Targeting the O-GlcNAcase Transferase in Leiomyosarcoma and Desmoid-type Fibromatosis

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Introduction

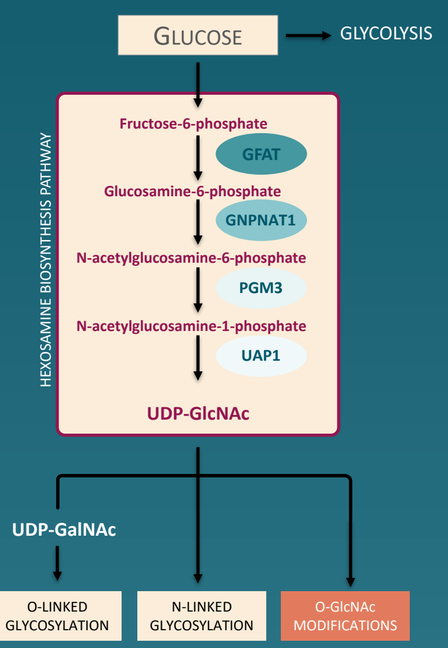
Different histopathological subtypes of sarcoma exhibit distinct aggressive properties and clinical behavior. **Desmoid-type fibromatosis** (DTF) originates from fibroblasts, is **locally aggressive**, and often recurs post-surgery but typically does not metastasize. In contrast, **leiomyosarcomas** (LMS) arise from smooth muscle cells, are **malignant**, and can metastasize to distant organs.

The current standard treatment options for sarcoma includes surgery, radiation, and chemotherapy, but these have **unsatisfactory response rates and resistance issues**. Targeted therapies and immunotherapies in clinical trials show variable outcomes. **Therefore, exploring new therapeutic targets for sarcoma is essential.**

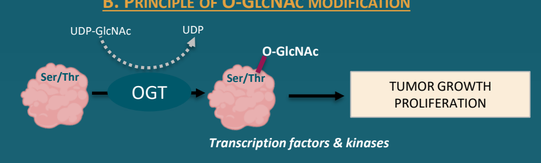
Our team focuses on onco-metabolism, specifically the glycoproteome—proteins modified by glycosylation. The **hexosamine biosynthesis pathway** (HBP) produces precursors of glycosylation (**A**). We found that GFPT2, the first and rate-limiting HBP enzyme, is linked to poor prognosis in LMS patients (PMID: 33941787). We are particularly interested in studying O-GlcNAcylation, the addition of an O-GlcNAc residue to proteins by **O-GlcNAc transferase (OGT)** (**B**)**.** In other cancers, O-GlcNAcylation affects oncogenes, tumor suppressors, and protein kinases, contributing to tumor development and chemotherapy resistance (**B**).

Our research aims to understand OGT regulation in LMS and DTF, and how OGT inhibitors can **impede tumor growth and overcome treatment resistance**. Comparing LMS and DTF is intriguing as it highlights the clinical spectrum of sarcomas. We aim to elucidate the mechanisms of action of OGT inhibitors, the regulation of OGT in these sarcomas, and identify O-GlcNAcylated proteins contributing to tumor progression and treatment resistance.

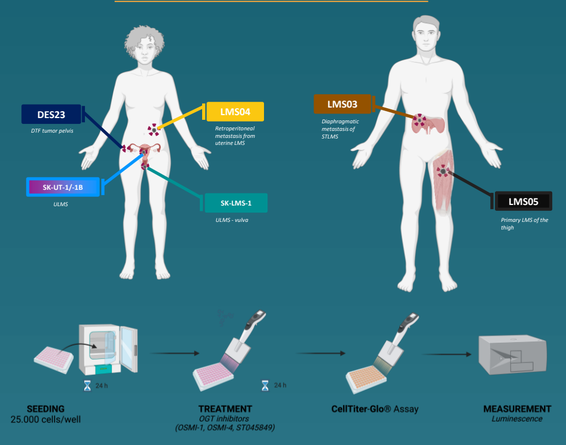
A. The Hexosamine Biosynthesis Pathway (HBP)



B. Principle of O-GlcNAc modification



C. Cell lines & Experimental approaches



Hypothesis & objectives

- We hypothesize that treatment of LMS and DTF cell lines with OGT inhibitors will **reduce cell viability**. To address this question, we will evaluate cell viability after 24-h treatment with with 3 OGT inhibitors (OSMI-1, OSMI-4, ST045849).
- We aim to **combine OGT inhibition with standard LMS and DTF therapies** (doxorubicin) to assess potential combinatory effects or decreased resistance. This study will be the first to combine HBP-targeting drugs with standard chemotherapies and targeted therapies for LMS and DTF.

