Contribution of lysine deacetylases to the therapy of malignant pleural mesothelioma

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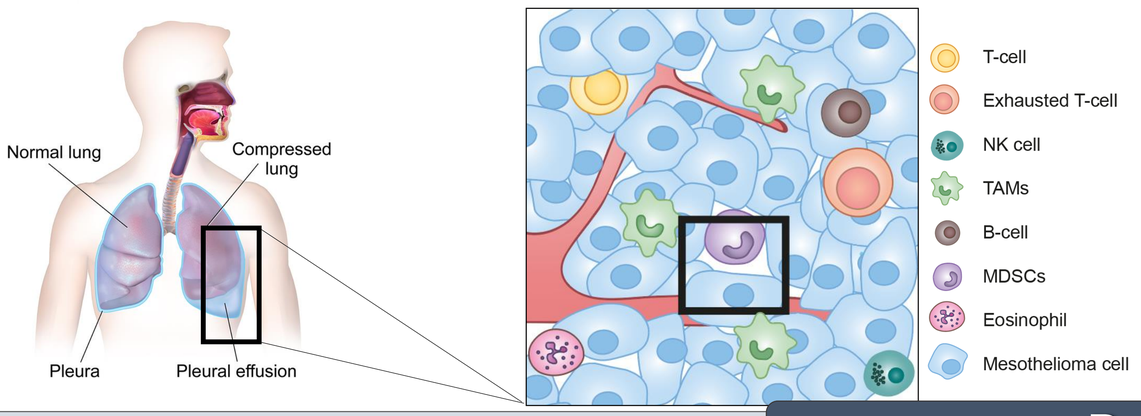
Introduction

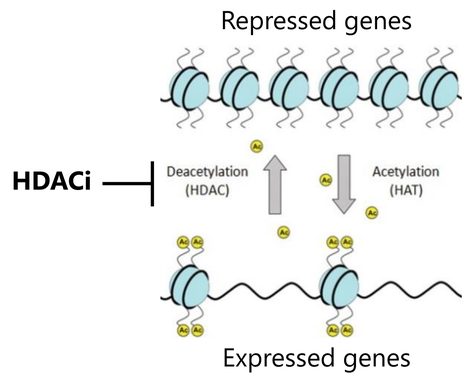
Mesothelioma (MPM) microenvironment



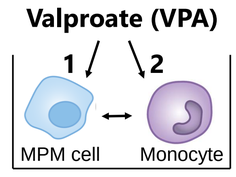
Lysine deacetylase (HDAC) involvement

Proliferation, differentiation and apoptosis of tumor cells

Development and activation of immune cells



How HDAC inhibitor (HDACi) influences MPM microenvironment?



