Physio-pathological properties of colon cells are regulated by the nucleocytoplasmic OGT

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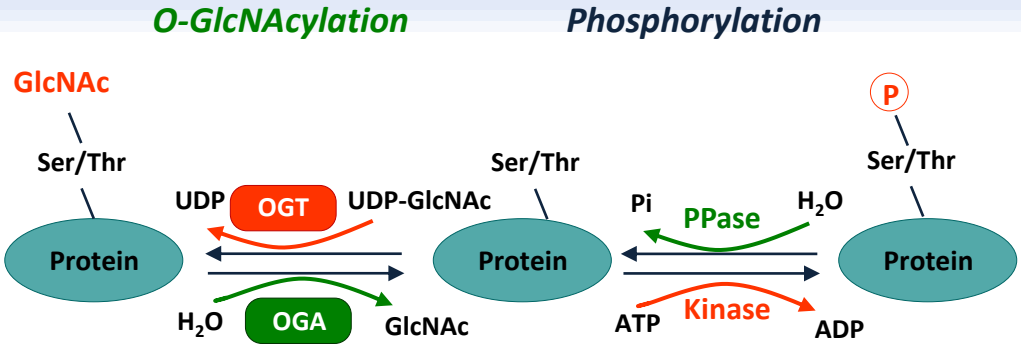
O-GlcNAc modification

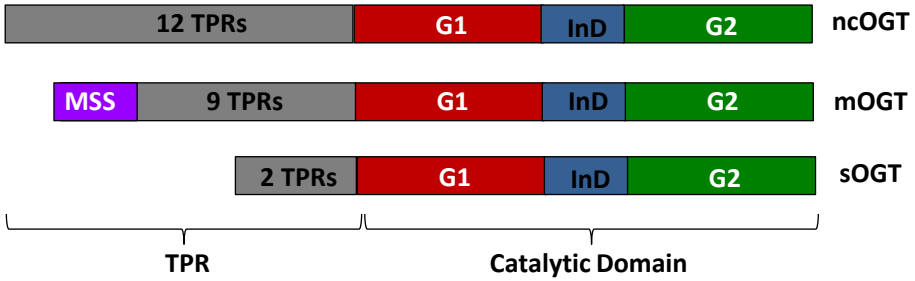
- ***O-GlcNAcylation*** **- dynamic and reversible PTM nucleocytoplasmic and mitochondrial proteins by addition of *O*-GlcNAc onto Ser/Thr residues**



- ***O-GlcNAcylation*** **- controled by OGT and OGA**

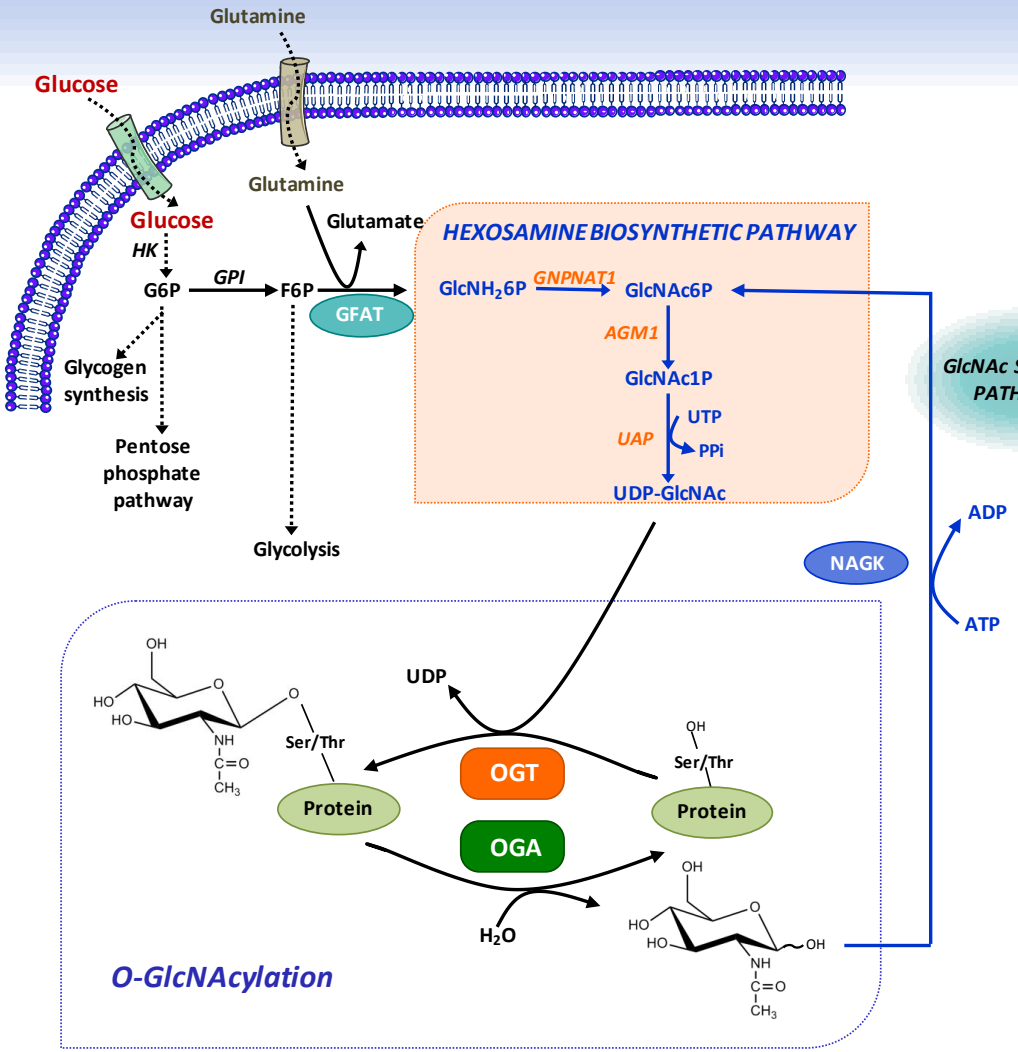
- ***O*-GlcNAcylation interplays with Ser/Thr phosphorylation and regulates fundamental cellular processes eg. cell cycle, cell signaling and protein trafficking**

