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Cell line study of nucleosome-based biomarkers in the diagnosis and detection of relapses in glioblastoma

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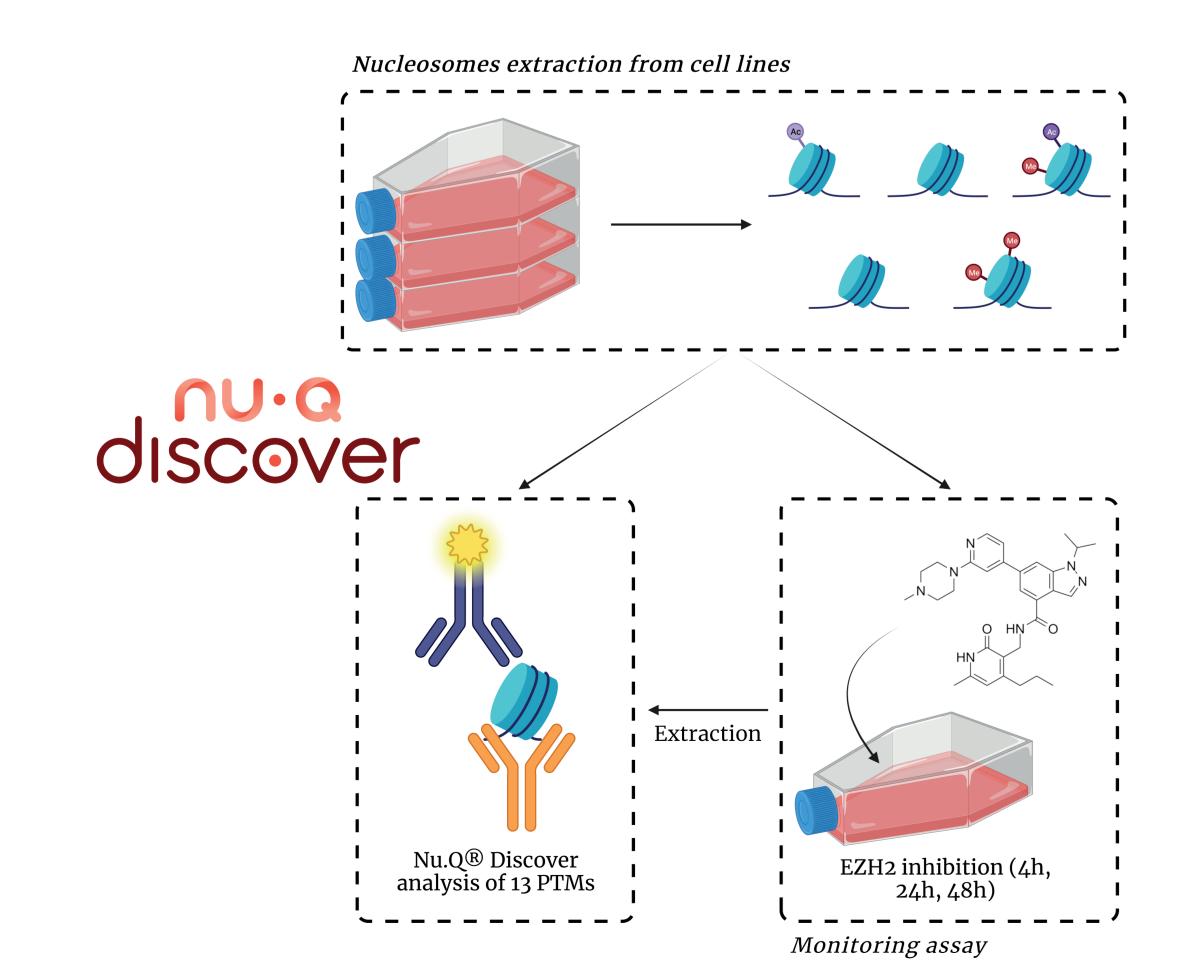
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INTRODUCTION

