Signaling Pathways in Uterine Smooth Muscle Cells

Romana A. Nowak, Department of Animal Sciences, University of Illinois, Urbana, IL 61801.

Uterine leiomyomas, or fibroids are characterized by an increase in proliferation of smooth muscle cells (SMCs) and an increase in production of specific extra-cellular matrix (ECM) proteins primarily collagens type I and type III. Leiomyomas share a number of similarities with other pathological conditions such as keloids, renal fibrosis, pulmonary fibrosis and restenosis following angioplasty. All of these conditions involve abnormal proliferation and collagen deposition by mesenchymally derived cells such as fibroblasts, SMCs and mesangial cells. Most of these pathologic conditions arise in response to some type of injury such as the physical denudation of the vascular endothelium that occurs during angioplasty, a wound or surface burn of the skin, or chronic exposure to inhaled particles in the lung. We hypothesize that leiomyomas develop in the uterine myometrium as a response to injury in a manner similar to what occurs during the formation of SMC lesions in the vasculature. We believe that the injury may occur as local ischemia or hypoxia during the time of menstruation, injury due to the presence of bacterial or other pathogens, or perhaps foreign material in the uterus such as talc.

An important observation for all of the fibrotic diseases and pathologies mentioned above is that the increase in cell proliferation by SMCs or fibroblasts is clearly linked to an increase in collagen production, particularly collagen type I. Our group and others have shown that collagen type I and type III mRNA and protein are upregulated in uterine fibroids. This upregulation of expression does not appear to depend on ovarian steroid hormones since leiomyomas from women who had received gonadotropin-releasing hormone agonist (GnRHa) treatment for three months still showed elevated levels of collagen type I and type III mRNAs when compared to autologous myometrium. Transforming growth factor-ß is a growth factor that has been shown to regulate collagen production by leiomyoma SMCs and its expression is upregulated in both leiomyomas and vascular SMC lesions. In light of the many similarities between leiomyomas and other fibrotic pathologies we have begun to focus more closely on the intracellular signaling pathways utilized by growth factors in leiomyoma SMCs and comparing these to what is already known in other fibrotic cells.

Reactive oxygen species (ROS) are some of the newest additions to the family of second-messenger molecules. Although one ROS, nitric oxide ($NO^{\cdot}$), has been known for years to serve as a signaling molecule by activating guanylate cyclase, it has only recently become apparent that other ROS can alter the function of specific proteins and enzymes as well. Virtually all types of vascular cells including SMCs produce O_2^- and H_2O_2. In addition to mitochondrial sources of ROS, O_2^- and H_2O_2 can be derived from xanthine oxidase, cyclooxygenase, lipoxygenase, NO synthase, and NADPH oxidases. Several investigators have shown that these latter enzymes, the membrane-associated NADPH oxidases are the primary physiological producers of ROS in vascular cells including SMCs. While it has been known for years that these ROS and RNS are produced at high levels in cells undergoing oxidative stress, only recently have we come to understand that ROS and RNS play an important role as signaling molecules in a variety of normal physiological processes. Much of this recent work has focused on vascular SMCs and in particular how ROS act as signaling molecules for growth factors coupled to tyrosine kinases, inflammatory cytokines, and ligands tranduced by G-protein coupled receptors. The major source of oxygen intermediates in the vascular SMCs is nonphagocytic NADPH oxidase, which is regulated by vasoactive agents (angiotensin II, endothelin-I, serotonin), cytokines (interleukin-1, tumor necrosis factor-alpha), growth factors (PDGF, EGF,

TGF-β) and mechanical factors such as sheer stress. The importance of a rise in ROS within SMCs in response to specific ligands was first demonstrated several years ago in a study by Sundaresan et al. They showed that in vascular SMCs the addition of PDGF produced a rapid rise in ROS levels. This ROS production peaked within minutes of ligand addition and returned to baseline after approximately 30 minutes. By increasing the intracellular levels of the peroxide scavenging enzyme catalase, they were able to block the ability of PDGF to induce a rise in intracellular ROS. Blocking the rise in intracellular ROS in response to PDGF had a profound inhibitory effect on the level of overall tyrosine phosphorylation and, in particular, on the activation of specific mitogen-activated protein kinase (MAPK) pathways. Subsequent studies using different cell types, different ligands, and different strategies to inhibit ROS levels or production resulted in similar interpretations, namely that the ligand-stimulated production of ROS is essential for downstream signaling. Several of the growth factors that have been shown to increase intracellular ROS production as part of their intracellular signaling pathways including PDGF, EGF and TGF-β are known to be important in the regulation of leiomyoma SMC proliferation and differentiation.

Our laboratory is investigating the role of reactive oxygen species (ROS) as signaling molecules in the signaling pathways for PDGF and EGF in leiomyoma and myometrial SMCs. We are utilizing primary cultures of these cells for our studies. The cells are loaded with dihydroethidium dye, an intracellular dye that fluoresces upon oxidation by ROS. Time course and dose response studies have shown that both PDGF and EGF cause a detectable increase in ROS production in leiomyoma and myometrial SMCs within 2-5 minutes. Furthermore, treatment with exogenous H2O2 causes an increase in DNA synthesis in leiomyoma SMCs. Treatment of cells with growth factor in the presence of the ROS inhibitor diphenyleneiodonium chloride blocks the increase in proliferation in response to PDGF.

The goal of our studies is to gain a better understanding of how leiomyomas develop and what signaling pathways are involved in the regulation of proliferation and collagen production by uterine SMCs. Reactive oxygen species appear to be an important component of these pathways. Further studies are underway to evaluate anti-fibrotic compounds to determine whether these act by inhibiting the ROS signaling pathway in leiomyoma SMCs.

References:

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