Public Abstract
First Name:Huang
Middle Name:
Last Name:Huang
Adviser's First Name:Gerald
Adviser's Last Name:Meininger
Co-Adviser's First Name:
Co-Adviser's Last Name:
Graduation Term:FS 2017
Department:Physiology (Medicine)
Degree:PhD
Title:Integrin Adhesion Response to Chemical and Mechanical Stimulation

It is well recognized that arterial stiffness increases with aging and aging-related diseases, such as hypertension. The mechanisms for the increase in stiffness have been largely thought to be the result of changes in the composition and structure of the extracellular matrix (ECM). However, recent evidence suggests that intrinsic mechanical properties of vascular smooth muscle cells (VSMCs) may also play an important role. The changes noted in VSMCs include an increase in cell stiffness and enhanced cell adhesion to the ECM protein fibronectin (FN). The stimuli that provoke these changes are not well known, nor are the underlying causes of these changes. In addition, previous work from our laboratory revealed that there is coordination between cell stiffness and cell adhesion to FN of VSMCs treated with vasoactive agents. VSMCs adhesion to ECM is largely mediated by the transmembrane receptors, integrins, which provide a physical connection between the cytoskeleton and ECM proteins. This unique molecular axis allows integrins to act as an ideal transducer for initiating signaling from both outside-in and inside-out signaling pathways. Integrin-mediated cell adhesion is known to play an important role in VSMCs normal function and it is also involved in various pathological conditions. Despite the growing body of evidence for the importance of integrins in vascular function and dysfunction, there are gaps in our knowledge concerning how integrin adhesion is linked to changes in VSMC mechanical properties and how integrin adhesions respond to dynamic mechanical stimulation. Therefore, my overall research goal was to better understand integrin adhesion behavior in VSMCs response to cellular and mechanical stimuli. Atomic force microscopy (AFM) was used to measure VSMC mechanical properties and adhesion to ECM as well as to provide a tool for applying mechanical stimulation to the VSMC.
The first part of this research focuses on clarifying the mechanism of coordination between VSMC stiffness and adhesion to FN. We hypothesized that enhanced cell adhesion to FN is mediated by changes in the level of intracellular calcium ([Ca2+]i). To test this hypothesis, confocal imaging of fluo-4, a fluorescent calcium indicator, combined with AFM force spectroscopy were used simultaneously to record levels of [Ca2+]i and force-distance curves to measure VSMC mechanical properties and adhesion. The cell mechanical properties and adhesion to FN were correlated with levels of [Ca2+]i. KCl and BAPTA-AM were used to modulate the level of [Ca2+]i. KCl-treated VSMCs showed a rapid transient increase in cell stiffness as well as cell adhesion to FN, and these two events were synchronized with the superimposed transient increase in the level of [Ca2+]i. In contrast, VSMCs incubated with an intracellular calcium chelator, BAPTA-AM, exhibited decrease in stiffness and cell adhesion to FN as well as reduced levels of [Ca2+]i. These findings suggest that in VSMCs integrin activation is linked to the level of [Ca2+]i. Further studies with ML-7 pretreated cells to inhibit myosin light chain kinase showed KCl induced changes were not abolished, suggesting that calcium-induced integrin activation is not dependent on mechanical events associated with contraction or signaling events downstream of contraction.
In the second part of my research integrin adhesion behavior was studied in VSMC focal adhesions subjected to oscillating mechanical stimulation. VSMCs from the aorta, a large elastic conduit artery, exposed to cyclic strain stress induced by heart rate-associated changes in pulse pressure, were selected for study. We worked together with applied mathematician scientists from the University of Nottingham. Through collaborative discussions, they developed a mathematical model to predict interactions between integrins and ECM during dynamic changes in mechanical stretch. In this study, my goal was to provide biological data to test and inform the model. We used the AFM with FN-coated probes and measured

VSMC adhesion to the FN by applying vertically oscillating stretch to integrin focal adhesions. Our experimental data provided evidence to support model predictions that changes in the degree of mechanical stretch applied to an integrin adhesion would behave in a bistable manner. The bistability was manifest as a breakpoint or failure point at which integrin adhesions rupture and reform. The simulation model and experimental data indicate that the bistable behavior occurs during intermediate amplitude stretches between full detachment and no detachment. The data also indicated that the failure point for adhesion was dependent on the initial conditions of the adhesion and influenced by whether the adhesion was pre-existing or newly formed. These data suggest this bistability behavior could be an indication of a unique switch point in the nature of integrin signaling.

In conclusion, this research has provided new information on integrin adhesion in response to inside-out cellular stimulation and outside-in dynamic mechanical stimulation. These data indicate the involvement of a calcium-related signaling pathway in VSMC integrin activation. In addition, these data show unique integrin adhesion behavior in response to a dynamic vs static physical environment. It is clear from this work that further studies will be needed to develop a whole picture and to understand the functional and pathological implications of mechanisms coordinating integrin adhesion with cell mechanical properties and the dynamic behavior of integrins.