SEX-DEPENDENT EXPRESSION AND FUNCTION OF eNOS IN HUMAN ENDOTHELIAL CELLS

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Atherosclerosis and cardiovascular disease (CVD) are classical diseases where sex/gender differences have been described. Clinical and epidemiological data show that CVD is less prevalent in women than men until midlife, and the female advantage is lost with menopause. An impaired endothelial function, characterized by an imbalance in endothelial Nitric Oxide Synthase (eNOS) activity, precedes and accelerates the development of CVD. However, whether there is any sexual dimorphism in eNOS activity and function in human endothelial cells (ECs) is still unknown. For that reason, we investigated eNOS expression and function in human male and female ECs obtained from umbilical cords. We found that female ECs expressed higher levels of eNOS mRNA and protein in comparison to male ECs. These differences were maintained when eNOS expression was analyzed in twin-derived ECs and ex vivo in ECs directly collected from cords. Searching for a biological significance for the increased female expression of eNOS, we compared proliferative and migratory capabilities of male and female ECs. Our results demonstrated that female ECs possessed higher migratory capabilities in comparison to male ECs. These properties showed a morphological relationship with the presence of lamellipodia, and a functional correlation with the localization of eNOS at the leading edge of polarized female ECs. Importantly, pharmacological and genetic modulation of eNOS activity showed that the enzyme is strictly required for migration only in female ECs. On the contrary, eNOS was not involved in the control of both male and female EC proliferation. Angiogenesis is the result of a coordinated process where the migration of specialized cells – the tip cells – and the proliferation of the so-called stalk cells both contribute to the growth of new vessels. Therefore, we evaluated in an in vitro 3-D angiogenesis assay the relative contribute of motility and proliferation to the male and female EC sprouting process. Strikingly, sprouting from spheroids relied mostly on eNOS-dependent migration in female ECs while it was the result of eNOS-independent proliferation in male ECs. This different behavior was confirmed in mixed spheroids where female ECs were located as tip cells in most of sprouts.

In conclusions, we found that female ECs constitutively express higher amount of eNOS than the male counterpart, and that this increase has important consequences on cell behavior. At variance with male ECs, female ECs show a greater motility and are strictly dependent on eNOS activity for migration and sprouting. Our study highlights some potential relevant sex-dependent differences in EC molecular and functional properties that should be further evaluated to develop more effective and precise sex-oriented strategies for the prevention and therapy of CVD.