- ***OGT*** **(*O*-GlcNAc transferase), key and unique regulator of PTM**



Hexosamine biosynthetic pathway and O-GlcNAcylation

- 2-3% cellular glucose enter the hexosamine biosynthetic pathway (HBP) to produce UDP-GlcNAc
- *OGT* uses *UDP-GlcNAc*, from the nutrient dependent HBP
- *UDP-GlcNAc*: nutritional sensor
- *Upregulation of HBP by increased glucose flux directly elevates UDP-GlcNAc levels as well as intracellular protein O-GlcNAcylation to modulate their localization, stability and activity (β-catenin & CRC)*



Glucose flux, UDP-GlcNAc pool are implicated in global O-GlcNAc levels through OGT

What is the role of OGT in cancer development ?

Role the OGT expression in cancers

Overexpression of OGT

Breast cancer :

↗O-GlcNAcylation in cancer tissues/adjacent tissues and in metastatic lymph nodes /breast cancer tissues.

shOGT⊣ cell migration/invasion and number of metastatic lung nodules (Gu *et al*., 2010)

→ important roles in cancer initiation and metastasis.

Prostate cancer :

↗ OGT in cancer tissue/normal epithelium , associated with poor prognosis

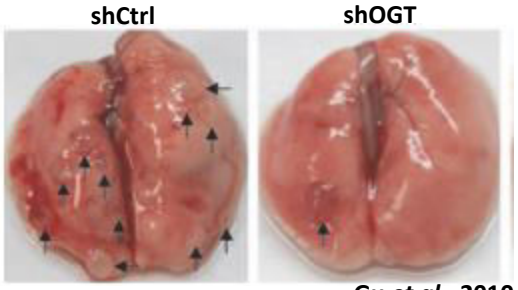
↗ OGT and *O*-GlcNAc levels in cancer cell lines/non-transformed prostate cells

siOGT⊣growth, invasion, angiogenesis and metastasis of prostate cancer cells (Lynch *et al*., 2012)

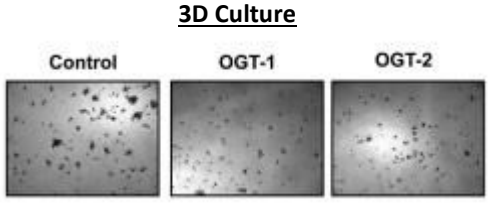
Colon cancer :

↗ O-GlcNAcylation of colon cancer tissues /adjacent tissues.

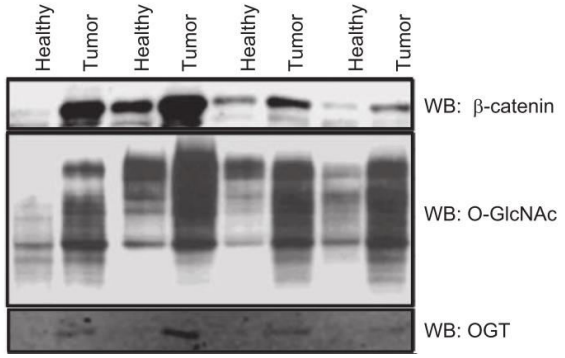
siOGT ⊣ colony formation of HT29 cells (Mi *et al*., 2011)



Gu *et al*., 2010



Lynch *et al*., 2012

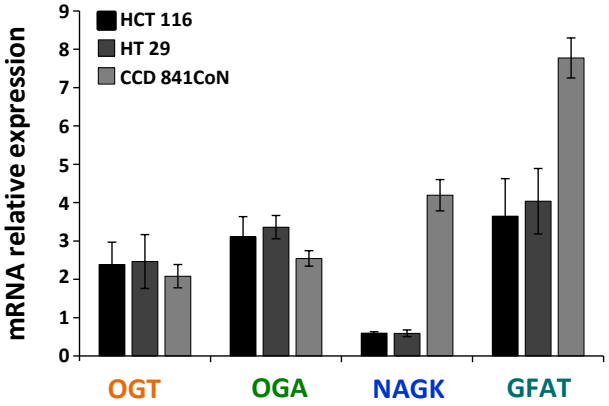


Olivier Van-Stichelen *et al*., 2014

What is the impact of OGT knock-down on biological properties of colon cell lines?

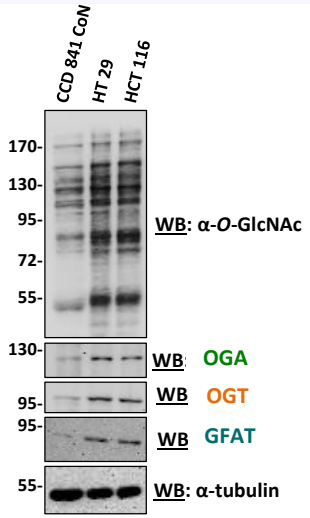
OGT expression and O-GlcNAcylation level in colon cell lines

