ABSTRACT

Introduction

Uterine fibroids (UF) are the most common benign pelvic tumors in women. Progesterone (P4) plays a crucial role in their pathogenesis. UFs consist of changed myometrial cells (MYO) and abnormal extracellular matrix (ECM), which major components are collagens. The tumor growth factor β (TGF-β) signaling pathway plays an important role in the regulation of ECM production. Until recently, selective progesterone receptor modulator – ulipristal acetate (UA) has been used to treat patients with UFs, but its mechanism of action is still poorly understood.

Aim

The goal of the study is to assess the effect of P4 and UA on the expression of collagens in the extracellular matrix and to investigate whether the TGF-β signaling pathway mediates the P4 and UA mechanism of action by regulating the collagens production in uterine fibroids.

Material and methods

In this study expression of collagens and TGF-β pathway genes were investigated in myometrial tissues (N=50), uterine fibroids tissues from patients not-treated with UA (N=100) and uterine fibroids tissues from patients treated with UA (N=50). Additionally, experiments were carried out on cell and explant cultures of the myometrium and uterine fibroids. RT-PCR was used to evaluate the expression level of collagen genes - *COL1A1*, *COL1A2*, *COL3A1*, *COL4A4*, *COL4A5*, *COL5A1*, *COL5A2*, *COL5A3*, *COL11A1* and TGF-β pathway genes –*TGF-β1*, *TGF-β3*, *TGF-βR1*, *TGF-βR2*, *SMAD2* and *SMAD3*. Immunocytochemistry and immunohistochemistry were used to determine the localization of SMAD3 protein. The concentration of produced proteins was measured by the ELISA method.

Results

Increased expression of *COL1A1*, *COL3A1*, *COL5A1*, *TGF-β1*, *TGF-β3*, *SMAD2*, *SMAD3* was observed in UF not-treated with UA in comparison to normal myometrium. In UF treated with UA expression of *COL1A1*, *COL5A1*, *TGF-β1*, *TGF-β3*, *TGF-βR2* and *SMAD3* was lower than in UF not treated with UA. In UF cell and explant cultures, P4 stimulation caused an increase of *COL1A1*, *COL3A1*, *COL5A1* expression and UF cells viability. OU stimulation decreased the *COL1A1*, *COL4A4* and *COL5A1* expression levels as well as UF cell viability. In myometrial explant cultures, no differences between stimulated groups and controls were observed. In UF cell cultures UA decreased the *TGF-β1*, *TGF-β3*, *TGF-βR2* and *SMAD3*

expression levels. Next, the measurement of TGF-β1 and TGF-β3 concentration was performed. In UF cell cultures the production of TGF-β1 and TGF-β3 was higher than in myometrial cells. UA stimulation decreased the TGF-β1 and TGF-β3 levels compared to control. Immunolocalization of SMAD3 protein demonstrated cytoplasmic and nuclear SMAD3 localization in not-treated UF, whereas mainly in the cytoplasm in UA treated UF. In immunocytochemical studies, SMAD3 was localized only in the cytoplasm in UA treated UF cells. To better characterize the role of the TGF-β pathway in UF we used TGF-βR1/R2 and SMAD3 inhibitors. SMAD3 and TGF-βR1/R2 inhibitor suppressed UF cells viability up-regulation induced by P4. TGF-βR1/R2 inhibitor increased the negative action of UA on cell viability, but iSMAD3 did not change the UA action. In UF cell cultures stimulated with P4 in presence of iSMAD3 statistically significant decrease in *COL1A1* expression was found in comparison to cells stimulated only with P4. There was no difference in *COL1A1* expression in UF cells stimulated only with UA or UA with iSMAD3. The measurement of proCOL1A1 alpha concentration showed lower production in UF cells after UA stimulation compared to the control and P4 group.

Conclusions

P4 stimulates the growth of UF by increasing the UF cell viability and expression up-regulation of some ECM genes via activation of the TGF-β signaling pathway. UA may inhibit this pathway and therefore UA treatment leads to a decrease in ECM protein production.