Glioblastoma (GBM) is the most frequent and most aggressive brain tumor in adults. Given the impossibility of removing the entire tumor, relapse is observed in almost all patients, leading to a 5-year survival rate of around 7%. To date, the diagnosis of GBM mainly relies on imaging techniques (MRI) and tissue biopsies. Tissue biopsy is required to guide clinical making. However, biopsies have limitations: not only are they high risk but there also only represent a snapshot of the whole tumor complexity. Conversely, liquid biopsy (CSF or blood) have the potential to diagnose and monitor the tumor in a dynamic way given the presence of tumor material. To date, no biomarkers have been identified and used in clinical routine for the diagnosis, monitoring and prognosis of GBM in blood or CSF. Within the circulome, this project focuses on epigenetic modifications of nucleosomes, such as post-translational modifications of histones. This approach has already been proven in colorectal, lung or blood cancer.

METHODS

- 4 glioblastoma (GBM) cell lines : U-87MG, U-118MG, U-138MG and SF-126
- 4 control cell lines :
  - 3 non-GBM cancer controls : pancreas (MiaCaPa-2), liver (HepG2) and uterus/cervix (HeLa)
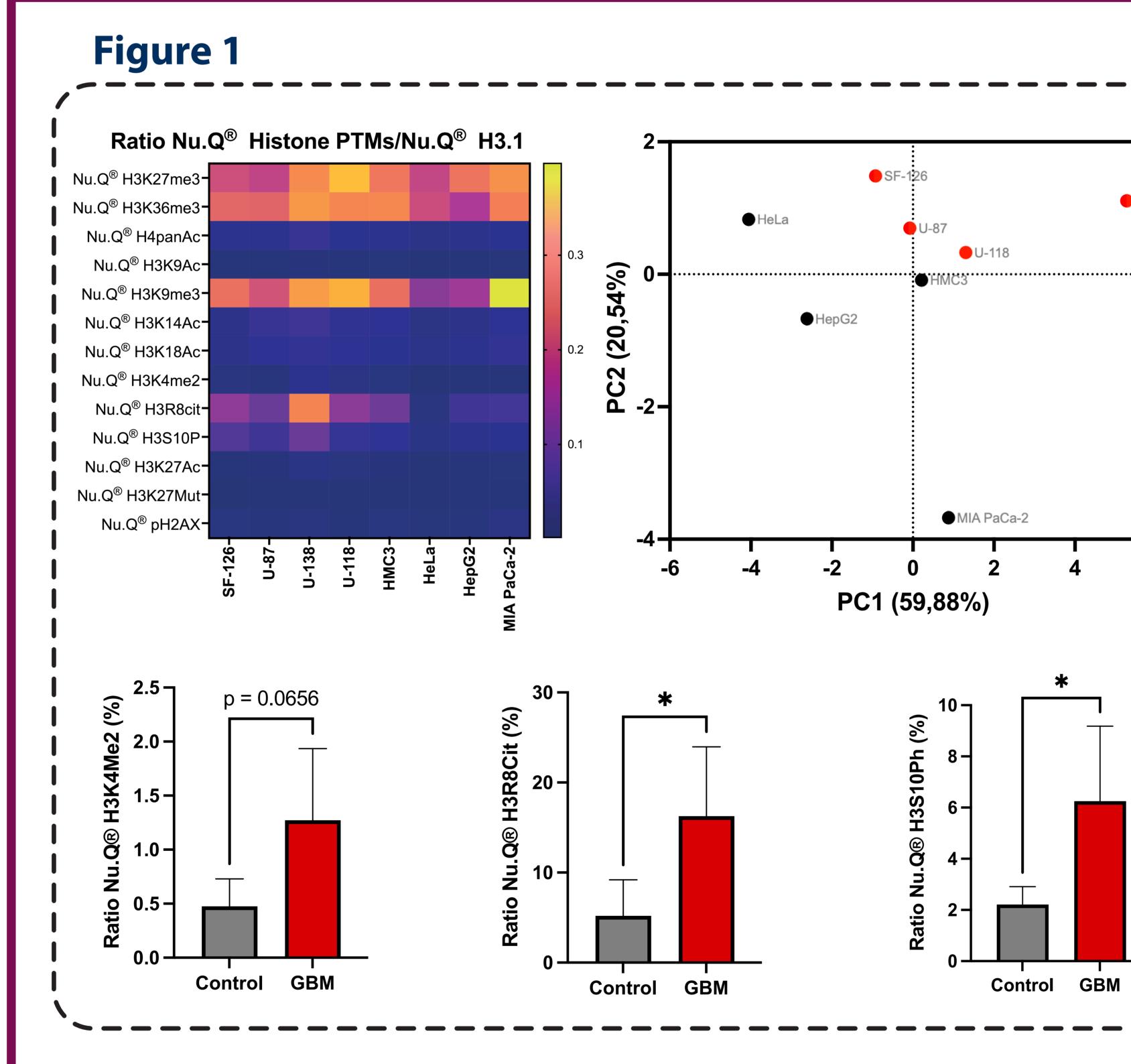 - 1 healthy brain cell line : HMC3

