G-protein coupled estrogen receptor (GPER) and phosphofructokinase-2/fructose-2,6-bisphosphatase 3 (PFKFB3) are involved in estrogen-mediated angiogenesis

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Inhibition of angiogenesis by blocking phosphofructokinase-2/fructose-2,6-bisphosphatase 3 (PFKFB3), an activator of glycolysis, has already been reported (Schoors et al., 2014). Although, estrogen's role in promoting angiogenesis is well known, often these effects are tied to its action of nuclear hormone receptors estrogen receptor α (ERα) and/or ERβ (Arnal et al., 2010; Iwakura et al., 2006; Sanchez et al., 2011). The possible contribution of the membrane G-protein coupled estrogen receptor (GPER) to such responses, however, remains to be determined. We tested the hypothesis that the pro-angiogenic effect of 17β-estradiol (E2) is also mediated by GPER and involves PFKFB3. Using an in vitro system of human umbilical vein endothelial cells (HUVECs) we assessed the effects of E2, the GPER agonist G1 and antagonist G15 on PFKFB3 expression, endothelial cell migration and formation of tube-like structures. Treatment of HUVEC with E2 (10-100 nM) for 0.5-3h did not affect PFKFB3 mRNA levels. By contrast, E2 induced PFKFB3 protein expression in a time- (peak at 3h) and concentration-dependent manner as measured by Western blot. Similarly, the selective GPER agonist G1 (1-100 nM) enhanced the amount of PFKFB3 at 3 h while the GPER antagonist G15 (1 μM) blocked E2-mediated PFKFB3 expression. G1 treatment for 6 h also induced HUVEC migration using a microchemotaxis chamber and promoted wound healing. Furthermore, G1 promoted the formation of tube-like structures through Matrigel assay at 4 h, while the GPER antagonist G15 counteracted E2-mediated effects. Selective inhibitors of PFKFB3 (3PO and PFK15) abolished GPER agonist (E2 or G1)-mediated angiogenesis, indicating the role of GPER-PFKFB3. Finally, we tested the knockdown effect of GPER and showed that treatment with a GPER-specific siRNA reduced E2-mediated HUVEC migration, tube-like structure formation and PFKFB3 protein levels. In conclusion, E2 and G1 treatment increased PFKFB3 expression independent of mRNA transcription, and induced a pro-angiogenic effect which was reduced by selective GPER antagonist, indicating the involvement of GPER. These findings unravel a previously unrecognized mechanism of estrogen-dependent endocrine-metabolic crosstalk in HUVECs via GPER and may have implications in angiogenesis occurring in ischemic or hypoxic tissues.

References

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