The Effects of Age and Sex of Megakaryocyte Secreted Factors on Endothelial Cell Growth and Function

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Abstract: With an aging population, the risk of fractures and compromised healing increases. Angiogenesis and vasculogenesis are impaired with aging. Vascularization at the callus plays a significant role in bone healing, and we have previously shown the important role of megakaryocytes (MKs) in regulating bone healing. Notably, MK-derived conditioned media (CM) contains factors known to promote angiogenesis. Whether differences exist with aging and/or based on sex was the primary focus of this study. Here, we examined the effects of CM secreted from MKs derived from young (3-4-month-old) and aged (22-24-month-old) male and female C57BL/6J mice on bone marrow endothelial cell (BMEC) growth and function. Specifically, BMEC proliferation, vessel-like formation, wound/transwell migration, and RNA expression were examined. Both young and aged female MK CM saw a >65% increase in BMEC proliferation ($p<0.001$ and $p<0.05$, respectively). In addition, female MK CM, regardless of age, improved all four parameters of vessel-like formation by >115% ($p<0.05$). Likewise, young male MK CM increased vessel-like formation in all parameters by more than 160% ($p<0.001$). Although aged male MK CM resulted in higher vessel-like formation parameters, including significant >150% increases in the formation of nodes and meshes, 62% fewer vessel-like structures formed compared to that observed with young male MK CM treatment ($p<0.05$). Additionally, aged MK CM, irrespective of sex, improved transwell migration by over 2500% ($p<0.01$). On the other hand, aged female and male MK CM inhibited wound closure by 46% and 17%, respectively ($p<0.05$). RNA analysis found MK CM yielded significantly different levels of expression in CXCR4, CXCR2, CD36, CD74, PDGFRB, and TGFBRB not only relative to controls, but also between sexes and ages. Further testing to identify the mechanisms responsible for these age-associated differences may allow for novel treatment strategies to improve MK-mediated angiogenesis, vasculogenesis, and bone healing, particularly within the aging population.