Locally Produced Estrogen Metabolites Regulate Airway Smooth Muscle Proliferation

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Rationale: Airway remodeling due to increased airway smooth muscle (ASM) mass is one of the hallmark features of asthmatic airways. Clinical data reveals the higher prevalence of asthma in women compared to men, suggesting the importance of sex steroids especially estrogen in asthma. Previously, we reported differential effects of 17β-estradiol (E_2) towards estrogen receptors (ERs) with ERβ as a protective while ERα showing a detrimental role in regulating ASM proliferation. However, it is not clear whether these differential effects are due to E_2 itself or through its local metabolites produced by inherent ASM cytochrome-P450 (CYP's) during asthma and/or inflammation. Considering these facts, we hypothesize a change in CYP's expression and activity during asthma/inflammation, results in a differential E_2 metabolites profile and their net effect modulates ASM proliferation.

Methods: Asthmatic and non-asthmatic primary ASM cells (male/female) were isolated from human lung tissue (Mayo Clinic, IRB-approved), cultured in DMEM-F12. The expression and activity of ASM CYP's (CYP1A1, 1B1, and 3A4) with/without cytokines (TNFα) were evaluated using Western, RT-qPCR, and Glo-luminescence assays. Further to confirm the effect of TNFα, we measured the activity of CYP1A1 and CYP3A4 in the presence of NFκB inhibitor, SN50. To assess the role of CYP's in regulating overall effects of estrogen metabolites on ASM proliferation, cells were treated with CYP1A1 inhibitor (Rhapontigenin) and CYP3A4 inhibitor (PF49) prior to E_2 (1nM) exposure with/without PDGF. Additionally, we used CYP's specific siRNA for molecular inhibition of CYP's in the ASM cells, followed by E_2 treatment with/without PDGF and proliferation rates measured using MTT and Bright-field assays.

Results: We observed higher expression and activity of CYP3A4 in asthmatic ASM compared to non-asthmatics. TNFα exposure increased the expression of CYP3A4 in non-asthmatic ASM cells, with no changes observed in CYP1A1 and CYP1B1. Further, TNFα exposure increased the activity of CYP3A4, CYP1A1 and CYP1B1 in asthmatic and non-asthmatic ASM cells, with greater change in 3A4 in asthmatics; effects of TNFα were inhibited by SN50. PF49 in presence of E_2 significantly inhibited PDGF-induced ASM cell proliferation, possibly due to decreased CYP3A4 mediated local production of E_2 metabolite 16αHE_2. Interestingly, Rhapontigenin with E_2 showed significant increases in ASM cell proliferation. These effects were further confirmed by molecular inhibition of CYP1A1 and 3A4 using specific siRNA with/without E_2.

Conclusion: Overall, these data show an alteration of ASM CYP's regulates the local production of estrogen metabolites and thereby dictating the net effect of E_2 in regulating ASM proliferation.

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