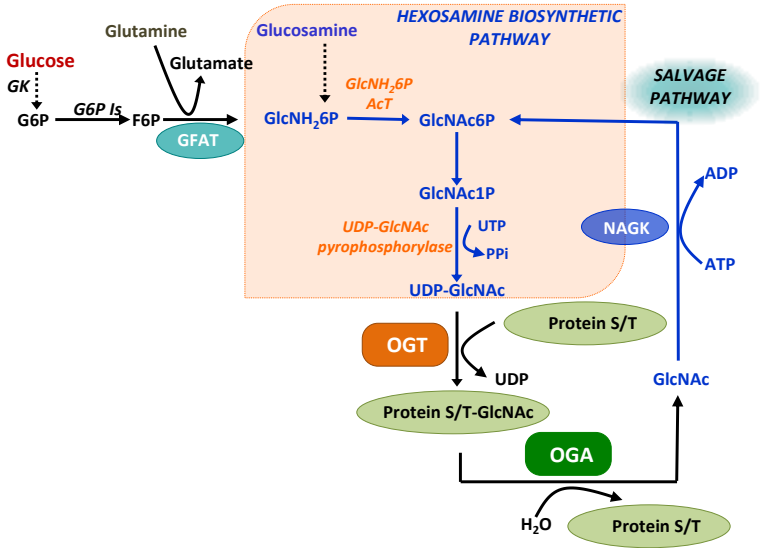
1.qPCR analysis to the mRNA levels of O-GlcNAc cycling enzymes



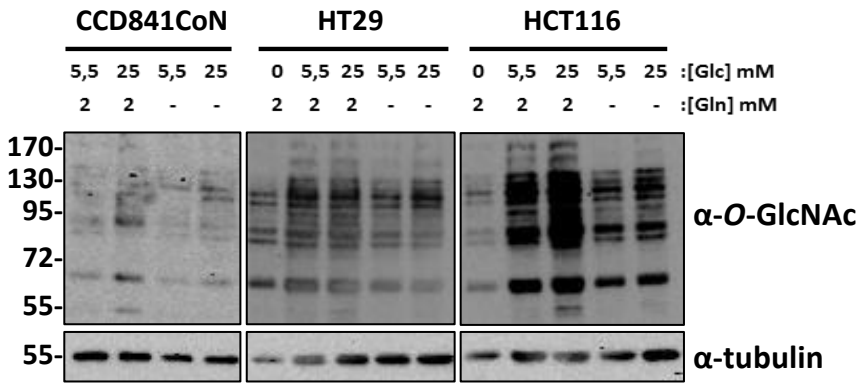
No correlation between mRNA profile expression and protein pattern

2. Western Blot of O-GlcNAc cycling enzymes





3. O-GlcNAcylation rate depends on glucose concentration and glutamine

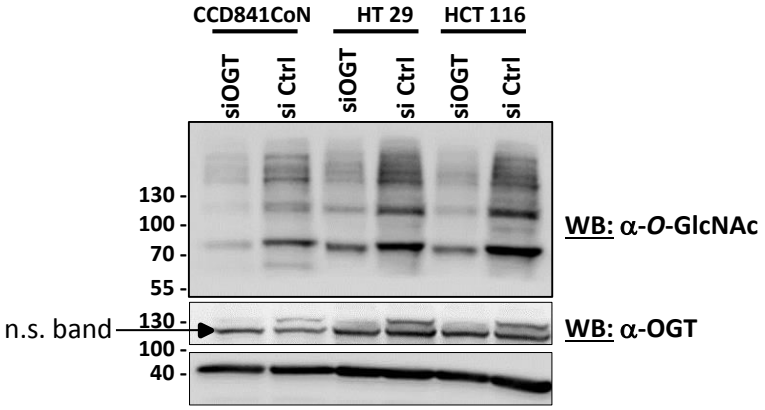


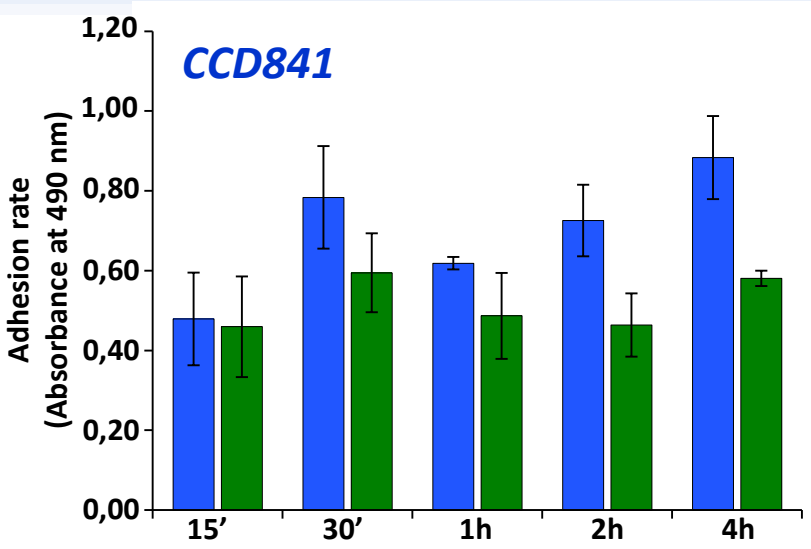
HCT116 colorectal carcinoma, HT29 colorectal adenocarcinoma, CCD841 fetal

