University of Utah
UNDERGRADUATE RESEARCH JOURNAL

THE EFFECT OF DIFFERENTIAL RASGRP2 ISOFORM EXPRESSION ON MEGAKARYOCYTE ACTIVATION

Olivia Lee (Jesse Rowley's)
Department of Molecular Medicine

RASGRP2 is a critical signaling molecule in platelets, and defects in RASGRP2 result in impaired hemostasis. Preliminary studies in Dr. Rowley's lab identified multiple predominant RNA isoforms of RASGRP2 that are variably expressed between individuals, including isoforms with signficant retention of intron 5, an 80bp splice removal of part of exon 5, or both. However, the functional consequences of differential isoform expression to RASGRP2 and platelet function are unclear. Lentiviral vectors were created for 4 predominant transcript isoforms of RASGRP2 with a V5 c-terminal epitope tag. To test their effect on RASGRP2 protein expression, vectors were transfected into HEK-293 cells. Western blot was then used to detect the amount and size of RASGRP2 protein. The isoform with ORF + intron 5 had the greatest amount of protein expression. The isoform with ORF also displayed a strong signal of protein expression. However, isoform with ORF + intron 4 + intron 5 showed a significant decrease of protein expression when compared to intron with ORF and intron with ORF + intron 5. The isoform with ORF + Intron 5 -80bp in Exon 5, displayed the weakest signal of protein expression. Constructs were then used to create lentivirus to transduce CD34+ megakaryocytes. Vectors contained a GFP tag driven by a second promoter to mark transduced cells. Following transduction, megakaryocytes were activated with collagen related peptide (CRP) to induce αIIbβ3 activation and degranulation. Megakaryocytes were stained with antibodies for P-selectin and activated αIIbβ3. The amount of surface P-selectin and active αIIβ3 quantified on GFP+ cells using flow cytometry. Isoform ORF + intron 5 showed the greatest amount of activation. Isoform with ORF showed the second greatest amount of activation, followed by empty vector control. Meanwhile, isoform with ORF + Intron 5 -80bp in Exon 5 had a significant decrease in activation when compared to the other isoforms. From these results, we conclude that RASGRP2 transcript isoforms differentially affect RASGRP2 protein production, and potentially influence megakaryocyte activation. Future studies will assess whether RASGRP2 transcript usage differences may explain differences in platelet reactivity between individuals.