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Sommario	Lysine-Specific Demethylase 1 (LSD1) is a protein lysine demethylase that catalyzes the removal of methyl-groups from the mono- and di- methylated forms of lysine 4 and lysine 9 of histone H3 (H3K4me1/me2 and H3K9me1/me2), thus playing an important role in gene expression modulation. This enzyme is aberrantly expressed in various types of cancer and has emerged, in particular, as a promising target for the epigenetic therapy of Acute Myeloid Leukemia (AML). In my thesis project, I implemented and applied a panel of quantitative mass spectrometry (MS)-based approaches to characterize the molecular effects of two potent and selective LSD1 inhibitors under development in our Institute both on the LSD1 interaction network and on global histone post-translational modifications (PTMs), in Acute Promyelocytic Leukemia (APL) cell models, a subtype of AML. Preliminary results acquired by the group of Prof. Saverio Minucci had previously demonstrated that these inhibitors sensitize NB4-APL cells to physiological doses of all-trans retinoic acid (ATRA) independently from the inhibition of LSD1 catalytic activity. These data suggested that the phenotypic effects elicited by these compounds could be due to an effect on the LSD1 interaction network. By SILAC-based quantitative MS-interactomics

	we first identified the complete set of LSD1 interactors, most of which involved in chromatin remodelling and transcription regulation activities. The subsequent analysis of the dynamic changes of the LSD1-interactome upon pharmacological inhibition of the enzyme with the drugs under focus led us to the identification of GFI1 and GSE1, two interactors that displayed a decreased binding to LSD1 upon drug treatment, independently from the specific inhibition of the catalytic activity of the enzyme. Functional and mechanistic follow-up experiments allowed demonstrating that the inhibition of the LSD1-GFI1 interaction sensitizes NB4 cells to physiological doses of ATRA, thus promoting reduction of cell proliferation and differentiation of the cells. The LSD1-GSE1 interaction was basically uncharacterized before this study; the experiments carried out during my PhD project demonstrated that the two drugs lead to the down-regulation of GSE1 expression, both at the transcript and protein levels, rather than disrupting the physical interaction with LSD1. To mimic the effect of the drugs, we performed GSE1 knock-down (KD) in NB4 cells and analysed the consequence of this depletion on both cellular phenotype and transcription. We found that GSE1-KD reduced NB4 cell proliferation by triggering apoptosis. Before the initiation of cell death, GSE1 down-regulation also induces the expression of various differentiation markers and leads to cell cycle arrest by enhancing p21 expression. The comparative analysis of transcriptomic changes upon GSE1-KD and LSD1 pharmacological inhibition in NB4 cells unveiled a set of genes in common, which are involved in “cytokine-mediated signalling” and “regulation of cysteine-endopeptidase activity involved in the apoptotic process”. This result suggests the existence of a regulatory LSD1-GSE1 axis controlling the transcription of these genes. To complete the molecular inspection on the effects of LSD1 inhibition in APL, we carried out a systematic MS-based profiling of histone post-translational modifications (PTMs) changes upon drug treatment in NB4 cells. We observed not only the increase of the well known LSD1 target H3K4me2, but also a reproducible increase of H3K27me2 and H3K27me3, paralleled by the decrease of the H3K27me1 in combination with H3K36me1. UF-1 cells, an APL cell model more sensitive to pharmacological inhibition of LSD1 than NB4, did not present any drug-induced change in H3K27 methylation levels. Interestingly, the basal levels of H3K27me2/me3 measured by quantitative MS are much higher in NB4 than UF-1, which may suggest that the bulk levels of these modifications may be critical for the sensitivity of the cells to the LSD1 inhibitors. In line with this hypothesis, we found that pre-treating the NB4 with EZH2/1 inhibitor sensitizes the cells to LSD1 inhibition. Collectively the data presented in this thesis provide novel insights into the molecular activity of LSD1 and its inhibitors in APL cells.
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