Crosstalk between transforming growth factor-β3 and microRNA-29c in leiomyoma: are we stepping forward?

Uterine fibroids (UFs) are benign monoclonal neoplasms, representing the most common solid tumor of the female genital tract in reproductive-aged women. Despite the incidence being generally high worldwide (70%–80% in women by the age of 50 years), UFs affect $\leq$80% of women in some particular populations (African-American women). Furthermore, UFs are relative common in pregnancy and might negatively influence its outcomes (1). Uterine fibroids are generally asymptomatic; however, when symptoms occur, they might impair normal functioning and affect quality of life, most widely causing abnormal uterine bleeding and iron deficiency anemia, abdominal and pelvic pain, and compression to the surrounding organs thus urologic and gastrointestinal symptoms. However, being typically encountered during the reproductive age, UFs may also have a detrimental effect on women's fertility. Although submucosal UFs certainly affect fertility, intramural UFs more than a certain size (>4 cm) may have a negative effect on early pregnancy outcomes. With regard to the subserosal UFs, the effect on fertility is still on debate. Different mechanisms have been proposed to explain this effect. Except for the obvious alteration of uterine structure, some investigators (1, 2) have been focusing on functional changes of the myometrium and endometrium, and the interfering with the endocrine and paracrine signals that play paramount roles in gametes recognition and encounter, embryo tubal migration, and nidation and its development. These interferences could be related to the recent conception that UFs arise from an inflammatory background in genetically predisposed women. Several uterine irritations, such as ovulation, menstruation, infections, tissue injury (cesarean section, uterine surgery, surrounding peritoneal irritation), talc use, intrauterine device (IUD), male reproductive proteins, stress, mechanical forces, hypoxia, and cellular oxidative stress, may have been the initial triggers for cellular and extracellular modification in UFs formation. Changes in the extracellular matrix (ECM) is a marked sign of UFs. As it occurs in all fibrotic disease, histopathology analyses report an increasing number of smooth muscle cells, collagen, proteoglycans, and fibronectin. These triggers can promote monocytes and macrophages migration and activation and secondarily, the release of growth factors (transforming growth factor [TGF]-β, activin-A, and platelet-derived growth factor) and cytokines (tumor necrosis factor [TNF]-α). This leads to fibroblast activation and differentiation into myofibroblasts, a process that is increased by steroid hormones (estrogen [E] and P). During chronic inflammation, myofibroblasts become resistant to elimination by apoptosis and produce excessive amounts of ECM components, leading to fibrotic transformation. The ECM accumulation is supported not only by growth factors, cytokines, steroid hormones, but also by the interaction between cells and modified ECM, affecting cell behavior and producing epigenetic modifications (2). Among the three main epigenetics mechanisms in modulating the gene expression in fibroids formation, and more specifically DNA methylation, histone modifications and microRNAs (miRNAs) interfering, the last seems to have a pivotal role in UFs formation and maintenance. Among miRNAs (miR-29 family, miR-200c, and miR-93/106b) that have been shown to affect the regulation of ECM accumulation, the miR-29 family seems to have a central role. All members of these microRNAs are down-expressed in UFs, acting on a different level of the interaction between cells and ECM. Down-expression of miRNA-29a and miRNA-29b supports the accumulation of collagens, including COL1A1 and COL1A3, whereas down-expression of miR-29c is associated with high expression of COL3A1, elastin, and matrix metalloproteinases (3). Consistent data have established that TGF-β and its family of ligands induce abnormal proliferation in UF cells but not in healthy myometrial cells. This action depends on its concentration and the high levels recorded in fibrotic tissues that are able to promote tumor growth. Particularly, TGF-β3 isoform is up to five times more expressed in UFs than in surrounding healthy tissues. Through activation of Smad 2/3 and ERK 2/3 signaling pathways, TGF-β3 increases messenger RNA expression of ECM components and decreases the production of matrix metalloproteinases (2, 3). Because TGF-β3 is a target of miR-29c and both promote fibrosis, Chuang and Khorram (4) in an in vitro study cultured cells obtained from UFs and surrounding normal myometrium. They determined not only the expression of miR-29c and TGF-β3, but also the mechanisms of their reciprocal regulation in healthy and UFs tissues. They showed that miR-29c expression was lesser whereas TGF-β3 was increased in UFs compared with paired healthy myometrium. Interestingly, no significant racial/ethnic differences in expression of miR-29c and TGF-β3 was noted. This is in contrast with the epidemiological data where UFs are more common and have more morbidity in African-American women, suggesting the presence of other factors that interact at the paracrine/endocrine and epigenetic levels. More remarkable, the investigators demonstrated that miR-29c directly interacts with the 3′ untranslated region of TGF-β3 thereby regulating its expression in UF cells. Gain-of-function of miR-29c in UF cells down-regulated the expression of TGF-β3 at protein and messemger RNA levels, whereas knockdown of miR-29c, using anti-miR-29c, had the opposite effect. Culturing UF cells with TGF-β3 (5 ng/mL) for 48 hours, Chuang and Khorram (4) also highlighted how the expression of miR-29c was inhibited. Assuming the epigenetic effect of miR-29c on these cells, they evaluated whether TGF-β3 had an effect on epigenetic enzymes involved in DNA and histone methylation (DNMT1, DNMT3A, and EZH2). They demonstrated that TGF-β3 induced DNMT1 expression, but had no significant effect on DNMT3A and EZH2 expression.

Interestingly, the DNMT1 suppression resulted in suppression of TGF-β3 and increase of miR-29c expression. Thats study (4) pointed out the presence of a crosstalk between growth factors and miRNAs interfering at the epigenetic level. However, this is one of the possible mechanisms of crosstalking, opening new perspectives of similar interactions. For instance, an important aspect that should be investigated is the E and P roles in modulating these possible crosstalkings. New evidence demonstrated that ulipristal acetate (UPA), a selective P receptor (PR) modulator with proven anti-UF effect changes the ECM production and matrix metalloproteinase expression. To better understand this mechanism, Lewis at al. (5) analyzed the effect of UPA on TGF-β3 canonical and noncanonical signaling pathways in UFs. They proved that UPA affects ECM protein production through the attenuation of transcription and translation of TGF-β3. After UPA treatment significant reductions in canonical pathway through the TGF-receptor I and II/Smad-dependent cascade were recorded, whereas no inhibition of noncanonical (MEK1 and MEK2 signaling) pathway was noted. They (5) also saw a dose-dependent reduction of some ECM proteins, including collagen 1 after 72 hours of UPA treatment. However, there is no evidence how the activation of Smad 2/3 leads to upregulation of collagen 1 gene in UFs because at present, no Smad response elements have been identified within the promoter of this gene. Several in vivo and in vitro studies (3) have shown that miR-29 regulate the messenger RNAs encoding ECM proteins, such as collagen type I, alpha 1 and 2, collagen type III alpha 1, elastin, and fibrillin 1. A similar crosstalk model between miR-29 family and other growth factors, hormones, and paracrine factors could be taken into account to elucidate better the fibrotic mechanisms behind UF formation and growth and, for instance, the interracial differences, as noted in the literature.

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