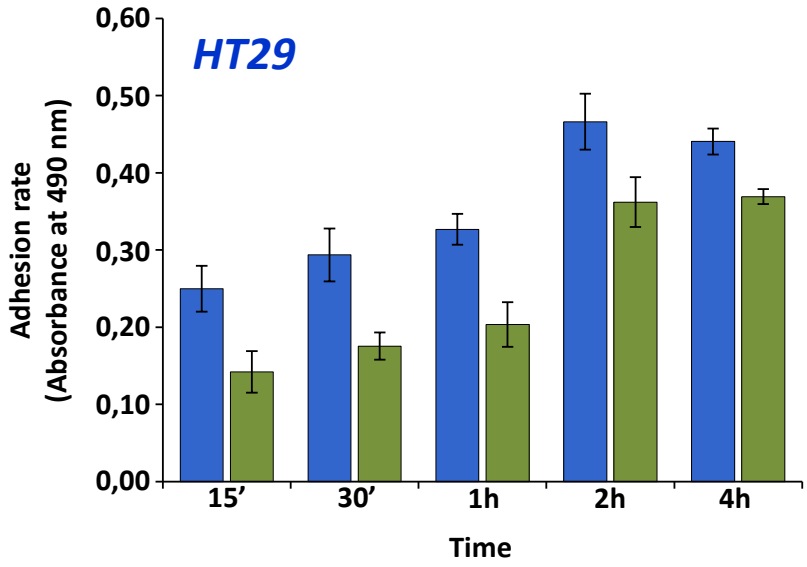
OGT silencing decreased cell adhesion

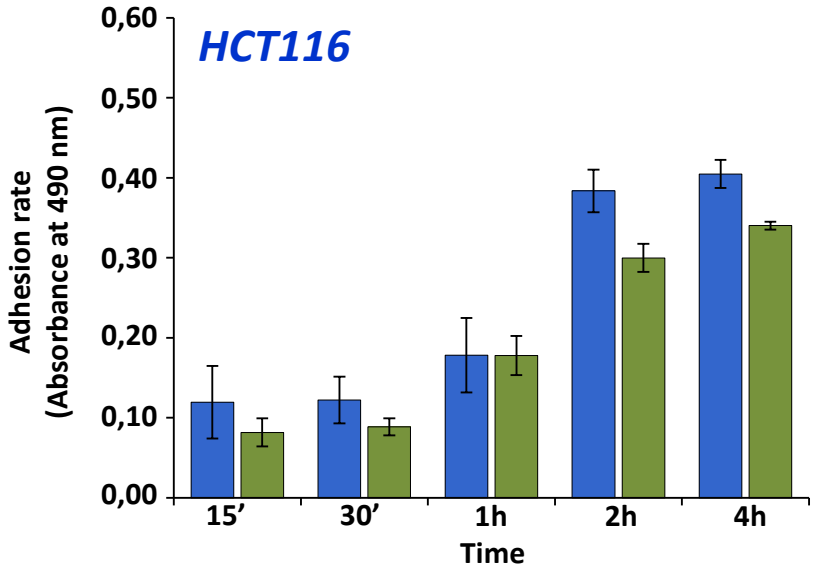
siCrl
siOGT

OGT silencing reduced OGT expression as well O-GlcNAcylation in the three colon cell lines

