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PTBP1 Is a Novel RUNX1 Interactor in Leukemia

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Runt-related Transcription Factor 1 (RUNX1) is a transcription factor that, along with its obligate binding partner CBFβ, plays a key role in hematopoiesis. RUNX1 is frequently mutated in leukemia and wild type RUNX1 also promotes leukemogenesis by cooperating with other common mutations. The interaction of RUNX1 and CBFβ is well characterized but we are only now unraveling the role of other interacting partners for RUNX1. We recently identified a novel RUNX1-interacting protein called Polypyrimidine Tract Binding Protein 1 (PTBP1) whose interaction with RUNX1 may be dependent on HDAC1 activity. PTBP1 is an RNA binding protein, known to regulate alternative splicing of mRNA transcripts. Pediatric leukemia is associated with a favorable prognosis, however, a large percentage of patients relapse. This may be because leukemia is a highly heterogeneous disease and current treatment strategies like chemotherapy are non-specific. Therapies targeting key leukemogenic players is the need of the hour. However, to develop targeted therapies we need to learn more about the mechanisms by which leukemia cells are established and maintained. The novel association of PTBP1 and RUNX1 is dependent on HDAC1 activity and promotes proper splicing of RUNX1 target genes. To identify RUNX1 interacting proteins that may be dependent on HDAC1 activity, we treated primary mouse leukemia cells with an HDAC1 inhibitor (entinostat), immunoprecipitated the RUNX1 complex and performed mass spectrometry analysis. We observed a statistically significant decrease in the immunoprecipitation of the splicing regulators polypyrimidine tract binding proteins 1 and 3 (PTBP1/3) with RUNX1 in cells treated with entinostat by mass spectrometric analysis. We confirmed association of PTBP1 with RUNX1 via immunoprecipitation and western blot in both mouse primary leukemia cells and human leukemia cell lines. Additionally, using proximity ligation assay we confirmed that this association occurs only within the nucleus of mouse and human leukemia cells. Finally, we observed that high PTBP1 levels correlate with increased colony formation in mouse primary leukemia cells. We have discovered a novel RUNX1 interacting protein, PTBP1 in both mouse and human leukemia cells. We are currently in the process of characterizing the interaction of PTBP1 with different members of the RUNX1 complex and determining the implication of this association in leukemia and normal cells. Our work will test a potential novel mechanism of RUNX1 activity in leukemia cells via HDAC1 mediated recruitment of the splicing regulator, PTBP1. This will have important implications for not only leukemia, but normal hematopoiesis, as well.