The role of exosomes in pediatric precursor B acute lymphoblastic leukemia

Childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most prevalent childhood hematological malignancy, that arises from an expansion of malignant B cell precursors in the bone marrow (BM). Despite overall survival in pediatric ALL has improved to roughly 80-85%, prognosis of relapsed patients is still very poor and outcome are much worse with second or later relapses. In leukemia context, the BM microenvironment has a key role in chemotherapy resistance, sustaining cell survival and protecting leukemia blasts from environmental stress signals. Additionally, leukemic cells can transform the BM niche into a leukemia-permissive one through exosomes secretion, nano-sized membrane vesicles (30–150nm) that have a key function in tumorigenesis, due to their role in intercellular communication. Exosomes cargo includes small RNA, which can be released in the circulatory system and can affect many pathophysiological functions of target cells, such as modulate the cellular microenvironment to promote the metastatic niche and the tumor formation.

Purpose of this project is to investigate exosome cargo of BCP-ALL and to explore their role in cell-cell communication, leukemia survival and tumor progression. In particular, the aims of this research proposal are:

1. To characterize the small RNA profile of BCP-ALL plasmatic exosomes at diagnosis and during follow-up of patients with different molecular response kinetics to chemotherapy and healthy donor (HD);
2. To investigate the exosome-mediated small RNA transfer within microenvironment and the crosstalk between tumor cells and microenvironment host cells.

Interestingly, our preliminary data showed that exosome cargo derived from BCP-ALL patients is different from the healthy donors one. Our group has already isolated the plasmatic exosomes of 4 pediatric BCP-ALL and 8 HD. Plasma were processed for exosomes isolation, RNA extraction and sequencing by Illumina HiSeq 4000 with a target depth of 15M reads per sample. sRNA-seq data was analyzed by miR&moRe pipeline, for the identification of specific miRNA and small RNA in exosomes.

We detected 1402 small RNAs in samples (BCP-ALL and HD) after removing low confidence newly predicted pre-miRNAs; in detail among the 1402 we identify 1035 miRNAs, 112 moRs, 184 new miRNAs together with other 28 small RNAs. 446 small RNAs were consistently expressed (i.e. with > 1 reads in 80% of samples of at least one condition) of which 96% of them were both expressed in HD and BCP-ALL (Fig.A-B). Differential analysis between BCP-ALL and HD exosome revealed a total of 18 sRNA deregulated ((FDR<0.1); 11 out of 18 are up-regulated in BCP-ALL.

For the first aim, we will propose to enlarge patient cohort up to a total of 16 BCP-ALL patients (at diagnosis; 8 high risk and 8 low risk) cargo exosome plasma derived characterization.

Moreover, we would like to extend our analysis and plasma exosome characterization during therapy follow-up (+33 and +78 and after complete remission) to evaluate longitudinally the expression of smallRNAs previously identify to be aberrant in leukemia patients compare to healthy donor (top 10 significantly different expressed miRNAs according to FDR and p value < 0.05).

Moreover, RNA extracted from BM at diagnosis will be used to back-tracking the expression of exosome miRNA.

In addition, to investigate the possible crosstalk between leukemic and microenvironment cells towards exosome circulation we co-cultivated healthy donor mesenchymal stromal cells (HD-MSC) and BCP-ALL cell lines (NALM-6 and MHH-CALL-4) and we demonstrated the passage of biomolecules through the Boyden chamber by confocal microscopy (Fig.C).
Furthermore, we have already isolated MSC from bone marrow of the 4 patients analyzed for smallRNAs cargo. With HD-MSC we will investigated whether leukemic exosome cargo could interfere with their differentiation potential. After cell seeding, 2μg/ml of extracted EVs from tumor cell lines will be administrated every 2 day with Osteogenic/Adipogenic Differentiation Medium (Lonza) for 21 days and then, osteoblasts and adipocytes will be fixed and stained with Alizarin Red S and Oil Red O, respectively. We aim to investigate also osteogenic/adipogenic differentiation of MSC derived from 4 BCP-ALL patients.
For aim 2 we will propose to obtain BM derived MSC from at least 10/16 patients already characterized for their exosome cargo.

Overall this project will prompt us to deeply investigate the role of exosome in the leukemogenesis and disease dynamics, as well as to flush out their potential role in the cell-cell communication and bone marrow niche modulation.