Review Article

Roles of the WHHL Rabbit in Translational Research on Hypercholesterolemia and Cardiovascular Diseases

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Conquering cardiovascular diseases is one of the most important problems in human health. To overcome cardiovascular diseases, animal models have played important roles. Although the prevalence of genetically modified animals, particularly mice and rats, has contributed greatly to biomedical research, not all human diseases can be investigated in this way. In the study of cardiovascular diseases, mice and rats are inappropriate because of marked differences in lipoprotein metabolism, pathophysiological findings of atherosclerosis, and cardiac function. On the other hand, since lipoprotein metabolism and atherosclerotic lesions in rabbits closely resemble those in humans, several useful animal models for these diseases have been developed in rabbits. One of the most famous of these is the Watanabe heritable hyperlipidemic (WHHL) rabbit, which develops hypercholesterolemia and atherosclerosis spontaneously due to genetic and functional deficiencies of the low-density lipoprotein (LDL) receptor. The WHHL rabbit has been improved to develop myocardial infarction, and the new strain was designated the myocardial infarction-prone WHHL (WHHLMI) rabbit. This review summarizes the importance of selecting animal species for translational research in biomedical science, the development of WHHL and WHHLMI rabbits, their application to the development of hypocholesterolemic and/or antiatherosclerotic drugs, and future prospects regarding WHHL and WHHLMI rabbits.

1. Introduction

According to WHO, the major cause of death within member nations is cardiovascular diseases which account for about 30% of all deaths [1]. This report has indicated that cardiovascular diseases are one of the most important classes of diseases to be overcome. As main risk factors for cardiovascular diseases, hypercholesterolemia, hypertension, disorders in glucose metabolism, smoking, aging, male gender, and social stress are listed. Particularly, control of serum lipid levels is thought to be most important for the prevention of cardiovascular diseases. Currently, in the Japanese population, the upper limits of the normal ranges for serum total cholesterol and LDL cholesterol levels are 220 mg/dL and 140 mg/dL, respectively, and the lower limit of the normal range of HDL cholesterol is defined as 40 mg/dL [2]. According to

studies conducted during the 1980s, the incidence of cardiovascular events increases as the serum cholesterol level increases and decreases with hypocholesterolemic treatments [3]. One potent hypocholesterolemic compound is statin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, a rate-limiting enzyme in cholesterol synthesis. The first statin (compactin) was initially developed by a Japanese pharmaceutical company, Sankyo Co. Ltd. [4], and this accelerated the development of cholesterol lowering drugs. The hypocholesterolemic effect of compactin was initially examined with rats. However, the anticipated cholesterol-lowering effect was not observed [5], and the development of this compound was ceased. On the other hand, since compactin showed a potent inhibitory effect on cholesterol synthesis *in vitro* and in chickens, researchers had been looking for other mammalian species applicable for

the assessment of this agent. They found a report of a mutant rabbit strain showing hyperlipidemia, written in a Japanese university's bulletin [6]. This rabbit strain contributed greatly to the development of this compound. The strain was the Watanabe heritable hyperlipidemic (WHHL) rabbit. This was in 1979. Currently, there are seven statins in widespread clinical use. It is estimated that statins are prescribed to more than 40 million patients worldwide and statin therapy has decreased mortality from cardiovascular diseases by 20–50% [7]. Thus statins became essential agents for the treatment of hypercholesterolemia and cardiovascular diseases. These results demonstrate the importance of selecting animal species and/or animal models for translational research to develop therapeutic agents.

This review raises the importance of selecting animal species and/or animal models for translational research by describing the history of the WHHL rabbit and its contribution to studies of hypercholesterolemia and atherosclerosis.

2. The Development of the WHHL Rabbit and Its Characteristics

The history and characteristics of the WHHL rabbit were described in a previous article [8]. In 1973, Dr. Yoshio Watanabe (1927–2008) found one male Japanese white rabbit showing hyperlipidemia. From this mutant, he established a strain, the WHHL rabbit, after seven years of selective breeding. At first, this strain was designated the hyperlipidemic rabbit (HLR) [9]. He submitted a study on this strain to an international journal and renamed it the Watanabe heritable hyperlipidemic (WHHL) rabbit [10], according to a suggestion by the editor.