Results

1 : Effect of VPA in MPM cells

Epithelioid : M14K **Biphasic : M38K** **Sarcomatoid : ZL34**

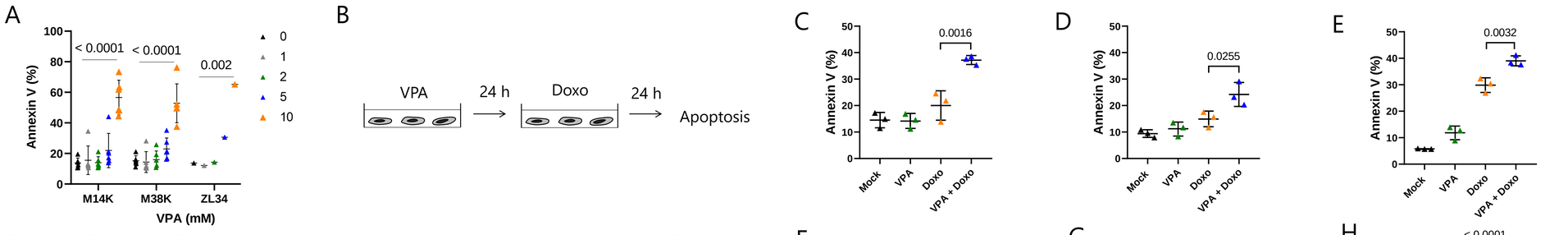
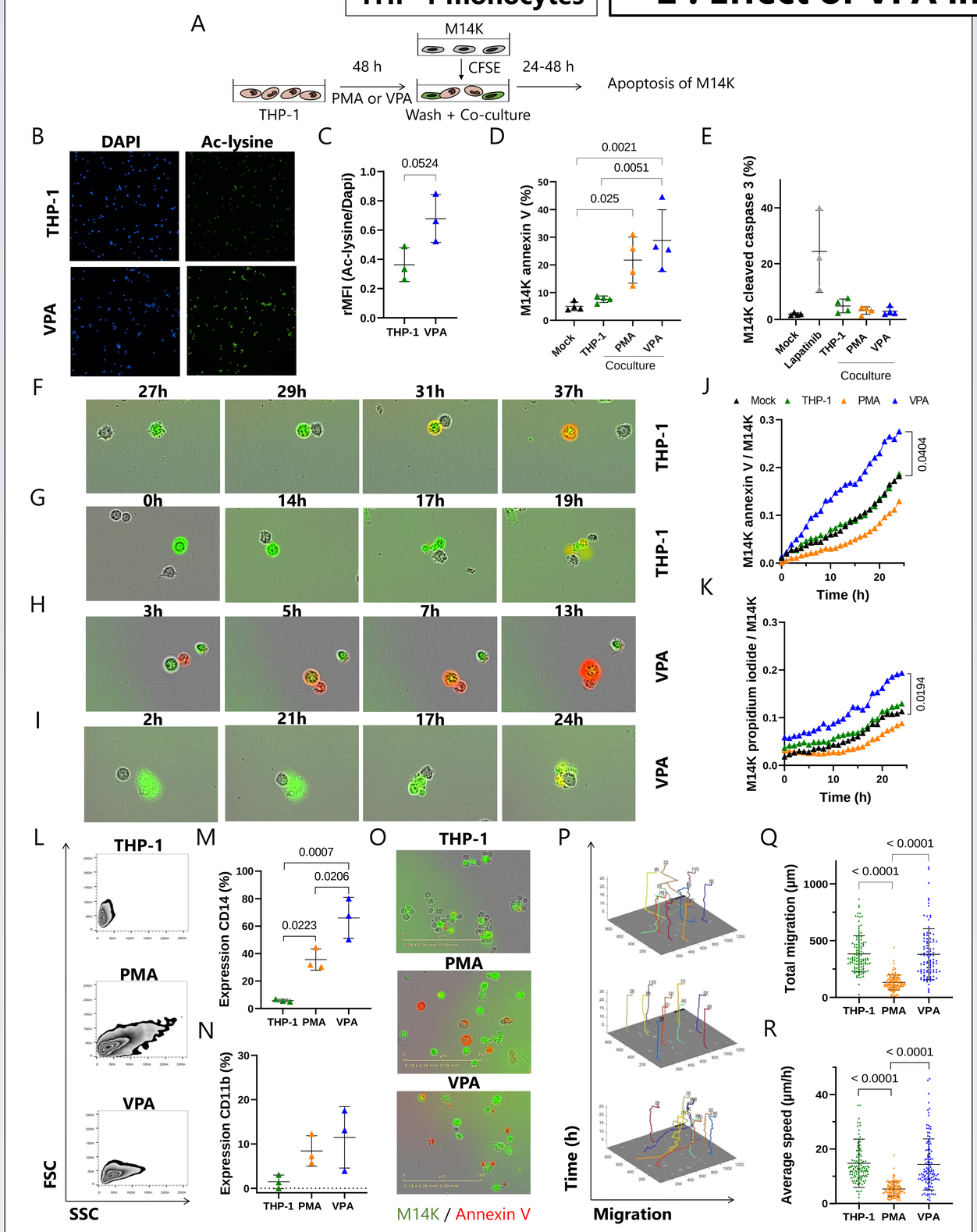


Figure 1 : Effect of VPA and doxorubicin on apoptosis and protein profiling in MPM cells