Analytical assay
Nu.Q® Discover assays (Belgian Volition, Belgium) was used for PTMs analysis.
Statistical analysis
The t-test was used for unpaired comparisons and two-way ANOVA Test was used for multiple comparisons using GraphPad InStat software (*: p < 0.05).

RESULTS

Figure 1

In comparison to the other non-GBM cell lines, GBM cells expressed a higher level of citrullinated histone H3 (**H3R8Cit**) (median; control=4.68% (n=4) vs GBM=13.88% (n=4); p<0.05), phosphorylated H3S10 (**H3S10Ph**) (median; control=2.31% (n=4) vs GBM=5.57% (n=4); p<0.05) and an increasing trend for **H3K4Me2**-nucleosome levels (median; control=0.43% (n=4) vs GBM=1.07% (n=4); p=0.066). The principal component analysis (PCA) indicated that the two first principal components (PC) were responsible for about 80.41% of the variance (PC1: 59.88% and PC2: 20.54%). Interestingly, those two **PCs revealed differences between cell lines** with the separation of non-GBM cells to the glioblastoma cells, mainly **driven by the identified PTMs**.
Altogether, these results suggest that **three histone PTMs composed by H3R8cit, H3K4Me2 and H3S10ph could be used as epigenetic pattern** to identify nucleosomes derived from glioblastoma in cell lines.
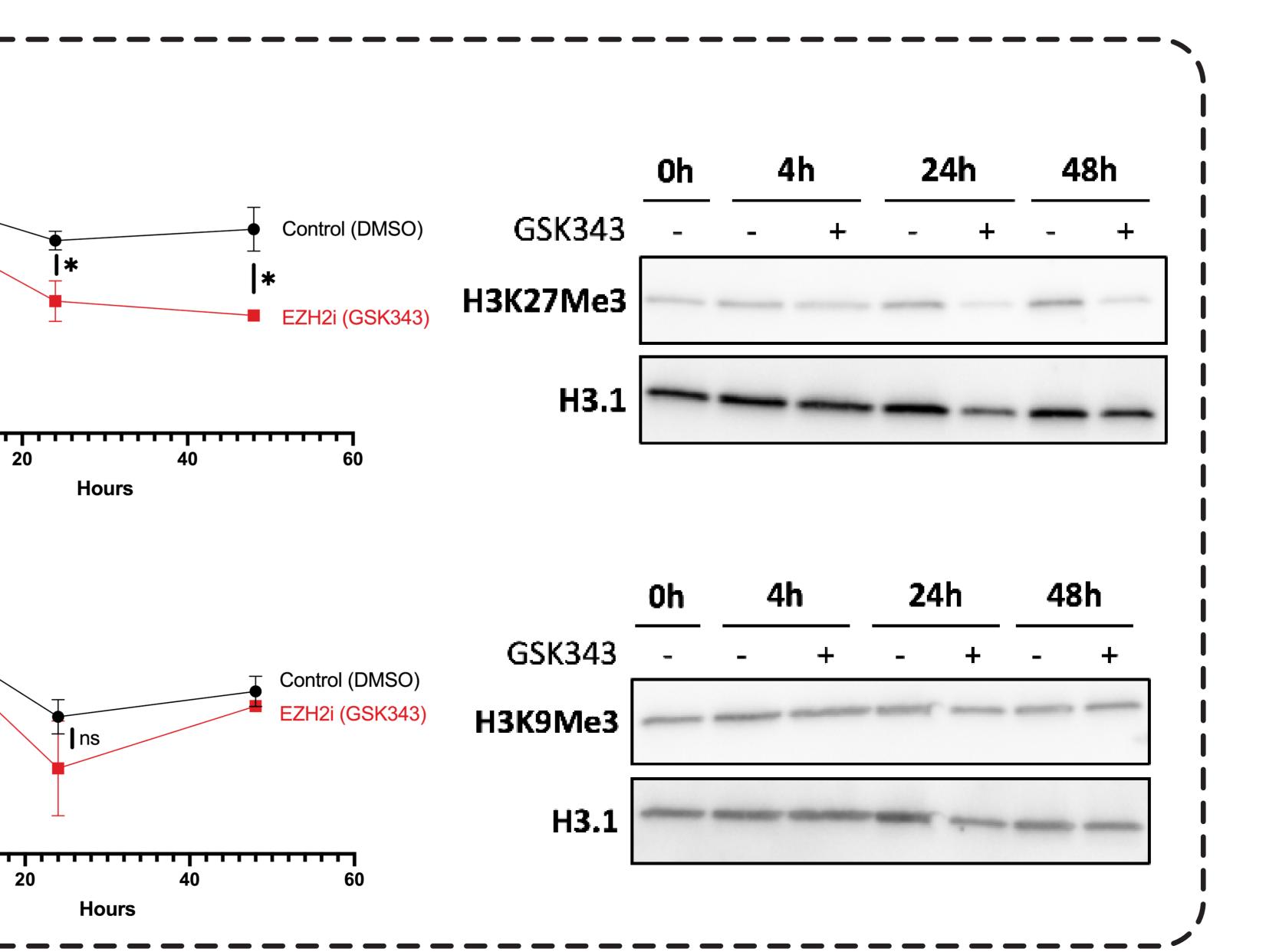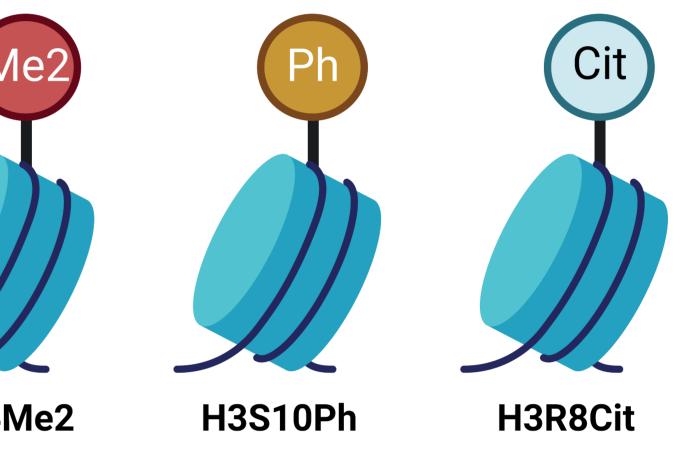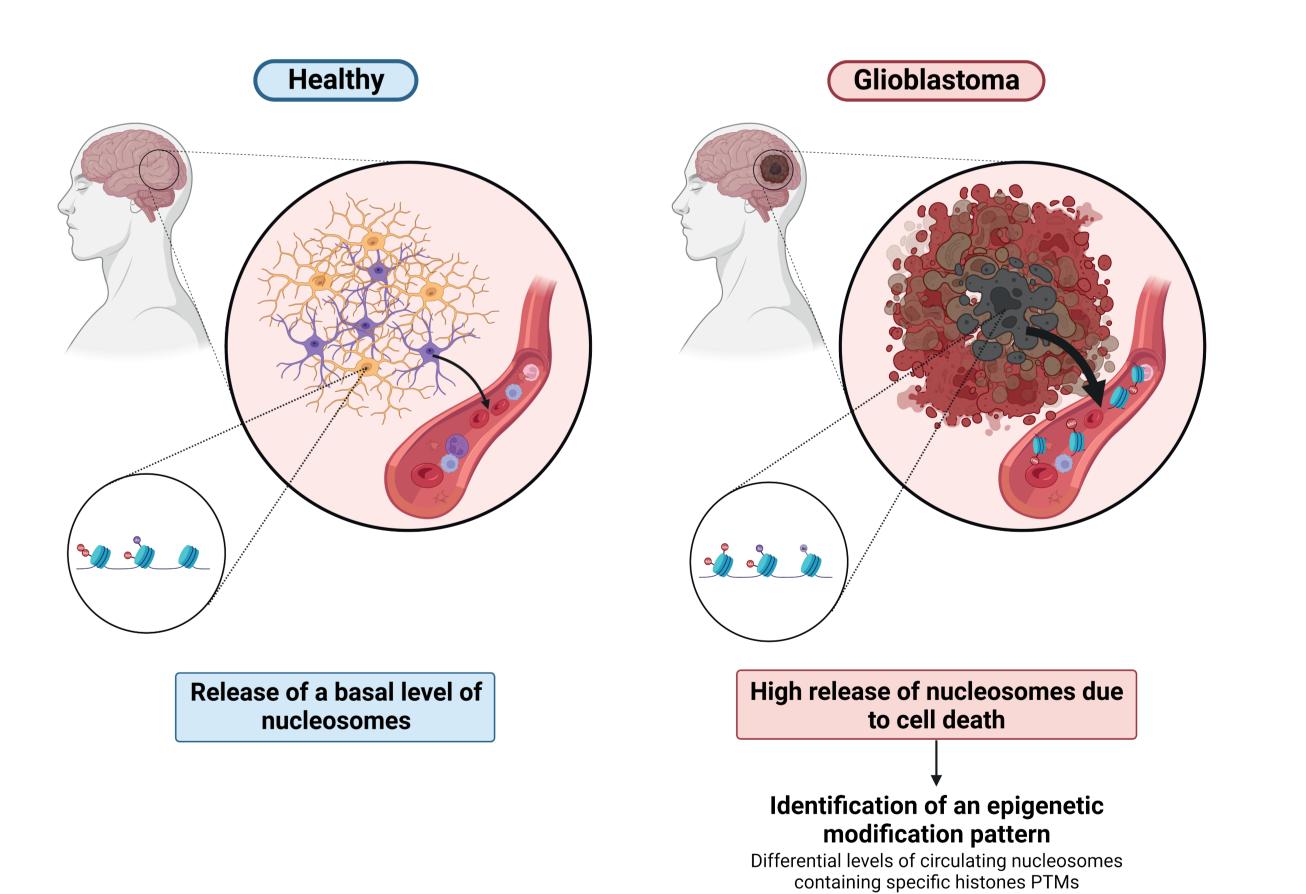
Figure 2

