Summary

Our results reveal a striking switch in the balance of myeloid-erythroid ratio in ΔMIR182-K562 cells demonstrating a clear role of this microRNA in contributing to lineage decisions and also implicating such choices with tyrosine kinase resistance. In this study we followed a step wise process and started by identifying the expression of miR-182 in both cell lines and primary cells from clinical cases of CML. Human cell lines are an invaluable resource to study the mechanisms of disease progression. Primarily, techniques have involved approaches which transiently either assess expression of a given gene or the consequence of lowering gene output. While important information about the relationship of various classical genes, miRs and their pathways with links to specific cellular phenotypes have emerged through these transient approaches, there are obvious limitations. The primary limitation, apart from their incomplete penetrance is the lack of a stable long standing genetic change with concomitant output. A potential approach to overcome these limitations is to use gene editing tools on specific loci.

Our initial analysis was using established methods that transiently interfere with gene expression and revealed an insight into both the role of miR-182 in regulating proliferation/viability and the lineage choices. In order to establish a robust stable system to define this observation and lay the foundation for subsequent analysis, we used CRISPR as a tool to generate a MIR-182 deletion. The cell line generated shows a clear switch with the emergence of myeloid cells. This is consistent with the clinical observation of erythroid cells in the presence of both tyrosine kinase resistance and miR-182 expression. To further validate the role of the miR-182 axis, we followed our initial bio-informatic analysis with experiments to probe the role of Hes1. The results with Hes1 modulation also are consistent with a key role for the miR-182 axis in modulating myeloid- erythroid ratio.

Other studies have also raised the possibility of an important role for miR-182. For example, miR-182 has been implicated in chemo resistance in breast cancer cells and in the progression of tumors such as melanomas. The molecular pathways that are implicated in mediating the effects of miR-182 are only partially characterized. Hes1 is a key molecular component of the Notch pathway, which has been implicated in both hematopoietic lineage determination and disease. The traditional view of the Notch pathway in this context is based on mutations which have been detected in leukemias, particularly ALL. The data generated in our study would suggest a rethinking of the role of this pathway in the context of late stage CML and particularly tyrosine kinase resistance in the absence of mutations in the TK locus. Several miRs such as miR-223, miR144/451 have been implicated in mediating a role in tyrosine kinase resistance. Our data in this study categorically adds an important role of miR-182, with a particular focus on TK independent cases. Both the use of the cell line that we have generated and clinical analysis should offer an opportunity to create novel therapies for the relapsing CML cases in the years ahead. As a wider implication, we also validate that gene editing of specific locus in established cell lines is a useful approach to create reagents for phenotypic analysis in the context of drug resistance.