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Titolo	IDENTIFICATION OF EPIGENETIC INHIBITORS OF PHYSIOLOGICAL CELLULAR PLASTICITY AS NOVEL TUMOR SUPPRESSOR [Tesi di dottorato]
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Sommario	Cellular plasticity, the inter-conversion of cells between differentiated cells and stem cells (SCs), can lead to tissue regeneration and restoration of homeostasis after injury. Conversely, inappropriate induction of cellular plasticity could be involved in tumor initiation and progression, through de-novo generation of cancer stem cells (CSCs) by de-differentiation of normal or non-tumorigenic bulk tumor cells. This intrinsically dangerous potential must be tightly controlled by genetic and epigenetic mechanisms, to prevent unscheduled de-differentiation. However, their systematic identification by large-scale screenings is just beginning to be exploited. In order to identify physiological inhibitors of cell plasticity, that could play a function as tumor suppressors, we performed short-hairpin RNA (shRNA) screens. In the screens, we used pooled lentiviral shRNA libraries targeting 234 epigenetic regulators to identify shRNAs endowing mouse mammary progenitors with the SC-specific ability to regenerate mammary gland tissue upon in vivo orthotopic transplantation. Sequencing of genomic DNA extracted from the regenerated mammary glands led to the identification of 38 hits/genes. We individually validated 7 hits by in vivo regeneration assays, showing that their down-regulation

	leads to conversion of mammary progenitors into stem cells able to regenerate mammary glands. We performed also in vitro mammosphere and phenotypic cell conversion assays to examine the hits’ function in self-renewal and cell plasticity, respectively. Next, among the validated hits, we showed that the inhibition of Cbx5 and Kmt2d induced efficient reprogramming of mammary progenitors. Therefore, we focused to investigate their mechanistic function in reprogramming at the transcriptome level, by RNA sequencing. We identified a specific enrichment for pro-inflammatory signalling pathways as an early transcriptional reprogramming response (ETRR), followed by up-regulation of Myc target genes. Finally, in order to set the basis for further characterization of reprogramming mechanisms induced by the validated shRNAs, we established an organoid assay using MycER, our positive control for mammary progenitors reprogramming, showing that MycER over-expression bestows mouse luminal cells with enhanced self-renewal ability and differentiation capacity. Moreover, we performed a single cell transcriptome analysis in mouse primary mammospheres that revealed considerable heterogeneity as 20 clusters identified and led to the identification of Cd36 (glycoprotein, collagen type I receptor) as a putative mammary SC-specific surface marker. We expect that further analyses of these data, together with single cell transcriptomic analysis of mammospheres interfered for the validated hits, will shed light on the mechanisms involved in physiological cell plasticity.
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