The strain has 300–700 mg/dL of total cholesterol and 300–400 mg/dL of triglyceride in plasma. There were atherosclerotic lesions in the aorta and xanthoma in the digital joints. The serum glucose level and blood pressure were in normal ranges. In WHHL rabbits, the function of low-density lipoprotein (LDL) receptors on the cell membrane was almost deficient and the clearance of LDL from the circulation delayed [11]. Such symptoms closely resemble human familial hypercholesterolemia (FH), which develops spontaneously, and thus the WHHL rabbit is recognized as the first animal model of this disease. Later, the Nobel Prize winners Goldstein and Brown used WHHL rabbits to verify their hypothesis of an LDL receptor pathway for the metabolism of lipoproteins and clarified human lipoprotein metabolism [12–15]. Their studies revealed that lipoprotein metabolism in the WHHL rabbit closely resembles human FH. Consequently, WHHL rabbits were used as an animal model for the development of cholesterol-lowering agents.

One of the most important features of an animal model for hyperlipidemia is the occurrence of myocardial infarction, the final event of human hypercholesterolemia. The development of severe atherosclerotic lesions in the coronary arteries is a prerequisite for the occurrence of myocardial infarction, but the incidence of coronary atherosclerosis in the WHHL rabbit was initially very low. To establish a new strain which develops coronary atherosclerosis, serial selective breeding was conducted and in 1985, the coronary atherosclerosis-prone WHHL rabbit was developed [16]. Further, a strain with severe coronary atherosclerosis was developed in 1992 [17]. Despite such long-term efforts, the incidence of myocardial infarction remained very low. After a further seven years of selective breeding with improved criteria, such as the use of descendents of rabbits with macrophage-rich coronary lesions, a new strain of WHHL rabbits was established; the myocardial infarction-prone WHHL (WHHLMI) rabbit that spontaneously develops myocardial infarction by progression of coronary atherosclerosis followed by occlusion of the coronary arteries [18]. The characteristics of WHHLMI rabbits are described in a previous review [19]. During their establishment, marked differences in the composition of atherosclerotic plaques were found between the aorta and coronary arteries [20], and the WHHLMI rabbit became an animal model with which to examine the inhibitory effects of drugs on coronary atherosclerosis. These studies suggested genetic factors other than hypercholesterolemia to be important to myocardial infarction and coronary atherosclerosis.

Figure 1 shows the changes in serum lipid levels with aging and the distribution of cholesterol in lipoproteins among WHHLMI rabbits [8]. Serum cholesterol levels are 900–1,400 mg/dL at weaning (3 months old) and at 6 months old, and then decrease gradually (700–1,200 mg/dL at 12 months old, 600–1,100 mg/dL at 18 months old, and 500–1,000 mg/dL at 24 months old). Serum triglyceride levels are 150–500 mg/dL and the change with aging is small. The HMG Co-A reductase activity (cholesterol biosynthesis) in WHHLMI rabbits does not decrease with aging and the precise mechanism of the age-related decrease in cholesterol is still unknown [21]. About 70% of cholesterol occurs in the LDL fraction, 16% in the very low-density lipoprotein (VLDL) fraction, 13% in the intermediate density lipoprotein (IDL) fraction, and 0.8% in the high density lipoprotein (HDL) fraction. Figure 2 shows the extent of atherosclerotic lesions in the coronary arteries and aorta of WHHLMI rabbits [8]. The main coronary artery is the left circumflex artery and the atherosclerotic lesion is more progressed compared to that in the left anterior descending artery and the right coronary artery. Therefore, the degree of coronary atherosclerosis (cross-sectional narrowing) has been evaluated using the left circumflex artery. The degree of aortic atherosclerosis was shown as the ratio of the surface lesion area to the lumen surface area of the aorta. Atherosclerotic lesions develop from 2 months old. At age 12 months, coronary cross-sectional narrowing was about 80% and about 60% of the aortic lumen surface was covered by atherosclerotic lesions. At 18 months old, coronary cross-sectional narrowing and aortic lesion increased to 90% and 80%, respectively [22].

