1. Record Nr. TD18002432

Autore	CAMMARATA, UMBERTO ANDREA
Titolo	CLONAL TRACKING AND HIGH THROUGHPUT SHRNA SCREENING IN AMLS [Tesi di dottorato]
Editore	Università degli Studi di Milano, 2016-03-18
Lingua di pubblicazione	Inglese
Formato	Tesi di dottorato
Livello bibliografico	Monografia
Note	diritti: info:eu-repo/semantics/openAccess In relazione con info:eu-repo/semantics/altIdentifier/hdl/2434/365771
Sommario	Acute myeloid leukemia (AML) is a malignant tumor characterized by the uncontrolled proliferation of immature hematopoietic cells (blasts) that colonize the bone marrow leading to a malfunctional hematopoiesis. Despite the efforts to search for new therapies and the identification of new targets, leukemias are still largely incurable. The main reason for treatment failure is the high frequency of relapses after remission induced by the first chemotherapy treatment. Recent studies suggest that the high rate of relapse is due to the presence of cells with limitless regenerative capacity, Leukemia Initiating Cells (LIC), that are very resistant to chemotherapy. Moreover, since the leukemic blasts are highly heterogeneous both from a biological and genetic point of view, AML evolves continuously under both environmental and external stimuli (eg, chemotherapy). In order to study the clonal evolution of AML and to find new therapeutic targets that allow to eradicate leukemic blasts, this study has set the following objectives: i) monitor LICs growth in vivo through the transplantation of traceable leukemic cells; ii) investigate the clonal evolution of the disease; iii) identify genes critical to tumor growth in vivo that can become new therapeutic targets. To address these issues, we have used two lentiviral libraries (Cellecta Inc.): i) a clonal tracking library composed

	by 30 million different barcodes; ii) an shRNA library composed by ~1000 different shRNA able to silence ~100 genes. The libraries were used to infect xenotransplants of patient-derived leukemias and murine leukemias obtained from mouse models. The infected blasts were transplanted in recipient mice and, following leukemia development in vivo, the DNA extracted from infected blasts has been used, through massive sequencing techniques, to investigate leukemia growth and evolution (clonal tracking library) and genes required for AML growth (shRNA screening). Our clonal tracking analysis highlighted a strong clonal selection troughout leukemia evolution with the disappearance even of clones that were present at very high frequency within the tumor population. Nonetheless, the clonal composition of all tumors appeared to be very similar in terms of number of clones, of their frequency distributions and even of 13 their identity. Taken all together, our data strongly support a model in which each LIC is endowed with an intrinsic and highly variable replicative potential, with the vast majority of LICs that are not immortal and do not possess an indefinite self-renewal ability As a consequence, each leukemia possesses a definite and small number of LICs able to propagate the disease long-term. These LICs tend to overgrow all other clones and to constitute a very large proportion of the tumor. Concerning the shRNA screening, we believe we set up an innovative in vivo approach that allowed us to identify putative therapeutic targets involved in the growth of primary tumors in their natural environment and that is mainly selective for targeting the CSC population. Most of the identified potential targets have been already reported to play a fundamental role in different types of cancer, including leukemia. Because we were able to perform our screening on several AMLs and identified both tumor-specific and tumor-wide putative targets, our study might offer solutions of therapeutic intervention in a wide group of AML patients, hopefully increasing their survival and decreasing therapy related side effects.
Localizzazioni e accesso	http://memoria.depositolegale.it/*/http://hdl.handle.net/2434/365771