Experimental design

- **Cell lines:** 6 LMS cell lines (**C**): 3 uterine LMS lines from ATCC (SK-LMS-1, SK-UT-1, SK-UT-1B)
  3 LMS cell lines obtained from Dr. Jonathan Fletcher (Harvard University)
  1 DTF cell line: DES23 obtained from Dr. Raphael Pollock (Ohio State University)
- **Experimental approach:** CellTiter-Glo® Assay (**C**) - Annexin V/7AAD Apoptosis Assay

Results

- **Targeting OGT with 3 OGT inhibitors in 5 LMS cell lines and 1 DTF cell line**

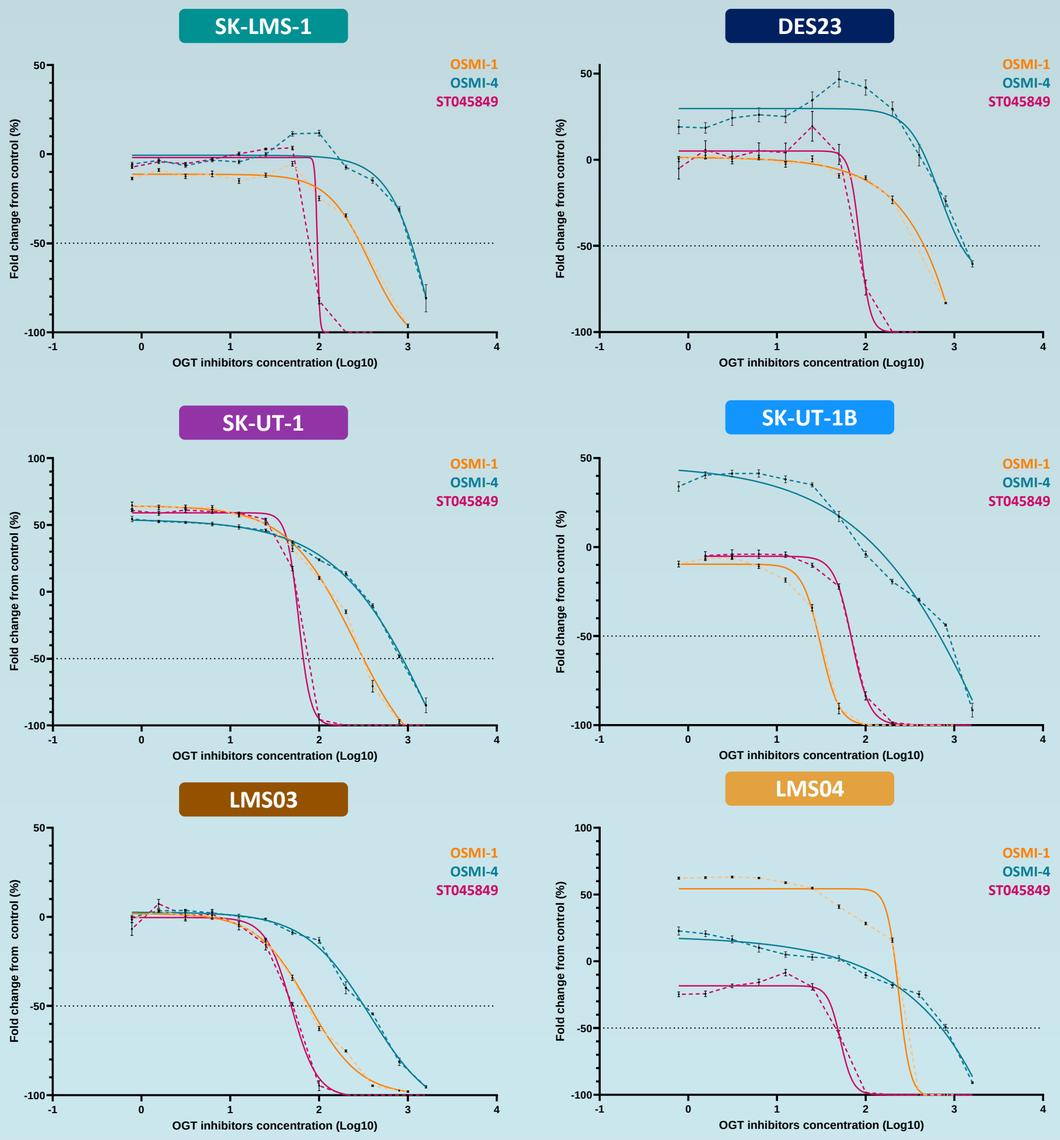


Figure 1 OGT inhibitors – Dose response curves to identify IC_{50}

*Cell viability was assessed after 24-hour treatment with OGT inhibitors within a range of concentration from 0.7 to 1600µM by Cell Titer Glo Assay. Viability (%) calculation is as follow ((Va-Vd)/Vd)*100 with Va= luminescence value of the treated well and Vd= luminescence value of control well. N= 6-8 biological replicates.*

