

Title: Unique microRNA Expression Patterns in Hematopoietic Stem and Progenitor Cells after Hemorrhagic Shock and Polytrauma in Young and Old Adult Mice

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Background: Older adults experience worse morbidity and mortality after severe trauma. Elderly mice are unable to sufficiently upregulate and sustain emergency myelopoiesis after severe injury, in part due to inadequate emergency myelopoietic response by bone marrow (BM) hematopoietic stem and progenitor cells (HSPCs) and circulating myeloid cells. MicroRNAs (miR) are small noncoding RNAs that regulate gene expression. There has been growing evidence of miR control of HSPC self-renewal and differentiation. Our main objective was to compare BM HSPC miR responses of old and young mice in a clinically relevant murine model of severe trauma and shock.

Methods: C57BL/6 adult male mice aged 8-12 weeks (young) and 18-24 months (old) underwent a model of polytrauma and hemorrhagic shock (PT) that engenders an injury severity score of 18. Pseudomonas pneumonia (PNA) was induced in a separate polytrauma cohort (PT+PNA) of young and old adult mice. miR expression patterns were calculated from lineage-negative enriched BM HSPCs isolated from PT mice at 24 hours and PT+PNA mice at 48 hours post-injury. Genome-wide expression analyses and Ingenuity Pathway Analysis (IPA) were also performed on bronchoalveolar lavage (BAL) leukocytes.

Results: miR expression patterns were significantly different among all conditions ($p < 0.05$), except for old-naïve vs old-injured (PT or PT+PNA) mice, suggesting an inability of old mice to mount a robust early response to severe shock and injury. Among the miRs with the highest differential expression between young and old PT (+/- PNA) mice were miR-494-3p, miR-223, miR196b-5p, miR-17-5p, miR-145a-5p, miR-132-3p, and miR-125b-5p,

and miR-125a-5p, which have been demonstrated to be important for renewal, expansion, or differentiation of myelopoietic stem cells (Table). Additionally, young adult mice had significantly more cells obtained from their BALs and were determined to have more PMNs compared to old mice (59.8% vs 2.2%, $p=0.0069$); however, there was no significant difference in the percentage of activated macrophages (6.0% vs 6.8%, $p=0.89$). Interestingly, despite increased gene expression changes, the old adult murine BAL leukocytes demonstrated a more dysfunctional transcriptomic response to PT+PNA than young adult murine BAL leukocytes, as reflected in IPA predicted upstream functional analysis with 119 pathways altered in young adult BAL leukocytes versus only 96 pathways in old adult BAL leukocytes.

Conclusion: Using our murine model of polytrauma and hemorrhagic shock, which emulates severe human trauma, we determined that the miR expression pattern in BM HSPCs after PT (+/-PNA) is significantly dissimilar in old versus young adult mice. In the acute phase, old adult mice are unable to mount a robust miR response in HSPCs following injury. HSPC miR expression in old PT mice reflects a diminished functional status as well as a blunted capacity for terminal differentiation of myeloid cells, as demonstrated by the decreased percentage of PMNs and dysfunctional gene expression pattern in the BAL leukocytes of old mice. This is important as miR expression is a modifiable epigenetic cellular component and miR modifications have been attempted in other disease states to improve outcomes.

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