(A) Three mesothelioma cell lines (M14K, M38K, ZL34) were cultivated with increasing concentrations of VPA (0, 1, 2, 5 and 10 mM) during 24 hours. Cells were then collected and apoptosis was evaluated by an annexin V labelling and flow cytometry analysis. **(B, C, D, E)** Three mesothelioma cell lines (M14K, M38K and ZL34) were incubated with 2mM of VPA during 24 hours. The cells were then treated with 0.5µM of doxorubicin during 24 hours. Apoptosis was evaluated by an annexin V labelling and flow cytometry analysis. **(F, G, H)** The mesothelioma cell lines were incubated with total pan caspase inhibitor (Z-VAD, pan-casp), caspase 8 inhibitor (Z-IETD, casp 8), caspase 9 inhibitor (Z-LEHD, casp 9), caspase 8 and 9 inhibitors (Z-IETD + Z-LEHD, casp 8 + 9), calpain inhibitor (calpeptin) or negative control (Z-FA, VPA + Doxo) for 1 hour before treatment with VPA and doxorubicin. **(I)** After treatment, cell lysates and cytosolic fractions of each cell line were collected. Protein fractions of each cell line were mixed in equal amount and were analyzed by western blot.

2 : Effect of VPA in monocyte activity

THP-1 monocytes



Primary monocytes

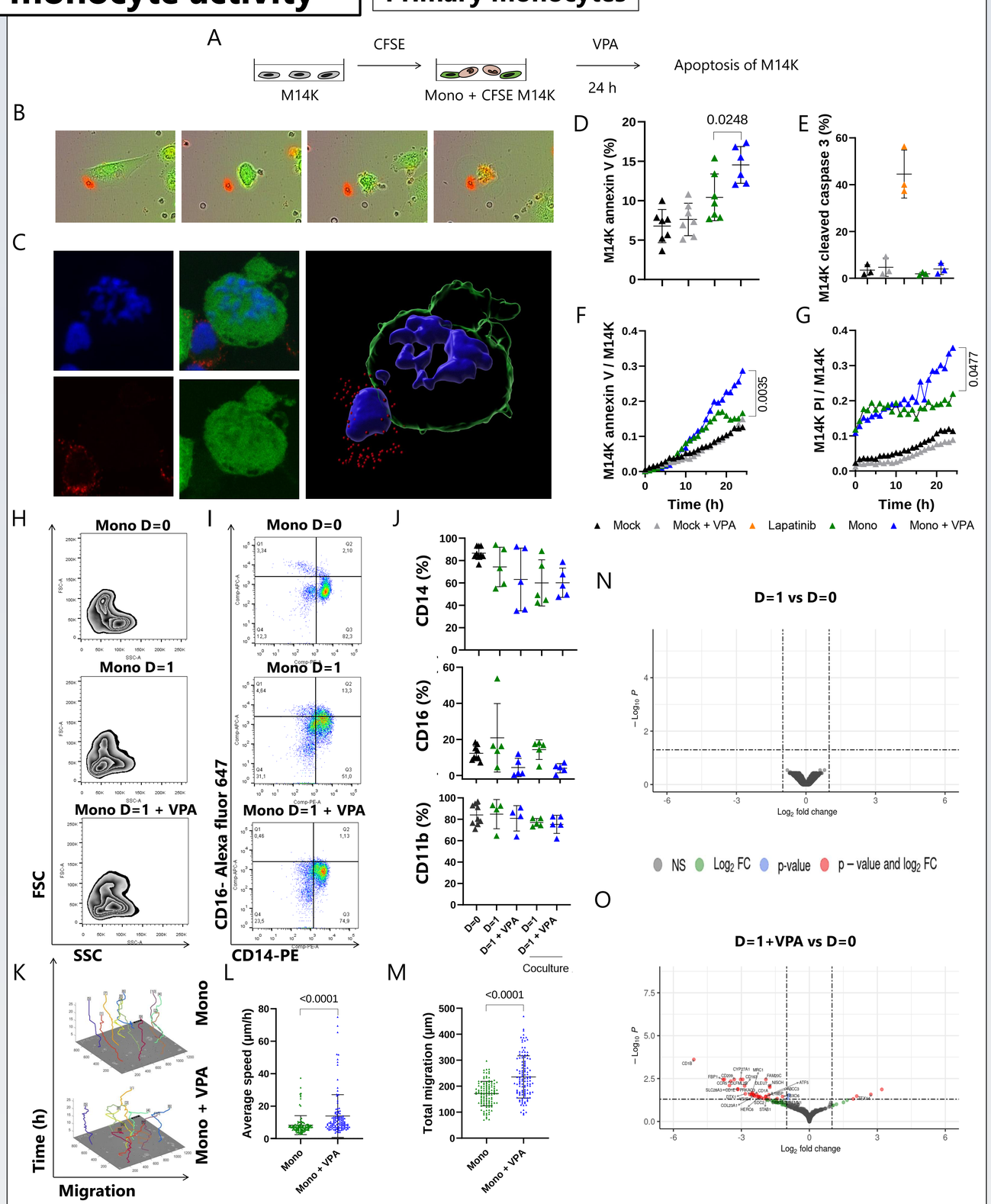


Figure 2 : Effect of valproate on activity and phenotype of THP-1 upon cell-to-cell contact with mesothelioma M14K cells. **(A)** Experimental design. THP-1 monocytes were treated with 100 ng/mL of PMA or 2.5 mM of VPA for 48h. After washing with RPMI, THP-1 monocytes were cocultivated with CFSE-labelled M14K cells during 24h or 48h. Apoptosis of M14K cells was analyzed by time lapse microscopy (Incucyte imaging) (ratio 1/1 during 24 hours) and flow cytometry (ratio 10/1 during 48 hours). **(B, C)** Level of total lysine acetylation related to DAPI after treatment with VPA **(D)** Apoptosis of M14K cells (%) was determined based on the double positive CFSE and annexin V labelling. **(E)** Cleaved caspase 3 of M14K cells (%) was determined based on the double positive CFSE and cleaved caspase 3 labelling. **(F, G, H, I)** Incucyte acquisitions. **(J, K)** Based on the monitoring of Incucyte imaging, apoptosis of M14K cells was determined by the ratio of the number of annexin V or propidium iodide positive CFSE-labelled M14K cells to the number of M14K