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Mechanical Stretch Induces Activation and Nuclear Translocation of Nuclear Factor-Kappa B (NF-κB) in Cardiac Fibroblast.

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NF-κB is a redox-sensitive transcription regulator consisting of hetero- and homo-dimers of the Rel family. In quiescent cells, NF-κB resides in the cytoplasm in a latent form bound to its inhibitor proteins, IκBs. Activation of NF-κB is preceded by phosphorylation of IκB by IκB kinases (IKKs), which targets the protein for ubiquitination and degradation by the 26S proteasome. Degradation of IκB unmasks the nuclear localization signal of NF-κB resulting in rapid translocation into the nucleus. NF-κB reaches maximal nuclear localization by 30-40 minutes following initial cell activation (Fig. 1). In the nucleus, activated NF-κB binds to specific consensus DNA sequences leading to the upregulation and expression of genes under NF-κB transcription control. NF-κB is activated by diverse agents including cytokines, viruses, immune modulators, and is considered to be oxidative responsive.

The kinetics of nuclear translocation of NFκB following activation by stress (mechanical or chemical) is analyzed by using indirect fluorescence immunochemical methods. All images used for analysis are captured at equal exposure times. The location, distribution and relative fluorescence intensity of cytoplasmic and nuclear NFκB is determined by using integrated morphometry and analysis dialog of MetaMorph (Universal Imaging) software. Briefly, the area and integrated optical density (IOD) are measured by individually thresh-holding the cytoplasmic and nuclear fluorescence of the p65 subunit of NFκB (p65- NF-κB), which is immunochemically labeled with FITC. Using the software's optical calipers, the measurements are refined by setting specific boundary conditions for area and IOD for signal acceptance from p65-NF-κB and eliminating non-specific fluorescence. The same boundary conditions are applied to all samples.

In response to 5% equilbiaxial, mechanical stretch, NF-κB in cardiac fibroblast undergoes activation and translocation into the nucleus (Fig.2). The magnitude of NF-κB activation in response to stretch is about 50% of that observed when cardiac fibroblasts are maximally activated by tumor necrosis factor–alpha (TNF-α, positive controls, Fig.3). The kinetics of NF-κB nuclear translocation in response to stretch is slower than that in the positive controls. Interestingly, two of the key cytostolic activators of membrane associated NAD(P)H oxidase, p47phox and Rac-1 are also activated by mechanical stretch undergoing morphological alterations and rapid translocation (completed within 5 minutes following activation) from the perinuclear cytoplasm to the peripheral cytoplasm and cell margins.

These data suggest that NF-κB in cardiac fibroblasts is activated and undergoes nuclear translocation in response to mechanical stretch. Additionally, stretch appears to activate the subunits of NAD(P)H oxidase suggesting that reactive oxygen species (ROS) may play a role NF-κB activation in cardiac fibroblast. Activation of NF-κB and expression of NF-κB-dependent genes may represent an early required event leading to phenotype transformation in cardiac fibroblast.

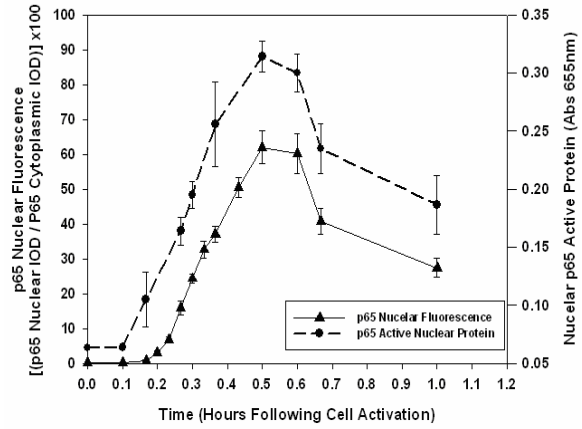

Figure 1. Typical maximal nuclear translocation response kinetics of NF-κB following activation in endothelial cells and fibroblast. Appearance of NF-κB in the nucleus is determined by measurement of the IOD fluorescence of the FITC-labeled p65 subunit and confirmed by measurement of the active p65 subunit in the nucleus by ELISA.

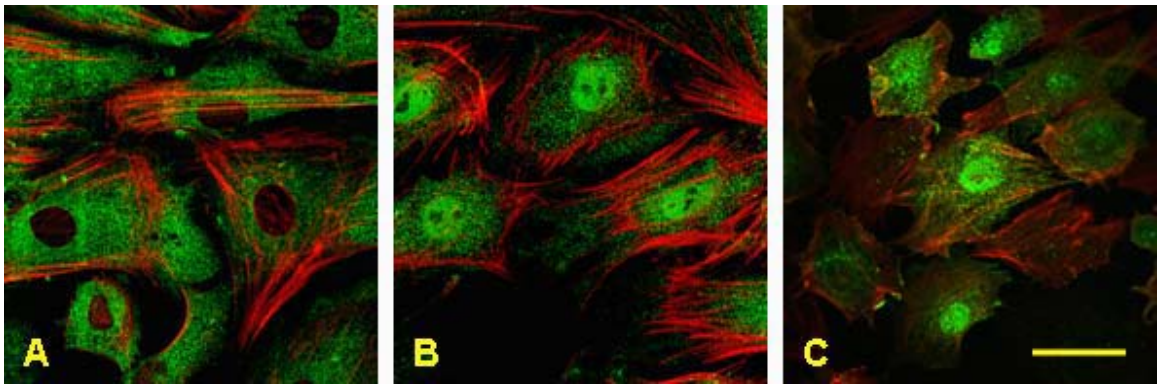

Figure 2. Activation of NF-κB in cardiac fibroblast by TNF-α and mechanical stretch.
A. Quiescent cardiac fibroblast, NF-κB is localized in the cytoplasm.
B. Cardiac fibroblast activated by TNF-α, NF-κB undergoes maximal nuclear translocation.
C. NF-κB is activated by mechanical stretch alone in cardiac fibroblast.
Green- p65-NF-κB. Red- Actin. Scale Bar = 50μm

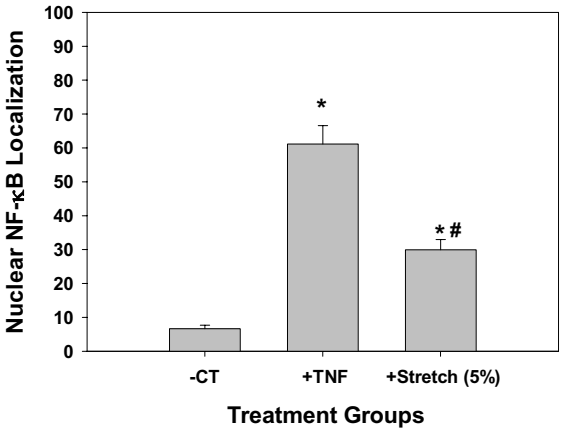

Figure 3. Mechanical stretch alone induces NF-κB activation and nuclear translocation in cardiac fibroblast which is about 50% of that seen following maximal activation by TNF-α.
* =Significant difference from the untreated control (-CT).
# = Significant difference from the maximal activated positive control (+TNF[20ng/ml]-60 minutes)

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