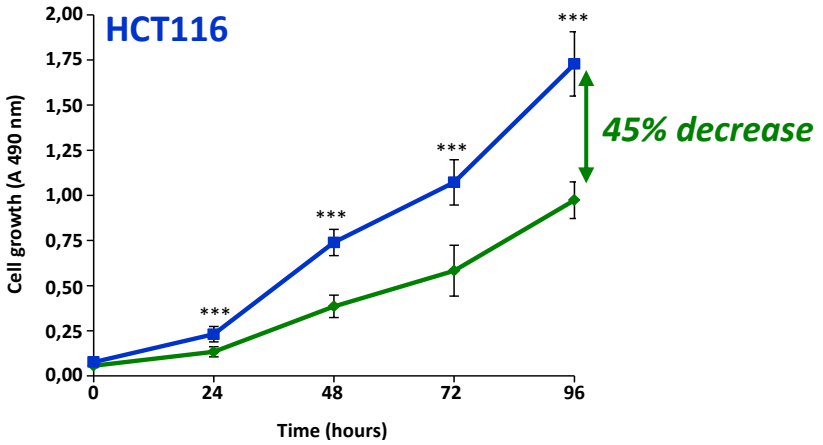


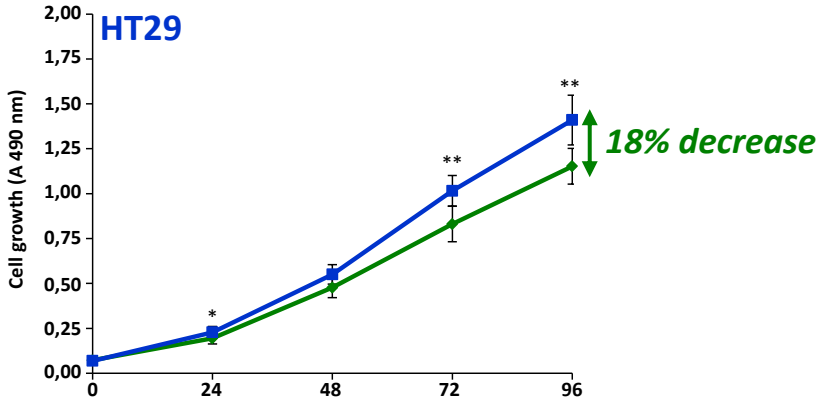


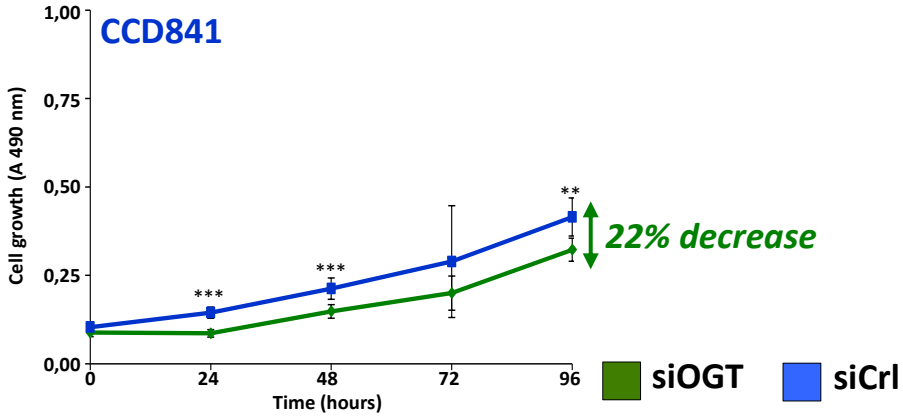


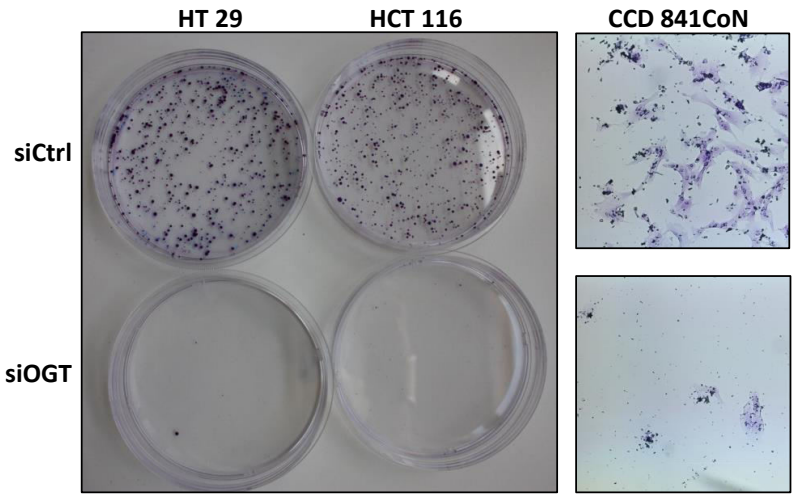


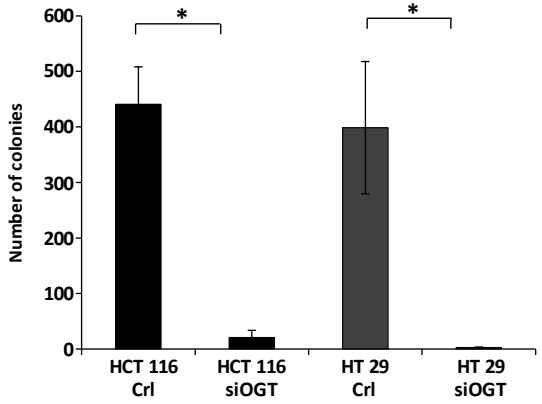
OGT silencing decreases cell proliferation and colony formation