Prior to the development of the WHHLMI strain, WHHL rabbits were used to investigate mechanisms of the development of atherosclerosis, and many aspects have been clarified: accumulation of oxidized LDL in the atherosclerotic lesions [23, 24]; antiatherosclerotic effects of antioxidants (inhibition of oxidized-LDL formation) [25, 26]; the expression of monocyte adhesion molecules on arterial endothelial
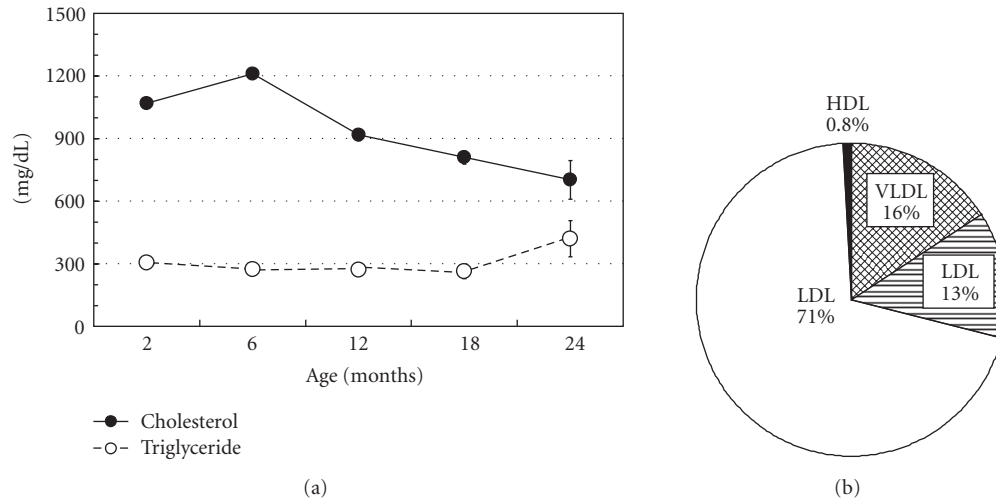
FIGURE 1: Changes in the serum lipid levels of WHHLMI rabbits with age (a), and the distribution of cholesterol in lipoproteins (b). Data are represented as the mean ± standard error of the mean. The serum cholesterol levels at 12 months old were about 900 mg/dL. Excess LDL cholesterol is atherogenic and HDL has antiatherogenic function. In WHHL rabbits, LDL is accumulated in the plasma and HDL-cholesterol is low, less than 20 mg/dL. The serum cholesterol levels decrease gradually with aging.
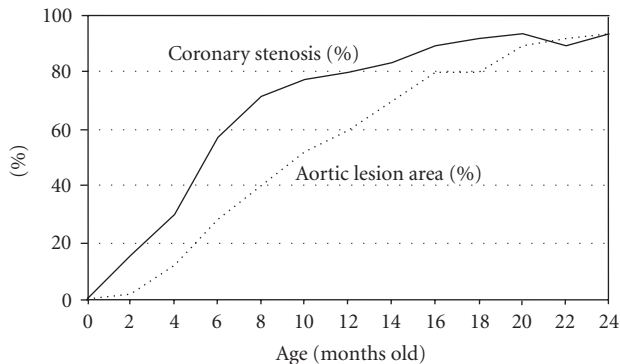

FIGURE 2: Development of atherosclerotic lesions in WHHLMI rabbits with age. The solid line denotes the degree of coronary atherosclerosis shown as coronary cross-sectional narrowing; lesion areas/area surrounded by the internal elastic lamina ×100 (%). The dotted line denotes the degree of aortic atherosclerosis; sum of the surface areas of the lesion/total surface area of the aortic lumen ×100 (%). Modified from Shiomi and Ito [8].

cells at the initiation of atherosclerosis [27]; scavenging of oxidized LDL at the lesions by macrophages through the scavenger receptors, VLDL receptors, and remnant receptors; accumulation of form cells derived from macrophages in arterial intima followed by further development of atherosclerotic lesions [28–32].