CELL LINES /OGT INHIBITORS	OSMI-1 IC_{50} (µM)		OSMI-4 IC_{50} (µM)		ST045849 IC_{50} (µM)	
	Experimental	*Calculated*	*Experimental*	*Calculated*	*Experimental*	*Calculated*
SK-LMS-1	200-1000	289.2 to 681.0	800-1600	>1022	50-100	95.43
DES23	200-800	>729.0	800-1600	494.0 to 1329	50-100	>74.83
SK-UT-1	200-400	215.5 to 302.5	≈800	1103 to 17968	50-100	56.68 to 60.31
SK-UT-1B	25-50	30.03 to 32.41	≈800	>688.9	50-100	67.34 to 73.16
LMS03	50-100	70.94 to 81.79	200-400	313.9 to 466.7	≈50	45.74 to 52.43
LMS04	200-400	<250.8	≈800	>41841	≈50	50.01 to 54.68

Table 1: OGT inhibitors IC_{50} values

→ Cell viability is significantly decreased in LMS and DTF cell lines after 24h of treatment with OGT inhibitors with the IC_{50} values around 800µM for OSMI-4 and 50µM for ST045849. OSMI-1 present variable IC_{50} values across tested cell lines

- **OSMI-1 increases apoptosis**

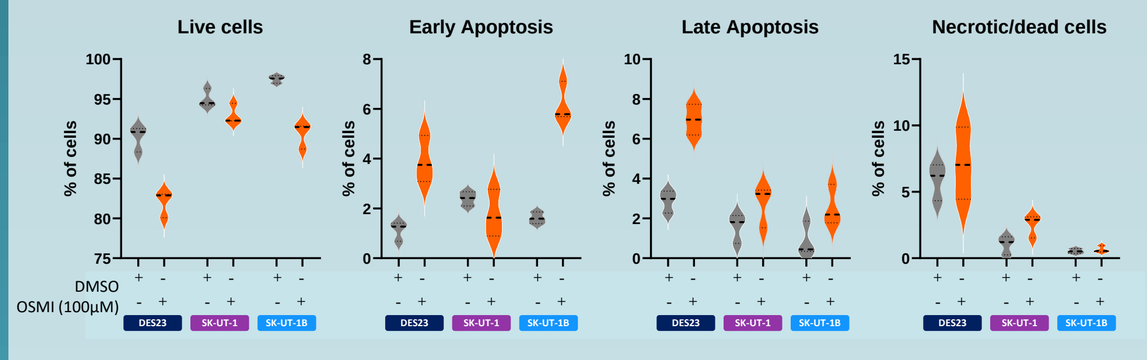


Figure 2 Apoptosis assay after OGT Inhibition

300,000 cells were seeded in 6-well plate and treated for 24 hours with 100µM OSMI-1 treatment or DMSO control. Apoptosis was evaluated by Annexin V/7AAD assay and analyzed with Canto II cytometer. N=3.

→ OSMI-1 treatment decreases cell viability and tends to promote early or late apoptosis in DES23, SK-UT-1 and SK-UT-1B cell lines

- **OSMI-1 potentiates doxorubin (DOX) treatment in selected cell lines**

Figure 3 Evaluation of cell viability after combination of OSMI-1 and DOX in LMS and DTF cell lines.

*Cell viability was assessed after 24-hours OSMI-treatment (100µM) combined or not with doxorubicin (1µM). n=2 individual experiment - 6 biological replicates per assay. Luminescence was normalized based on luminescence of untreated cells. Two-way ANOVA. * $p < 0.05$ *** $p < 0.001$; **** $p < 0.0001$.*

→ SK-LMS-1 are resistant to both OSMI-1 and DOX treatment
→ OSMI-1 alone has comparable effect as DOX in SK-UT-1, SKU-UT-1B and LMS05 cell lines and slightly potentiates DOX effect in combination.
→ In LMS03 and LMS05, OSMI-1 alone decreases cell viability while DOX has no effect
→ In DES23 cell line, OSMI-1 treatment decreases cell viability to a lesser extent than DOX (treatment was done with dose below the determined IC_{50}) and slightly potentiates the effect of DOX in combination.

Conclusion & Perspectives

Our results highlights the potential of targeting the Hexosamine Biosynthesis Pathway (HBP) through pharmaceutical inhibition of O-GlcNAc transferase (OGT). Using multiple leiomyosarcoma cell lines of different origin and one desmoid-type fibromatosis cell line, our results show that **OGT inhibition decreases cell viability in a dose dependent manner**. We also highlight that the OGT inhibitor **ST045846 has a higher potential to decrease cell viability at lower concentration** than OSMI-1 and OSMI-4 inhibitors. We also demonstrate that OSMI-1 treatment may increase apoptosis in selected LMS cell lines and DTF cell line.

Finally, our preliminary results shows that **OSMI-1 has a comparable effect as doxorubicin alone** but does not present a potentiator effect on doxorubicin efficiency.

Our future perspectives include the evaluation of apoptosis level after other OGT inhibitors (OSMI-4 and ST045849), in **combination with standard chemotherapies** (Doxorubicin, Gemcitabine, Docetaxel, Trabectedin - for LMS cell lines and Nirogacestat for DTF). We will also investigate the mechanisms of action underlying OGT inhibition effect on cell viability and apoptosis.

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