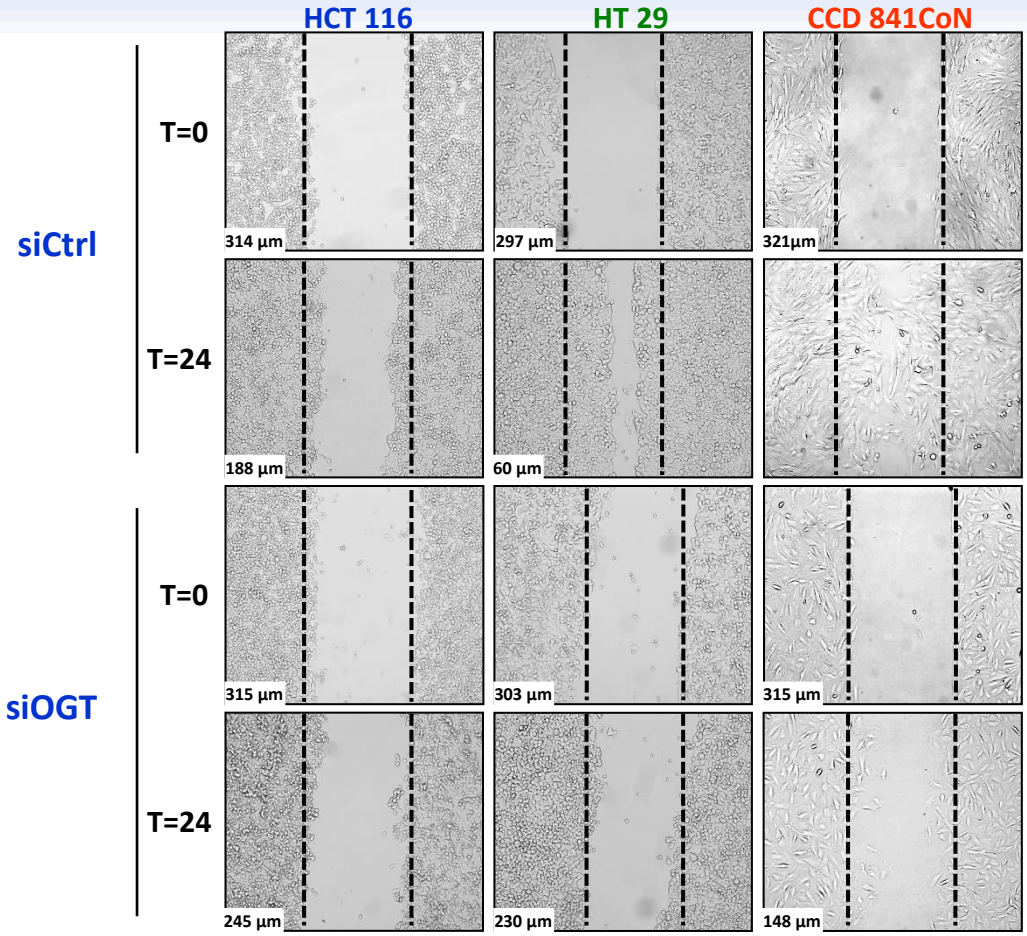




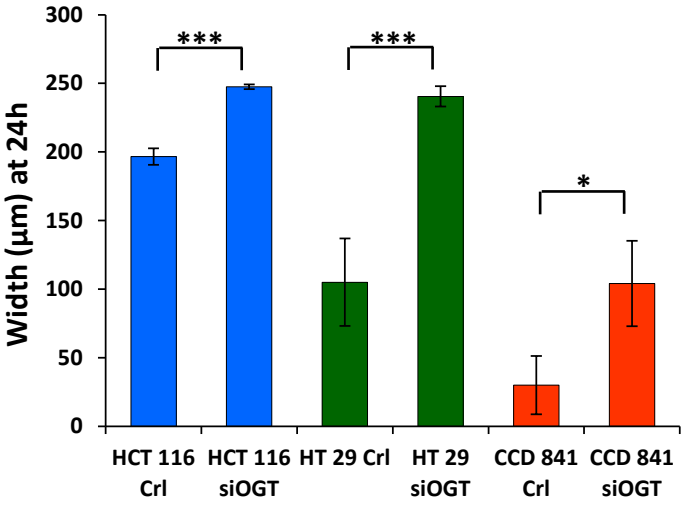




OGT silencing expression inhibited cell migration

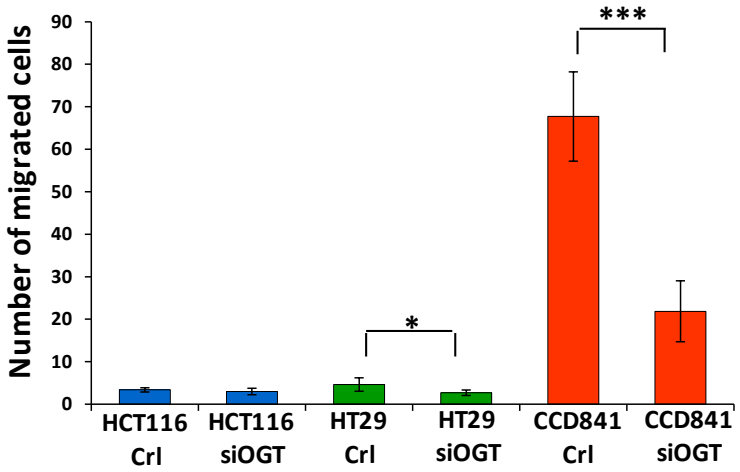


Wound healing assays

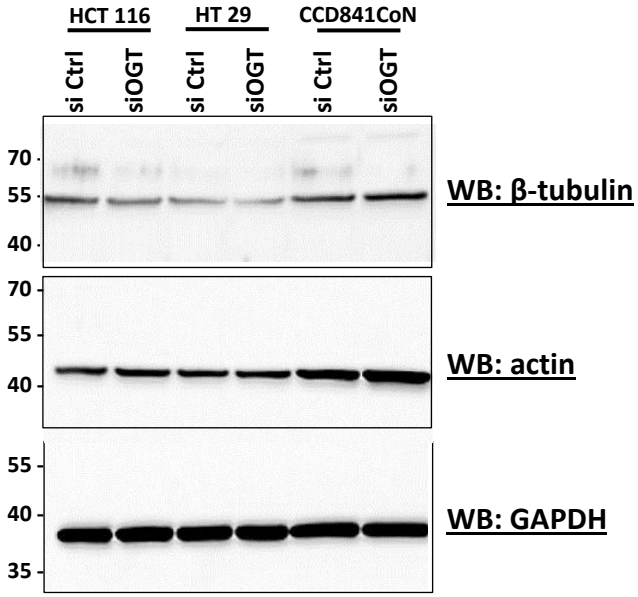


Cell migration and cytoskeletal proteins in colon cell lines

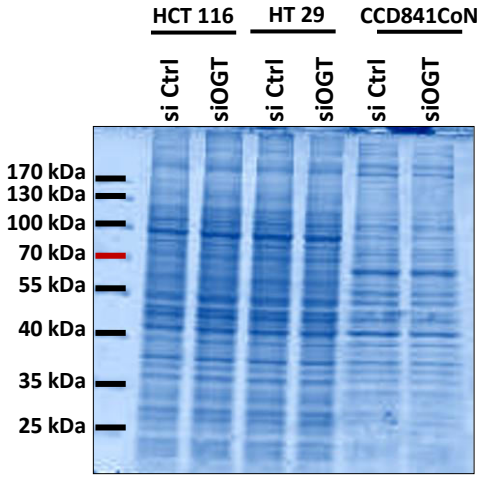
OGT silencing reduced migration of CCD841CoN compared to cancer cell lines using the Transwell system