3. Species Differences in Lipid Metabolism and Atherosclerosis

As mentioned, lipoprotein metabolism in rabbits closely resembles that in humans. However, representative laboratory animals such as mice and rats have very different lipoprotein metabolism from that in humans (Table 1). Some examples of major species differences in lipid metabolism are the following. (1) In mice and rats, apoB editing enzyme is observed in the intestine and in the liver, but in humans and rabbits, this enzyme is expressed only in the intestine [33]. In humans and rabbits, apoB-48 is a major apolipoprotein of chylomicron and chylomicron remnants, which carry exogenous lipids derived from foods and apoB100 is a major apolipoprotein of VLDL, IDL, and LDL, which are endogenous lipoproteins derived from liver. In mice and rats, however, endogenous lipoproteins as well as exogenous lipoproteins also contain apoB-48, because of the expression of apoB editing enzyme in the liver [34]. Since the metabolic clearance of lipoproteins containing apoB-48 is very rapid, apoB-48 containing VLDL particles disappear rapidly from the circulation in mice and rats. As a result, the LDL lipid levels in mice and rats are very low compared with those in humans. (2) Hepatic lipase is circulating in the blood stream in mice thus different from humans in degradation of neutral lipids and transportation of free fatty acids into the tissues [35]. (3) In mice and rats, there is no cholesterol-ester transfer protein (CETP) activity in plasma, which transfers cholesterol from HDL to VLDL, IDL, and LDL [36], although CETP plays an important role in humans and rabbits. As a result, in mice and rats, the proportion of cholesterol in the HDL fraction is high compared with other lipoprotein fractions. Therefore, lipoprotein profiles of mice and rats are markedly different from that of humans, even in knockout mice lacking apoE or the LDL-receptors [8]. (4) Competitive inhibitors of a rate-limiting enzyme for cholesterol synthesis, statins, showed potent hypocholesterolemic effects in WHHL rabbits [37–45] but not in mice and rats [5]. In humans, statins are the most effective hypocholesterolemic drugs. These results demonstrate how it is important to choose appropriate species in translational research. (5) C-reactive

TABLE 1: Comparison of lipid metabolism, atherosclerosis, and cardiac functions between genetically modified mice and WHHLMI rabbits.

	Genetically modified mice	WHHLMI rabbits
Lipid metabolism		
Major lipoprotein in the blood	X (Chylomicron, VLDL)	O (LDL)
Structural protein in theendogenous lipoprotein	X (apoB48)	O (apoB100)
Expression of apoB editing enzyme	X (The small intestine, liver)	O (The small intestine)
CETP activity in the blood	X (No)	O (Exists)
Hepatic lipase	X (Released to circulation)	O (Bound to vessel membrane)
Atherosclerosis		
The coronary lesion	X (Resistant)	Δ (Spontaneously develops)
Composition of the lesions	X (Over accumulation of macrophages)	O (Various lesions)
VLDL receptor	X (no expression)	O (expression)
Heart		
Electrocardiogram		
Limb lead	X (Largely different waveforms)	O (Similar to humans)
Chest lead	X (Difficult to monitor)	O (Similar to humans)
Myocardial ion channel	X (I_{to} and $I_{K,slow}$)	O (I_{kr} and I_{Ks})
Myocardial fibers	X (α-myosin heavy chain)	O (β-myosin heavy chain)
Others		
Inflammatory markers	X (SAP)	O (CRP)
The hypocholesterolemic effect of statins	X (Resistant)	O (Effective)

O: similar to humans; Δ: partly similar to humans; X: largely different from humans.

protein (CRP), a major inflammatory marker in humans and rabbits, which increases in patients with acute coronary syndrome [46], is not responsive to inflammation in mice and rats, due to a lack of complement activation [47]. The major inflammatory marker of mice is serum amyloid P component (SAP), instead of CRP. (6) The types of myocardial fibers in mice are also different from those of humans and rabbits [48]. (7) Moreover, the ECG waveforms in mice and rats are clearly different from those of humans, but rabbit ECG shows similar waveforms to humans [49, 50]. As such, mice and rats have greatly different sets of factors for lipoprotein metabolism and cardiovascular diseases. Therefore, to employ mice and rats for studies on cardiovascular diseases and lipid metabolism, great care is required with analyses and/or the interpretation of the results obtained from experiments.

