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hic sunt futura

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CORSO DI DOTTORATO DI RICERCA IN:
SCIENZE E BIOTECNOLOGIE MEDICHE

EVALUATION OF DIFFERENT HDACS INHIBITORS IN THE CONTEXT OF LEIOMYOSARCOMA

Department of Medicine, Udine

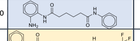
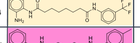
Compound	Molecular structure	IC50	MW	Energy Kcal/Mol	TS**
BML-210		29.9	339.43	-77.45*	1.000
NKL-54		15.5	393.41	-81.43	0.719
MC2991		51.3	333.44	-76.89	0.388
MC2983		N.A.	333.44	-80.25	0.521
MC2984		19.8	333.44	-79.69	0.723
MC2985		10.6	378.49	-79.37	0.510

Figure 1: Chemical structures of HDACi derived from PAOA.

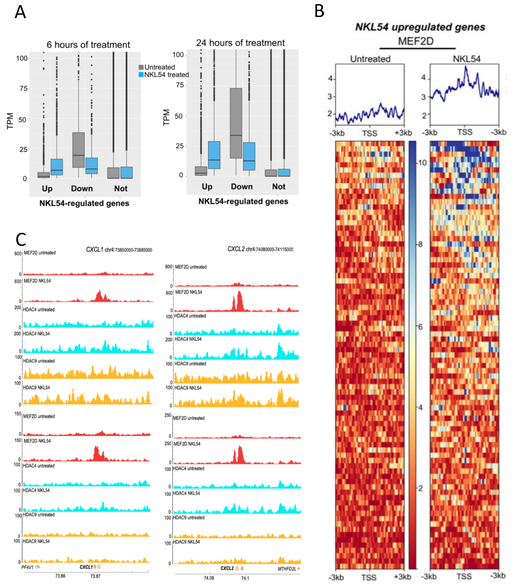


Figure 2A: TPM values are shown after treatment or not with NKL54. **B**: Heat-maps of the MEF2D signal distribution in a region of ±3 kb around the TSS of 90 genes upregulated by NKL54 treatment. Data derived from Chip-seq experiment. **C**: Detailed view of the MEF2D, HDAC4 and HDAC9 tracks at two representative loci (*CXCL1 and CXCL2*) upregulated by NKL54.

BACKGROUND:

Histone deacetylases (HDACs) are a group of 18 enzymes involved in the epigenetic and transcriptional regulation. HDACs are dysregulated in different diseases and many HDACs inhibitors (HDACi) have been generated. HDACs of class IIa (HDAC4,5,7,9) interact through an extended N-term domain with different transcription factors, such as myocyte enhancer factor 2 (MEF2) family members. We have investigated different HDACi in the context of leiomyosarcoma (LMS), a rare and aggressive cancer characterized by a poor immune infiltration and by dysregulations in the HDACs IIa-MEF2 axis.

AIM and APPLICATION:

The aim of our study is the evaluation of HDACs IIa-MEF2 axis in LMS cells where the complex resulted dysregulated. To do that, we have investigated different HDACi in order to restore the normal epigenetic landscape under the control of HDACs IIa-MEF2; we used some HDACi derivatives from pimeoloyanilide-o aminoanilides (PAOA), mainly NKL-54 (Figure 1A). Moreover, we compared SAHA, a pan-HDACi, and TMP-195, a specific class IIa inhibitor (Figure 3A). Our findings suggest the existence of a HDACs IIa-MEF2 repressive complex at the level of some genomic loci including some chemokines. The inhibition of this complex could potentiate the antitumor immune response in LMS, a cancer with a poor immune infiltration enhancing in this way the effect of immune-therapy.

Keywords: HDACS, LMS, inhibitors, MEF2, epigenetic regulation

RESULTS:

NKL-54 causes a resetting of gene transcription and in particular it leads to an increase of genes normally low expressed and a downregulation of the highly expressed ones (Figure 2A). Interestingly, NKL-54 induces an increased number of MEF2D peaks at the level of TSS of genes regulated by the inhibitor (Figure 2B). Analyzing in detail some of these genes, it emerged an effect on *CXCL1* and *CXCL2* loci, where the increased binding of MEF2D after the treatment with NKL-54 is evident (Figure 2C). These data pointed out an activation of MEF2D after the treatment with NKL-54 which has an effect also on genes codifying for chemoattractants.

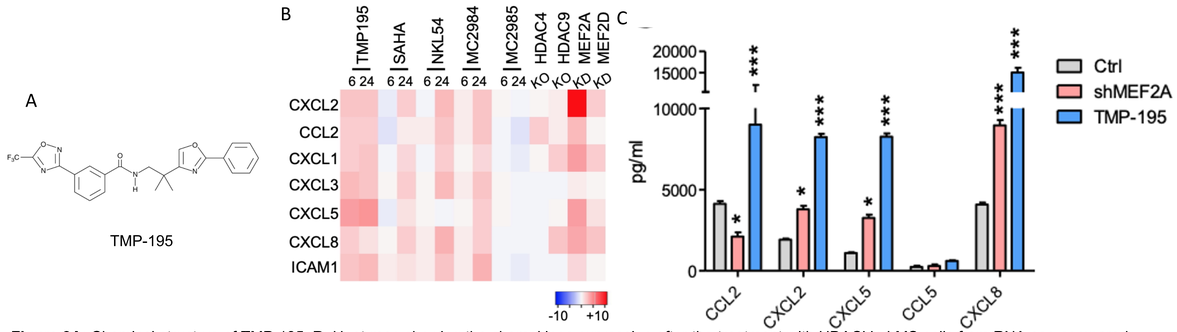


Figure 3A: Chemical structure of TMP-195. **B**: Heat-map showing the chemokines expression after the treatment with HDACi in LMS cells from RNA-seq compared with microarray of *HDAC4,9* ko and *MEF2A,* D kd. **C**: Levels of chemokines release in the supernatant of LMS cells treated with TMP-195 or sh for MEF2A.

We focused the attention on TMP-195 in order to better analyze the contribution of class IIa HDACs. Interestingly, we found that TMP-195 leads to an upregulation of some specific chemokines: *CXCL1, 2, 3* and *5*, in an early time point compared with other HDACi. We also compared the RNA-seq data with microarray of *HDAC4* or *HDAC9* knockout (ko) and *MEF2A* or *MEF2D* knockdown (kd) in LMS cells. A strong increase on *CXCL2,1* and *5* expressions after the downregulation of *MEF2A* was found (Figure 3B). Next, we evaluated the release of chemokines after the downregulation of MEF2A (short-hairpin MEF2A). As reported in figure 3C, the decreased level of MEF2A induces a high release of different chemokines in LMS cells supernatant and the effect is even stronger after the treatment with TMP-195.