Integration of exosome mirnome/proteome signature for identification of specific glioblastoma biomarkers

Exosomes are small extracellular vesicles that mediate intercellular communication in both normal and tumor cells (1). Exosomes derived from glioblastoma multiforme (GBM), one of the most aggressive and lethal forms of primary brain tumors, release molecules that alter the surrounding environment to favor tumor invasion (2). Important molecular effectors of this tumor promoting activity are microRNAs (miRNAs) and proteins. Exosomal miRNA signatures to be used as tumor biomarkers have been identified by various groups but show a high degree of variation, likely due to the high inter-tumor and intra-tumor heterogeneity. Therefore, new approaches are needed to identify more specific circulating biomarkers for tumor typing.

In collaboration with Regina Elena Institute in Rome (Dr. Giovanni Blandino), we studied the miRNA content of exosomes derived from three GBM cancer stem cell (CSC) lines originated from primary human tumors (3) showing different degrees of malignancy, in comparison with two established GBM cell lines (U87 and U373). By using Agilent microarray technology, we found a total of 854 miRNAs contained in the exosomes of our samples, of which 195 (23%) are in common among the three cell types. miRNA expression analysis identified 282 and 327 miRNAs differentially expressed in CSC-derived exosomes compared to U87- and U373-derived exosomes, respectively (FDR-BH < 0.05, |log2FC| > 1). Considering the first 20 most abundant miRNAs expressed exclusively in exosomes from CSCs and not in cell lines, we found a total of 1,927 experimental validated target genes according to miRTarBase database (v8.0). Functional enrichment analysis by ToppFun software evidenced that these genes are involved in many interesting processes, such as cell cycle, adhesion and cytoskeleton organization, as well as in several tumor-related pathways, including epithelial-mesenchymal transition (EMT).

In parallel, in collaboration with Istituto Superiore di Sanità in Rome (Dr. Federica Fratini), we performed the proteomic analysis of the exosomes derived from the same GBM CSCs and established cell lines. By mass spectrometry, we found a total of 3,103 exosomal proteins, of which 2,391 (77%) are in common among the three cell types. Interestingly, among the 1,927 miRNA target genes aforementioned, 466 were detected in exosomes at protein levels. Protein expression analysis identified 742 and 467 proteins differentially expressed in exosomes from CSCs as compared to U87 and U373, respectively (adjP < 0.05, |log2FC| > 1). Notably, also these differential proteins showed enrichment for adhesion, cytoskeleton organization, EMT and mesenchymal differentiation processes, among the others, as seen for miRNA target genes.

Further analysis of the specific miRNAs and proteins contained in the exosomes derived from the GBM CSCs and GBM cell lines will allow us to define a combined miRNA/protein signature peculiar of the different GBM tumor-derived cells. These findings will provide new insights on the molecular mechanisms underlying GBM pathogenesis and allow to identify additional, highly specific molecular signatures and markers for clinical applications in GBM.
References:

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