4. Translational Research on the Development of the Lipid-Lowering Agents

Figure 3 shows features of WHHLMI rabbits which resemble humans and applicable translational research fields. Since the WHHL rabbit is close to humans in lipoprotein metabolism, it was used for the development of various lipid-lowering agents and atherosclerosis-suppressing agents [8]. The hypolipidemic effects of various drugs have been investigated with WHHL rabbits (Table 2): cholesterol synthesis inhibitors, such as HMG-CoA reductase inhibitors and squalene synthetase inhibitors; inhibitors of microsomal triglyceride transfer protein, which works in the assembly of VLDL particles in liver; anionic exchange resins, which

block the enterohepatic circulation of bile acids; omega-3 fatty acids, which are a component of fish oil; fibrates, which lower serum triglyceride levels. In studies with a cholesterol synthesis inhibitor, statin, serum total cholesterol levels of WHHL rabbits were decreased dose-dependently by 10–30% compared with the control group [37, 39]. The mechanisms for the reduction in serum cholesterol levels by statins are an increase in expression of mRNA of LDL receptors in the liver [39] and, decrease in the excretion of VLDL cholesterol from the liver in cases of high-dose treatment [38]. The agents that inhibit squalene synthetase, another rate-limiting enzyme in cholesterol synthesis, also decreased the serum cholesterol level by similar mechanisms [51]. Since a small amount of LDL receptor protein can be processed from a precursor to a mature form in WHHL fibroblasts [52], inhibition of cholesterol synthesis in the liver is expected to cause LDL receptors to accumulate on the surface of hepatocytes. Anion exchange resins absorb bile acids at the duodenum and block the enterohepatic circulation [53]. As a result, cholesterol is utilized in the hepatocytes for the synthesis of bile acids, and then the hepatocytes, which was exhausted the cholesterol pool, increase the number of LDL receptor molecules to acquire external cholesterol [39]. Therefore, the combination of an inhibitor for cholesterol synthesis and an anion exchange resin can decrease the serum cholesterol level markedly, and this was proved using WHHL rabbits [40]. Since microsomal triacylglycerol transfer protein (MTP) inhibitors are also effective in WHHL rabbits [54], they may have potential benefit for human FH. The successful treatment in WHHL rabbits means that patients with FH, excluding the LDL-receptor negative type, can be treated with these agents.

TABLE 2: Drug development using WHHL/WHHLMI rabbits.

	Lipid-lowering effect	Lipid-lowering effect	
		Aorta	Coronary arteries
Cholesterol synthesis inhibitors			
Statins	O	X, O	O
Squalene synthesis inhibitor	O	O	O
Anion exchanger	O	O	
Statins + Anion exchanger	O	O	O
MTP inhibitor	O		
ACAT inhibitor	X, O	X, O	X, O
Antioxidants			
Probucol	O	O	
Vitamin E	X	X, O	
Colony stimulating factor			
MCSF	X, O	O	
GMCSF	X, O	O	
Apo E	X, O	O	
Fibrate	X		
Fish oils, ommega-3 fatty acids	X, O	X, O	
Thiazolidinedione	X	Δ	Δ
Thiazolidinedione + statin	O	O	O
Antihypertensive			
ACE inhibitor	X	O	
AT-II receptor antagonists	X	O	
Calcium antagonists	X	X	
Beta-blockers	X	X	
Gene therapy	O		