GBM cells were **exposed to GSK343, a selective EZH2 inhibitor** (EZH2i), over time. EZH2 catalyses H3K27 trimethylation without affecting the methylation state of H3K9. In control cells (untreated - DMSO vehicle), the global levels of H3K27Me3 (16.08%±1.59) and H3K9Me3 (22.92%±2.09) remained constant over time. Inversely, a **progressive decrease of H3K27Me3 ratio** was observed during the EZH2i treatment: a **significant decrease** was noted **after 24 hours** (ΔDMSO/EZH2i=32%, p<0.05) **and 48 hours** (ΔDMSO/EZH2i=41%, p<0.05). In parallel, no significant differences were observed between the control condition (untreated) and the EZH2i treatment groups for the expression of H3K9Me3. Those results were confirmed by western-blotting.

CONCLUSIONS/DISCUSSION

An elevation of the H3S10Ph, the H3R8Cit as well as an increasing trend of H3K4Me2 cells have been highlighted. Those PTMs have been linked with glioblastoma, notably H3S10Ph which has been observed in tumor resections of patients presenting a poor survival. One limitation is the use of a cellular model requiring nucleosomes extraction. Whereas, in vivo, there is a limited release of nucleosomes in the healthy subject's bloodstream while such a release will be observed in GBM patients due to a blood-brain barrier disruption. Moreover, this project highlighted the potential usefulness of the Nu.Q® Immunoassays platform in the monitoring of GBM.

ACKNOWLEDGEMENT / DOI

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