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Autore	M.E. Bicchieri
Titolo	A WHOLE-GENOME APPROACH TO IDENTIFY MICRORNA 'MODIFIERS' OF BREAST CANCER STEM CELL SELF-RENEWAL [Tesi di dottorato]
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Sommario	An emerging notion in the breast cancer field is that a rare subpopulation of cells within the tumor bearing stem cell (SC)-like properties, cancer stem cells (CSCs), are responsible for the degree of aggressiveness of the tumor, as well as the emergence of therapeutic resistance and disease relapse. Therefore, the development of novel therapeutic strategies that specifically target the CSCs population within a tumor could be the key to achieve an effective cure for breast cancer. One strategy for targeting CSCs could be to inhibit their altered self-renewal mechanism and induce a quasi-normal differentiation process in tumor cells. However, the mechanisms that control the replicative mode of SC division and the degree of “stemness” of tumours are poorly characterized. Recent research has highlighted the role of microRNAs (miRNAs), a class of small non-coding RNAs, as key regulators of gene expression in a variety of cellular processes, including SC self-renewal and differentiation. miRNAs negatively regulate gene expression at a post-transcriptional level and their expression is often deregulated in disease, making them ideal candidates as tumour biomarkers. Despite recent studies uncovered new microRNA molecules linked to stem cell biology, we definitively miss a defined picture of which microRNAs are involved in the regulation of breast cancer stem cell

	self-renewal and their contribution to tumorigenesis. The overall goal of this project was to identify key miRNA “modifiers” of breast cancer SC self-renewal that could either inhibit or enhance the self-renewal potential of cancer stem cells, with the purpose of identifying key molecules involved in the acquisition/regulation of stem-cell traits and bona fide novel therapeutic targets. We used a lentiviral microRNA library, composed of approx. 650 precursor microRNAs, to perform a functional whole-genome screening based on phenotypic competition assays on a very aggressive breast cancer cell line with stem-like properties (SUM159). Infected cells were challenged in an in-vitro 3D competition assay based on self-renewal ability of CSCs (mammosphere propagation). In the competition assays, miRNAs that supported stem cells expansion were positively selected during passages, while microRNAs that inhibited self-renewal were depleted overtime. In parallel we also performed a 2D assay based on cell proliferation to gain insights into the ability of miRNAs to alter the proliferation of cancer cells in adherent conditions. For each screening the positive and the negative selected miRNAs were identified by means of Next Generation Sequencing analysis. The screening yielded to 20 candidate microRNAs selected as potential modifiers of self-renewal: 18 presumably acting by decreasing self-renewal (and hypothetically with tumor suppressing functions) and 2 by increasing self-renewal (and potentially with oncogenic functions). A proof-of-principle validation revealed that 6 out of 10 tested cloned, confirmed their effects even when analyzed as single clones, underlining the potentiality of the whole-genome phenotype screening. We focused our attention on the two most promising candidates and, in order to search for the mechanism through which these microRNAs exert their function, we performed an RNA-seq analysis of transcriptional changes induced upon their overexpression. We revealed that one microRNA in particular was able to regulate hundreds of genes and control independently different pathway related to self-renewal, migration and proliferation, suggesting that this miRNA could effectively act at multiple levels to silence the self-renewal potential of cancer stem cells and, likely, inhibit the proliferation and migratory ability of the tumor, too. As further experiments, we will definitively need to completely understand the role of this microRNA in cancers with the potential of being effective even on the most aggressive breast cancer disease.
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