cells. **(L)** FSC and SSC. **(M, N)** Percentage of expression of membrane receptor CD14 and CD11b. **(O)** Incucyte acquisition showing annexin V labelling in THP-1 monocytes. **(P)** Incucyte images were processed with ImageJ. Mobility of the THP-1 monocytes was determined with the CellTracker software. **(Q,R)** Based on CellTracker analyses, total migration and average speed of the THP-1 monocytes were determined.

Figure 3 Effect of VPA on activity and phenotype of primary healthy monocytes upon cell-to-cell contact with mesothelioma M14K cell line. **(A)** Experimental design. M14K cells were labelled with CFSE and cultivated for 4 hours before being mixed with freshly isolated monocytes at a ratio of 20/1 during 24 hours. Apoptosis of M14K was analyzed by Incucyte imaging, confocal microscopy and flow cytometry. **(B)** Annexin V labelling during Incucyte acquisition. **(C)** CD33 and DAPI labelling of a primary monocyte and a M14K cell. Acquisition was analyzed by confocal microscopy and processed by Imaris. **(D)** Apoptosis of M14K cells (%) was determined based on the double positive CFSE and annexin V labelling. **(E)** Cleaved caspase 3 labelling in CFSE-positive M14K cells (%). **(F, G) .** Based on the monitoring of Incucyte imaging, apoptosis of M14K cells was determined by the ratio of the number of annexin V or propidium iodide positive CFSE-labelled M14K cells to the number of M14K cells **(H)** FSC and SSC profile of freshly isolated (D=0) and cultivated (D=1, D=1+VPA) monocytes. **(I).** Double labelling of CD16 and CD14 of freshly isolated (D=0) and cultivated (D=1, D=1+VPA) monocytes. **(J)** Expression of membrane receptors (%) before and after culture **(K)** Incucyte images were analyzed with ImageJ. Mobility of the monocytes was determined with the CellTracker software. **(L, M)** Based on the CellTracker analyses, total migration and average speed of the primary monocytes were determined. **(N,O)** Volcano plots based on RNA sequencing of monocytes cocultivated with M14K at day 1 (D=0), at day 1 (D=1) or at day 1 in presence of VPA (D=1 + VPA).

Conclusion

VPA induces apoptosis of MPM tumor cells and increases the cytotoxic activity of primary monocytes.

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