William L Willis
PhD Candidate

“YB-1 Stress-Response Protein Conformation Implicated in Post-transcriptional Control of Myofibroblast Differentiation”

August 14
424 BRT
9:00am
VITA

6/20/1978 ................................. Born – W. Palm Beach, FL

2004 ....................................... BSc., Chemistry The Ohio State University

2006-present ............................ Graduate Research Associate, The Ohio State University

COMMITTEE MEMBERS

Arthur R. Strauch, PhD

Mark T. Ziolo, PhD

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ABSTRACT

Differentiation of stromal fibroblasts into myofibroblasts is critical for wound healing and tissue repair. Normally a transient process, chronic myofibroblast activation is the leading cause of hypertrophic scarring, loss of tissue compliance, and dysfunctional tissue remodeling. Vascular smooth muscle α-actin (SMαA) is an indicator of myofibroblast differentiation, as well as one of several fetal contractile protein isoforms re-expressed in adult cardiomyocytes in response to mechanical stress-injury. The stress-response protein, Y-box binding protein-1 (YB-1) binds SMαA mRNA and regulates its translational activity. Our central hypothesis is that YB-1 drives maladaptive SMαA expression in injury-activated myofibroblasts by modulating the packaging, delivery, and translational efficiency of its cognate mRNA. In a mouse model for cardiac fibrosis, we observed that accumulation of fetal SMαA protein in cardiac sarcomeres was associated with accumulation of punctate YB-1 deposits which localized to perinuclear regions as well as polyribosome-enriched cytosol proximal to cardiac intercalated discs. Samples from both fibrotic mouse hearts as well as SMαA positive endomyocardial biopsies from human heart transplant patients were enriched with high molecular weight, heat-denaturing resistant YB-1 oligomers migrating in the range of 100-250 kDa during reducing SDS-PAGE. Notably, YB-1 oligomers exhibited selective affinity for an exon-3 derived translation silencer sequence in SMαA mRNA. Presence of p180 YB-1 oligomers in endomyocardial biopsies increased with SMαA protein expression and graft age, suggesting that in addition to monomeric YB-1 p50, p180 oligomers may be a preferred YB-1 size variant for storing/protecting fetal mRNA transcripts during myocardial remodeling.

Based on these intriguing observations, which suggested that YB-1 oligomer formation may be associated with the packaging and translation control of SMαA mRNA, we examined the regulatory
aspects of YB-1 oligomerization using a model system based on isolated human pulmonary fibroblasts. Activation of SMαA gene expression in human pulmonary myofibroblasts by TGFβ1 was associated with formation of denaturation-resistant YB-1 oligomers with selective affinity for the SMαA exon-3 translation-silencer sequence. We discovered that YB-1 is a substrate for the protein-crosslinking enzyme transglutaminase 2 (TG2) that catalyzes calcium-dependent formation of covalent γ-glutamyl-isopeptide linkages in response to reactive oxygen signaling. TG2 transamidation reactions using intact cells, cell lysates, and recombinant YB-1 revealed covalent crosslinking of the 50 kDa YB-1 polypeptide into protein oligomers that were distributed during SDS-PAGE over a 75 kDa to 250 kDa size range. In vitro YB-1 transamidation required nanomolar levels of calcium and was enhanced by the presence of SMαA mRNA. YB-1 crosslinking was inhibited by (a) anti-oxidant cystamine, (b) the reactive-oxygen antagonist, diphenyleneiodonium, (c) competitive inhibition of TG2 transamidation using the aminyl-surrogate substrate, monodansylcadaverine, and (d) transfection with small-interfering RNA specific for human TG2 mRNA. YB-1 crosslinking was partially reversible as a function of free-calcium concentration and TG2 enzyme availability.

Metabolic stress incurred during tissue injury may also promote conversion of resident fibroblasts to the myofibroblast phenotype, as temporary loss of tissue perfusion promotes a hypoxic, energy-deficient pro-oxidative cellular microenvironment. Stimulation of AMPK activity with AICAR activated TG2 transamidation and induced the formation of high molecular weight YB-1 oligomers with enhanced affinity for the SMαA mRNA exon-3 translation-silencer sequence. We found that AMPK and peroxide differentially regulated phosphorylation of the YB-1 cold-shock domain, which modulates YB-1 subcellular localization and SMαA mRNA binding efficiency. AICAR suppressed YB-1 phosphorylation, which prevented nuclear translocation and activated SMαA mRNA binding. In contrast, peroxide stimulation activated Erk/MAPK dependent phosphorylation of the YB-1 cold-shock domain and caused the dispersal of YB-1: SMαA mRNA complexes. Thus, we propose

AWARDS AND HONORS


FUTURE PLANS

Bill plans to pursue a post-doctoral position in cardiac and skeletal muscle biology.
RECENT PUBLICATIONS


RECENT ABSTRACTS AND PRESENTATION


7. William Willis, Seetha Hariharan, Arthur R. Strauch. Dynamic Interplay of Smooth Muscle α-Actin Gene-