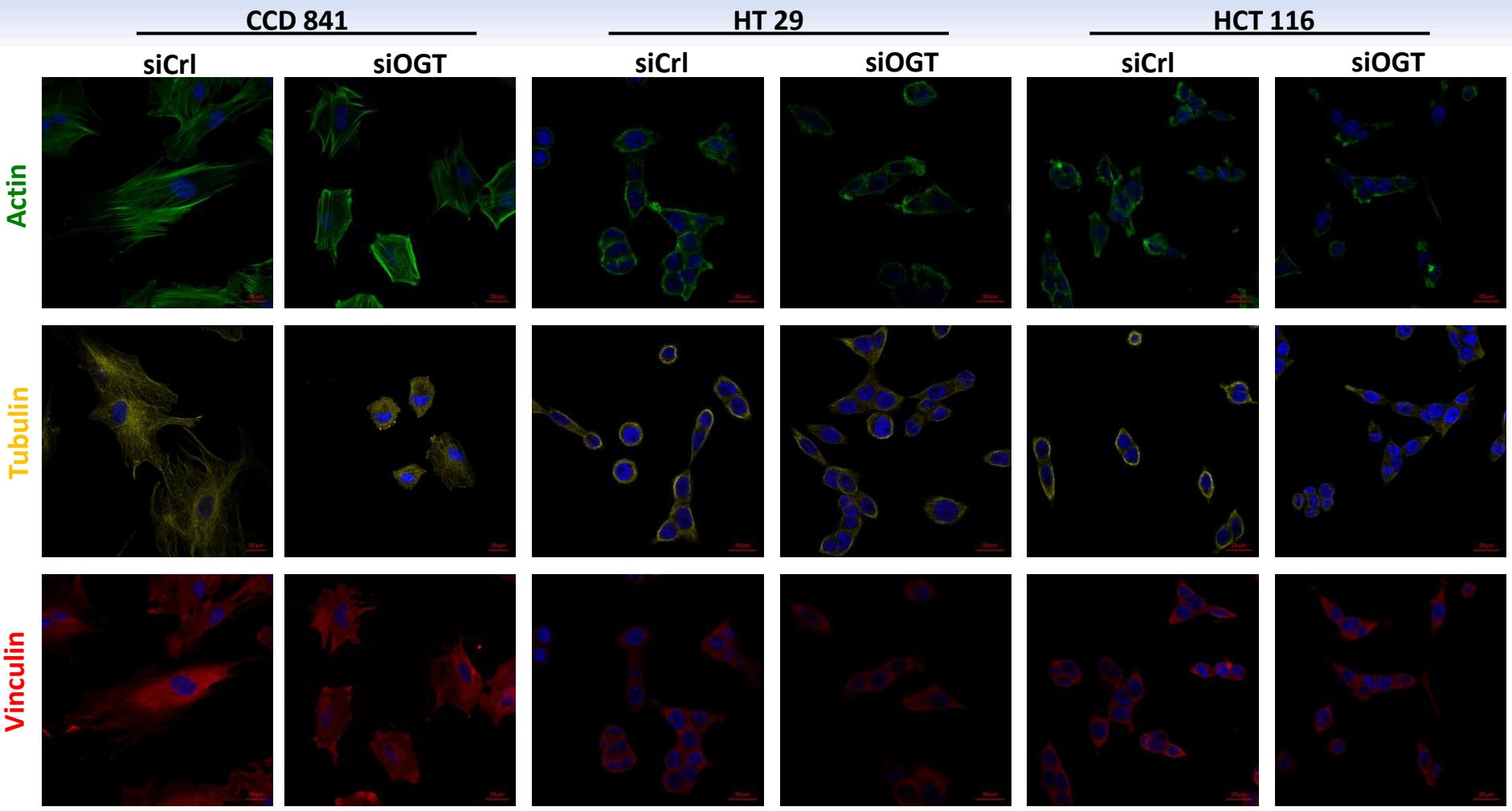
Cytoskeletal proteins profile



Visualizations the proteins by Bleu Coomassie

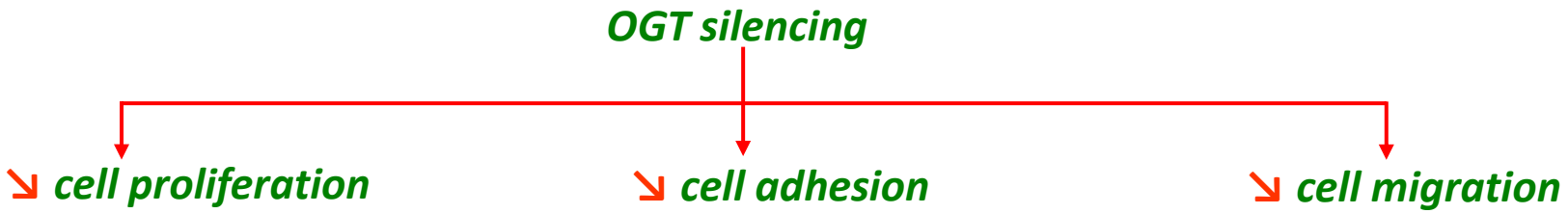


Cytoskeleton network in colon cell lines



OGT silencing greatly affected the cytoskeletal networks and cell morphology, particularly in CCD841CoN cells. The cell shape appeared stocky and stunted whereas the microfilament network, responsible for cell migration, was less extended.

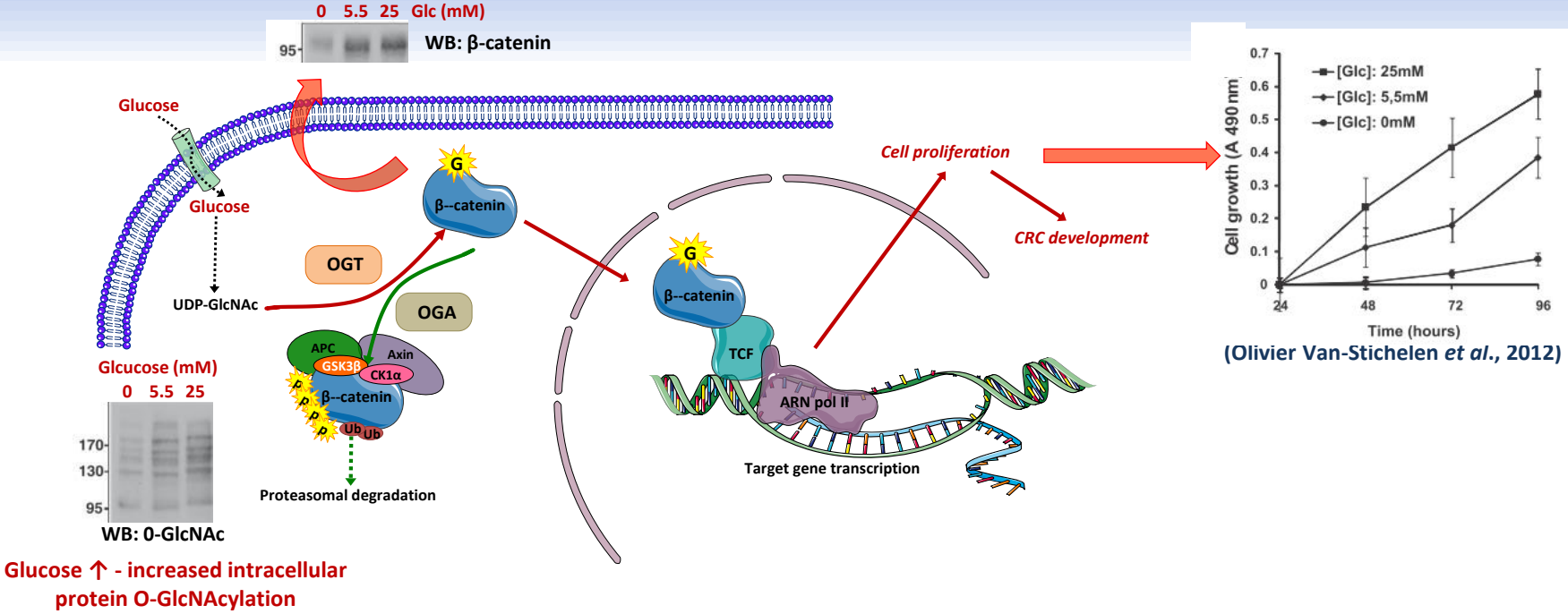
Conclusion



- ✓ *OGT silencing decreased O-GlcNAcylation level, proliferation, adhesion and migration of HT29, HCT116 and CCD841 cell lines*

