Molecular dissection of autophagy and glycolysis pathways in normal and leukemic myelopoiesis

Molecular disAutophagy is a catabolic self-degradation process with primarily pro-survival effects. It is characterized by the formation of double-membraned autophagosomes. Importantly, a dual, context-dependent role for autophagy in tumorigenesis is observed being tumorsuppressive in early stages of cancer development and rather oncogenic in later stages of cancer development where autophagy supports the survival of cancer cells in hostile microenvironment or upon chemotherapy. Our own findings describe an essential role for autophagy in myeloid leukemic cells depends on an alternative non-canonical mechanism, independent of Beclin1, but dependent on additional key autophagy genes such as VPS34, WIPI-1 and MAP1S as shown by knockdown experiments (Brigger et al., 2014; Haimovici et al., 2014; Orfali et al., 2015). We refer to this novel type of autophagy as myeloid differentiation-associated autophagy (MDAA). Since myeloid development is orchestrated by a panel of lineage specific transcription factors including the myeloid master regulators PU.1 and CEPBA, and since these transcription factors are often inactivated in AML, we asked if they might regulate autophagy genes. Indeed, we discovered that several autophagy genes (WIPI-1, MAP1S, DAPK2) are transcriptional targets of PU.1 and CEBPA (Humbert et al., 2014; Brigger et al., 2014; Haimovici et al., 2014). Thus, low autophagy gene expression in particular AML subtypes can be attributed to aberrant expression and function of PU.1 or CEBPA in this disease. Moreover, we linked the autophagy genes DRAM-1 and GATE-16 to neutrophil differentiation of APL cells (Humbert et al., 2012; Brigger et al., 2013). Lastly, we could show that the autophagy receptor p62/SQSTM1 positively affects cell survival of APL cells during neutrophil differentiation (Trocoli et al., 2014). Together, our results suggest a crucial role for autophagy in AML differentiation and cell survival.

Depletion of the glycolytic enzyme PKM2 allows neutrophil differentiation of ATRA-resistant APL cells

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A majority of cancer cells rely on metabolic intermediates from aerobic glycolysis to satisfy their increased demand for biosynthesis (Warburg effect). To this end, tumor cells frequently express the M2 isoform of pyruvate kinase (PKM2), which catalyzes the last step within glycolysis. Interestingly, murine normal hematopoietic and leukemic cells preferentially express PKM2 and this enzyme is needed for leukemia progression in mice. In addition to its metabolic function, nuclear PKM2 is involved in gene regulation, e.g. activation of cyclin D and C-myc via β-catenin. We now asked if PKM2 plays a role in cellular differentiation of APL cells.

PKM2 mRNA expression levels were measured by qPCR in primary AML patient samples, healthy neutrophils and in APL cell lines. NB4 APL PKM2 knockdown cells were generated using lentivirus encoding shRNA targeting PKM2. PKM2 knockdown efficiency and nuclear localization upon cell fractioning were determined by Western blotting. Cell differentiation and functionality was assessed by G-CSF-R mRNA levels, CD11b surface marker expression and nitroblue-tetrazolium reduction.

PKM2 is significantly downregulated in primary AML patient samples as compared to healthy granulocytes. Accordingly, PKM2 mRNA (10-15-fold) and protein levels were significantly induced upon ATRA-mediated neutrophil differentiation of parental but not ATRA-resistant NB4-R1/R2 APL cells. Increased expression of PKM2 was also seen during neutrophil differentiation of non-APL HL60 cells. Surprisingly, knocking down PKM2 in NB4 cells did not attenuate neutrophil differentiation. In contrast, depleting PKM2 in ATRA-resistant NB4-R1/R2 cells led to significantly enhanced neutrophil differentiation. Importantly, knocking down PKM2 caused minor, but significant neutrophil differentiation in NB4R1/R2 cells without ATRA treatment. Lastly, we found nuclear PKM2 expression in NB4-R2 cells and depleting PKM2 in these cells resulted in decreased c-Myc expression. Together, knocking down PKM2 partially rescues neutrophil differentiation of ATRA-resistant NB4 cells. Together, targeting PKM2 in differentiation impaired APL cells may resensitize these cells to retinoic acids.