O: effective; Δ: partly effective; X: no effect.
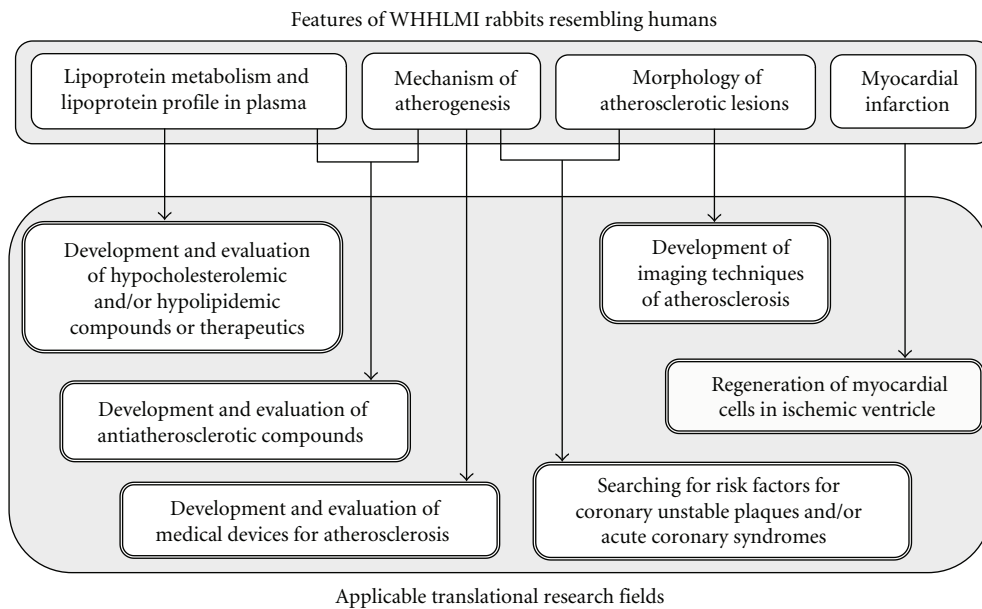Modified from Shiomi and Ito [8].

FIGURE 3: Features of the WHHLMI rabbit resembling humans and applicable translational research fields.

5. Translational Research on Antiatherosclerotic Effects

The purpose of lowering serum cholesterol levels is to inhibit atherogenesis and to circumvent the cardiovascular and cerebrovascular events. The WHHL rabbit contributed to prove the effects of cholesterol-lowering therapies on delaying the progression of atherosclerosis. Statin treatment resulted in a decrease in serum total cholesterol levels by 20–30%, and the cross-sectional narrowing of the coronary arteries was significantly decreased [41–45].

In several clinical studies, the incidence of cardiovascular events was significantly reduced in the statin-treated groups despite little or no improvement in coronary stenosis on evaluation by coronary angiography [55]. The WHHL rabbit contributed to the clarification of this paradoxical mechanism [42–45]. On the administration of statin to 10-month old WHHL rabbits for one year, in which coronary atherosclerosis had already developed to a mature stage, statin treatment showed not only the prevention of further progression of the coronary atherosclerotic lesions, but also various stabilizing effects on coronary plaques, such as reductions in the contents of macrophages and extra cellular lipids in lesions, and increase in the contents of collagen fibers and preservation of the smooth muscle cells in lesions. Thus it was clarified that, statin administration makes atherosclerotic lesions more stable, that is, less likely to rupture. With this study, it was confirmed that the stabilization of atherosclerotic lesions is important for the prevention of coronary events. Nowadays, more than 40 million patients worldwide are prescribed statins. Another type of cholesterol synthesis inhibitor, squalene synthesis inhibitors, that act downstream of the cholesterol synthesis pathway, also showed similar hypocholesterolemic and atheroma-stabilizing effects in WHHLMI rabbits [56].

Using WHHLMI rabbits, antiatherosclerotic effects have also been evaluated with other compounds such as omega-3 fatty acids, which decrease serum triglyceride levels by changing the composition of fatty acids [57–61]; antioxidants, such as probucol, vitamin C, and vitamin E [62–65]; agents that regulate the function of macrophages [66, 67]; drugs that inhibit the rennin-angiotensin pathway [68–71]. Interestingly, antiatherosclerotic effects of antihypertensive agents were unequal in WHHL or WHHLMI rabbits. Angiotensin converting enzyme (ACE) inhibitors and angiotensin-II receptor blockers (ARBs) showed antiatherogenic effects [69–72], but calcium antagonists and beta-blockers were not effective [73, 74]. Systolic blood pressure in WHHL and WHHLMI rabbits is 100–120 mmHg, which is slightly higher than normal [75]. This may be why calcium antagonists and beta-blockers did not show distinct antiatherosclerotic effects. In contrast, antihypertensive effects of ACE inhibitors and ARBs are mediated by suppressing the effects of angiotensin II. Angiotensin-II stimulates atherogenesis by impairing the function of arterial endothelial cells, proliferation of arterial smooth muscle cells, and inflammation [76]. These pleiotropic effects of angiotensin-II are considered to be mediated by reactive oxygen species. Thus, the WHHL rabbit is indispensable for studies on the antiatherosclerotic effects of the various compounds.

