Editorial

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Rho-Associated Kinase 2 Polymorphism of Vasospastic Angina in Korean Population

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Rho-associated kinases (ROCKs), the immediate downstream targets of RhoA, are ubiquitously expressed serine-threonine protein kinases, which are involved in diverse cellular functions, including smooth muscle contraction, actin cytoskeleton organization, cell adhesion and motility, and gene expression. Recent studies have shown that ROCKs may play a pivotal role in cardiovascular diseases, such as vasospastic angina, ischemic stroke, and heart failure.

Rho-associated kinases are important regulators of cellular apoptosis, growth, metabolism, and migration via control of the actin cytoskeletal assembly and cell contraction.[1] ROCKs phosphorylate various targets and mediate broad range of cellular responses that involve in actin cytoskeleton in response to GTPase-RhoA. ROCKs increase myosin light chain (MLC) phosphorylation through phosphorylating the myosin binding subunit on myosin light chain phosphatase (MLCP) and inhibition of MLCP.[2] In addition, MLC is one of the major downstream target proteins of ROCKs. ROCK2 phosphorylates Ser^{19} of MLC, the same residue that is phosphorylated by MLC kinase. Thus, ROCK2 may increase cellular contractility via dual effects on MLC kinase and MLCP. Indeed, ROCK2 can alter the sensitivity of vascular smooth muscle cell (VSMC) contraction, in response to the changes in Ca^{2+} concentration.[3] Based on the above experiments, we can speculate that an increased activity of ROCKs can be one of the important pathophysiologic mechanisms of vasospastic angina, and fasudil, a ROCK inhibitor has exerted beneficial effect on VA.[4]

Rho-associated kinases could regulate other cellular functions that are independent of their effects on the actin cytoskeleton. For examples, Rock inhibits insulin signaling, reduces cardiac hypertrophy and involves an in tissue differentiation from adipocyte to myocyte.

In addition to ROCK's effect on actin cytoskeleton system, inhibitory effect on endothelial nitric oxide synthase (eNOS) activity can be another mechanism involved in the pathogenesis of vasospasm. Enothelium derived NO plays an important role in the regulation of vascular tone, inhibition of platelet aggregation, and the suppression of smooth muscle cell proliferation. Increased bioavailability of NO is, in part, dependent on eNOS activity. Although various conditions and factors, such as laminar shear stress, oxygen tension, and transforming growth factor β-1, can regulate eNOS expression by transcriptional level, eNOS expression can be regulated at the post-transcriptional level. Chronic hypoxia,[5] tumor necrosis factor-α,[6] thrombin,[7] oxidized low density lipoprotein-cholesterol,[8] and cellular proliferation[9][10] are known to decrease eNOS messenger ribonucleic acid (mRNA) stability. Chronic hypoxia and cellular proliferation are known to activate RhoA and ROCKs,[11] thus, RhoA/ROCK inversely regulates eNOS expression, through an alteration in the eNOS mRNA stability. The RhoA/ROCK pathway may also be important in the regulation of eNOS activity. The regulation of eNOS activity can occur via eNOS phosphorylation. The phosphorylation of Ser1177 of eNOS leads to the rapid activation of eNOS by fluid shear stress, insulin, estrogen, bradykinin, and vascular endothelial growth factor.[12][13] Interestingly, inhibition of RhoA or ROCKs lead to the rapid activation of PI3K/Akt and phosphorylation of eNOS,[14][15] which suggests the potential role of ROCKs in the regulation of eNOS activation, in addition to the eNOS expression.

In the mammalian system, ROCKs consist of two isoforms, ROCK1

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and ROCK2. ROCK1 and ROCK2 are ubiquitously expressed in mouse tissues, from early embryonic development to adulthood. ROCK1 mRNA is preferentially expressed in the lung, liver, spleen, kidney, and testis, whereas, ROCK2 mRNA is highly expressed in the heart and brain.[16)17)]

In this issue of the journal, You et al.[18)] performed a novel study to evaluate the relationship between ROCK2 genetic polymorphism and VA in Korean the population. To my knowledge, this is the first study regarding ROCK2 gene polymorphism in Korean population. As the authors described, VA is a multifactorial disease, which has a diverse pathogenetic mechanisms, such as VSMC hypersensitivity, endothelial dysfunction, increased oxidative stress, and etc. In addition, smoking and heavy alcohol drinking is considered to be important risk factors. VA is generally considered to be a benign coronary artery disease, and most of the patients are treated well with calcium channel blocker and coronary vasodilator. However, some patients have trouble in ameliorating chest pain and severe VA can cause sudden cardiac death. Considering the function of ROCKs, already discovered in cellular and molecular researches, it could be expected to give us substantial information to perform genetic study, regarding Rho-ROCK pathway in VA patients.

According to this study, the genotype prevalence of all five interesting SNPs (rs978906, rs2271621, rs2230774, rs1515219 and rs3771106) of ROCK 2, in the VA group, was not significantly different from that in the control group. In haplotype analysis, G-T-C-T-G haplotype was found to be significantly more prevalent in the control group than in the VA group. These findings could have some clinical implications.

First of all, there were already some reports[19-21)] regarding eNOS polymorphism in coronary vasospasm. If this study result is combined with that of the previous genetic studies, the VA patients can be treated based on each patient's genotype. For example, the patients who have ROCK2 gene alteration might be treated with ROCK inhibitor, such as fasudil, and patients, who have eNOS gene alteration and decreased eNOS activity, might be treated well with l-arginine or nitrate. In a previous report, L-arginine improved symptoms and quality of life in the patients with eNOS T-786C mutation which was more frequently detected in VA than control group.[20)] However, the authors did not perform functional studies. Therefore, it remains uncertain how this G-T-C-T-G halotype prevents coronary vasospasm. Testing vasodilatory responses to acetylcholine, l-arginine or various coronary vasodilators in different genetic haplotypes might be helpful to discriminate the function of ROCK2 gene alteration.

Second, according to this study, the haplotype G-T-C-T-G in ROCK2 gene was significantly different in frequency, between the VA and the control group. However, this haplotype was detected only 3.45% in the control group and 0% in the VA group and the prevalent genetic haplotypes, consisting of more than 95% of study population, did not show any significant difference in haplotype distribution between the VA and the control group, whereas, other studies regarding eNOS genetic polymorphism have reported mutations having 25%[21)] to 50%[20)] of frequencies in VA population. In other words, the authors could not find high risk genetic mutations or haplotypes, which can be a major determinant of the VA pathogenesis. Therefore, we might speculate that other genetic alterations or environmental factors are probably more closely related to VA than ROCK2 genetic polymorphism is, and the studies regarding other SNPs of ROCK2 gene might be required to understand the exact role of ROCK2 genetic polymorphism in VA patients.

This study investigated the genotype difference between VA patients and that of the normal controls. In the clinical perspective, VA is not difficult to diagnose and the detection of high risk patient is important. The investigation of genetic polymorphism, between refractory VA and usual VA, would be more useful because most of the patients are treated well with the conventional coronary vasodilatory drugs, but the refractory patients whose genotype is not favorable for VA might require additional therapy targeting genetic alterations.

In spite of several limitations, this article suggested the association of genetic polymorphism of ROCK2 and VA in Korean population which has never been reported before. More large population study including functional study is needed to clarify and approve this hypothesis before clinical application.

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