brachiocepharic arteries. Lesions begin at 5 to 6 weeks of age with monocyte attachment to the endothelium in lesion-prone areas and transendothelial migration. Fatty streak lesions begin to appear at 10 weeks, and intermediate lesions containing foam cells and spindle-shaped smooth muscle cells appear at 15 weeks. Fibrous plaques appear after 20 weeks; these consist of a necrotic core covered by a fibrous cap of smooth muscle cells surrounded by elastic fibers and collagen (Fig. 2) (Ross, 1999). In some advanced lesions there is partial destruction of underlying medial cells with occasional aneurysm formation, and in others calcification occurs in the fibrous tissue. Extensive fibroproliferation can narrow the lumen, even to the point of occlusion of vessel. Complicated lesions characterized by thrombosis have not been found (Bleslow, 1996; Smith and Bleslow, 1997).

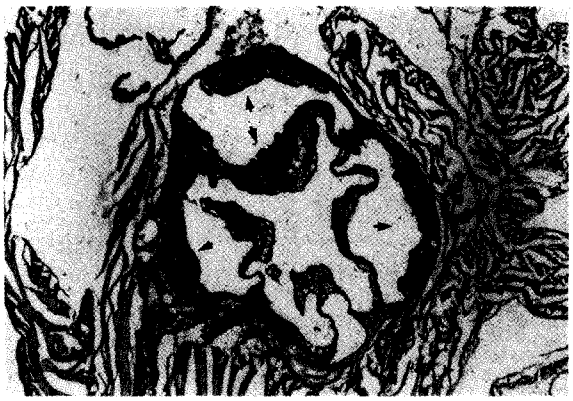

Fig. 2. Fatty streak lesions in aortic sinus of the ApoE-/- mice. Cross section of the aortic sinus from a 16-week-old male homozygous mouse fed the atherogenic diet for 8 weeks. Nine-micron-thick sections at the aortic sinus were stained for lipid with oil red O and counterstained with hematoxylin.

V. CONCLUSION

Considerable work still remains to be done in dissecting out, in a rigorous manner, the effects of alterations in single genes on the induction or progression of atherosclerosis and on the control of blood pressures. Perhaps even more exciting is the opportunity now becoming available to breed animals in which the effects of precise differences in more than one gene can be studied in combination. It would be overly optimistic to expect that all data from the mouse will directly apply to humans. Some species differences are to be expected, but it will be important not to dismiss unexpected findings too easily. Indeed, determining the basis of the unexpected findings with the help of mouse genetics may provide important insights into the causes and treatment of these complex multifactorial diseases. Finally, these mouse models can be used to test drugs that inhibit atherogenesis and protect hypertension.

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