6. Imaging Technology for Evaluation of Atherosclerotic Lesions

Although it is important to evaluate drug efficacy in clinical use, it is difficult to evaluate atheroma-stabilizing effects of drugs in clinical practice. With coronary angiography, it is possible to see the degree of stenosis but difficult to evaluate the severity of lesions, if the lesions are spread and extended in the coronary arteries, or if the coronary arteries are expanded due to the outward remodeling of the vessels. Furthermore, it is very important to develop noninvasive technologies and equipment to detect dangerous lesions, that is, vulnerable plaques that are prone to rupture, not only for the diagnosis but for the prevention of cardiovascular events. As vulnerable plaques that cause cardiovascular events, soft-type plaques rich in macrophages and large lipid droplets covered with a thin fibrous cap are important. To detect such soft-type plaques, computed tomography (CT) [77], positron emission tomography (PET) [77], CT plus PET [78], magnetic resonance (MRI) [78, 79], and intravascular ultrasound (IVUS) [80] have been applied to WHHLMI rabbits. One successful example was evaluation of the antiatherosclerotic effect of probucol, a potent antioxidant, in WHHLMI rabbits by imaging with CT plus PET [81]. Ogawa et al. demonstrated clearly that imaging with CT plus PET is powerful technology to detect antiatherosclerotic effects of compounds. Once imaging technologies for the evaluation of atherosclerotic lesions are established, they can be used not only for the assessment of drug effects, but also for the detection of dangerous coronary lesions that could lead to cardiovascular events such as acute coronary syndromes and consequently the prevention of ischemic heart diseases.

7. Perspectives

To overcome cardiovascular diseases, many research issues remain unresolved, despite diligent studies for the development of diagnostic methods and lipid-lowering agents. Particularly important is clarifying the mechanism of the disruption of coronary lesions (arterial plaque rupture and the following formation of a thrombus), which depress the trigger for the onset of acute coronary syndromes, and establishment of treatments. Still no suitable animal model, which is compatible with the study of human acute coronary syndromes, has been developed. To develop a suitable animal model for human acute coronary syndromes, trial studies/experiments such as the enhancement of vulnerable coronary lesions, and application of physical pressure to coronary lesions, are currently underway with WHHLMI rabbits. To destabilize coronary lesions, serial selective breeding with new criteria such as the formation of vulnerable plaques is also ongoing, in parallel with the development of genetically modified WHHLMI rabbits overexpressing matrix metalloproteinases (MMPs), and so forth. The established strain would be a subject of analyses for the identification of

the genes/loci responsible for the phenotype established. In the near future, with advances in gene-targeting technologies by using ES or iPS cells capable of germ-line transmission, in combination with the nuclear transfer technique, more precise manipulation of the rabbit genome may also be available. Since the lesion composition and severity of coronary lesions differ even in WHHLMI rabbits, despite no difference in the serum cholesterol levels, it will be important to explore marker proteins and/or risk factors affecting coronary lesions. Once markers and risk factors relating to vulnerable coronary atheromas are found, the mechanism of cardiovascular events may be clarified. Such findings would contribute to the development of new clinical diagnostics and thence to the prevention of cardiovascular events.

In conclusion, selecting appropriate animal model is important in translational research. WHHL and WHHLMI rabbits have contributed to development of hypocholesterolemic and antiatherosclerotic compounds and medical devices, such as imaging technologies for atherosclerosis, and diagnostic techniques for acute coronary syndromes, in addition to elucidation of the mechanisms of atherogenesis and coronary plaque rupture. These studies are helpful for progression of therapeutics.

Acknowledgments

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