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Clonal and epigenetic pathways in the development of pediatric acute lymphoblastic leukemia.

Pediatric acute lymphoblastic leukemia (pALL) is the most common malignancy in childhood. Nowadays successful therapy outcome is achievable in more than 85% of patients. However, due to high prevalence of leukemia, many patients relapse. Whereas leukemia recurrence is linked with very poor prognosis. Therefore, pALL is one of the most frequent causes of disease-related deaths among children. In this context, it is necessary to determine mechanisms of leukemia development and indicate new potential diagnostic and therapeutic targets.

At the day of diagnosis, leukemic blasts can constitute almost 90% of all bone marrow cells. Simultaneously, immunophenotypic and genetic analyses indicate that tumor tissue presents clonal structure. But clonal diversity in pALL cannot be fueled only by genetic mutations, as pALL presents a relatively low DNA mutational burden. Moreover, our preliminary analysis shows that hematopoietic stem cells from leukemic bone marrow present abnormal gene expression profile in the absence of driver DNA mutations.

Thus, the aim of this project is to identify the cell-of-origin and molecular mechanisms which contribute to malignant clonal evolution and expansion. Our central hypothesis is that in pALL epigenetic alterations among stem and progenitor cell (HSPC) pool are the first links in leukemogenesis chain and further contribute to clonal diversity. To achieve our goals, we propose three specific aims and advanced experimental strategy, based on analysis at single cell level. In aim 1. we will investigate clonal diversity and linked phenotype marks during pALL course. In aim 2. we will concentrate on the identification of epigenetic alterations within HSPC pool, and we will determine which fraction is a source of leukemic clone. In the first task we propose the single-cell approach with Mission Bio Tapestry Single-Cell DNA+Protein technology, to simultaneously determine the tumor clonal branching and the immunophenotype marks for individual cells from leukemic bone marrow. In the second part we will modify standard Mission Bio Tapestry protocol. Due to addition of methylation-sensitive restriction enzyme we will check aberrancies in methylation status of several genes. Simultaneously, we will perform clonal tracing with EPI-Clone algorithm. Finally, in aim 3. we will verify in prospective way if atypical epigenetics alone, without genetic mutations, is sufficient for inducing uncontrolled cell proliferation. Thereby, we will induce locus-specific methylation modifications in three ALL-related genes in mouse pro-B Ba/F3 cell line, using Crisper-dCas9 system conjugated with DNTM3A enzyme. Then we will observe if the cells are able to grow independently of IL-3 supplementation.

Our project is a reaction to still existing questions about the source and mechanisms of development of pediatric leukemias. After realization of proposed tasks, we will describe clonal architecture of tissue sample along with linked immunophenotypical diversity. We will determine whether hematopoietic stem or progenitor cells carry tumor-related epigenetic alterations, thus they may be the cell-of-origin in leukemia. Additionally, we will verify whether purely non-genetic mechanisms are sufficient to initiate malignant fate in targeted cells. We believe that our results will contribute to better understanding of cellular and molecular mechanisms in pALL and provide important implications for clinical management of childhood leukemias.