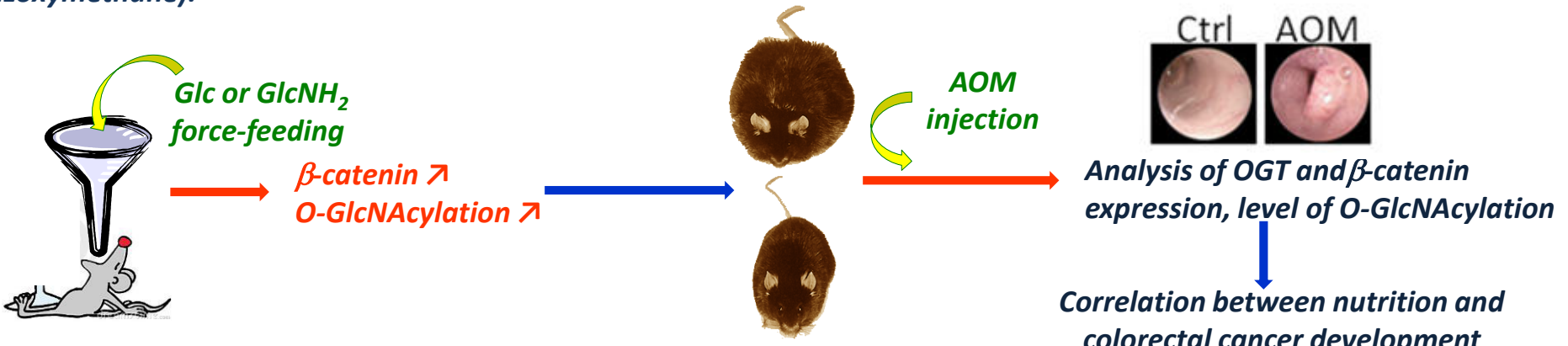
- ✓ *We showed that OGT expression is not only necessary for the biological properties of cancer cells but also for normal cells*

Previously : O-GlcNAcylation and β-catenin stability



Perspectives

In vivo model : fat mice (high carbohydrate diet) and C57Bl6 mice (regular diet) injected with AOM (azoxymethane).



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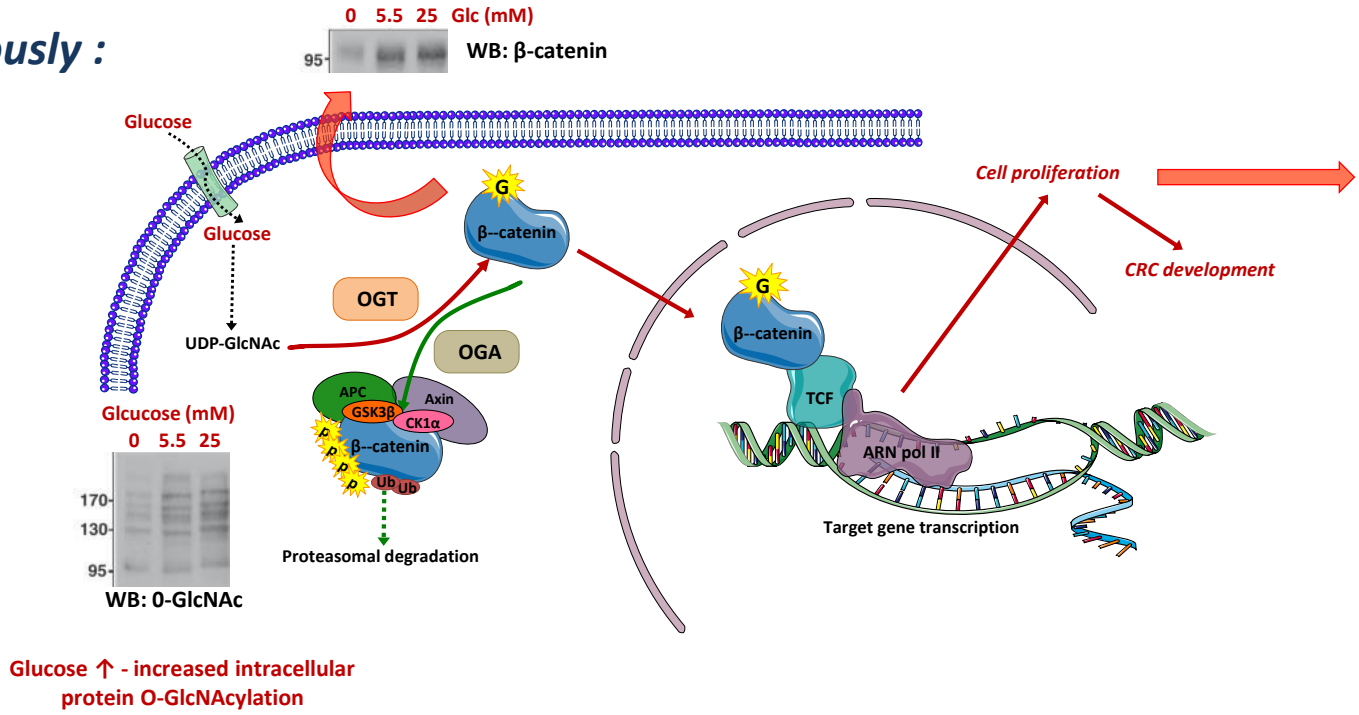
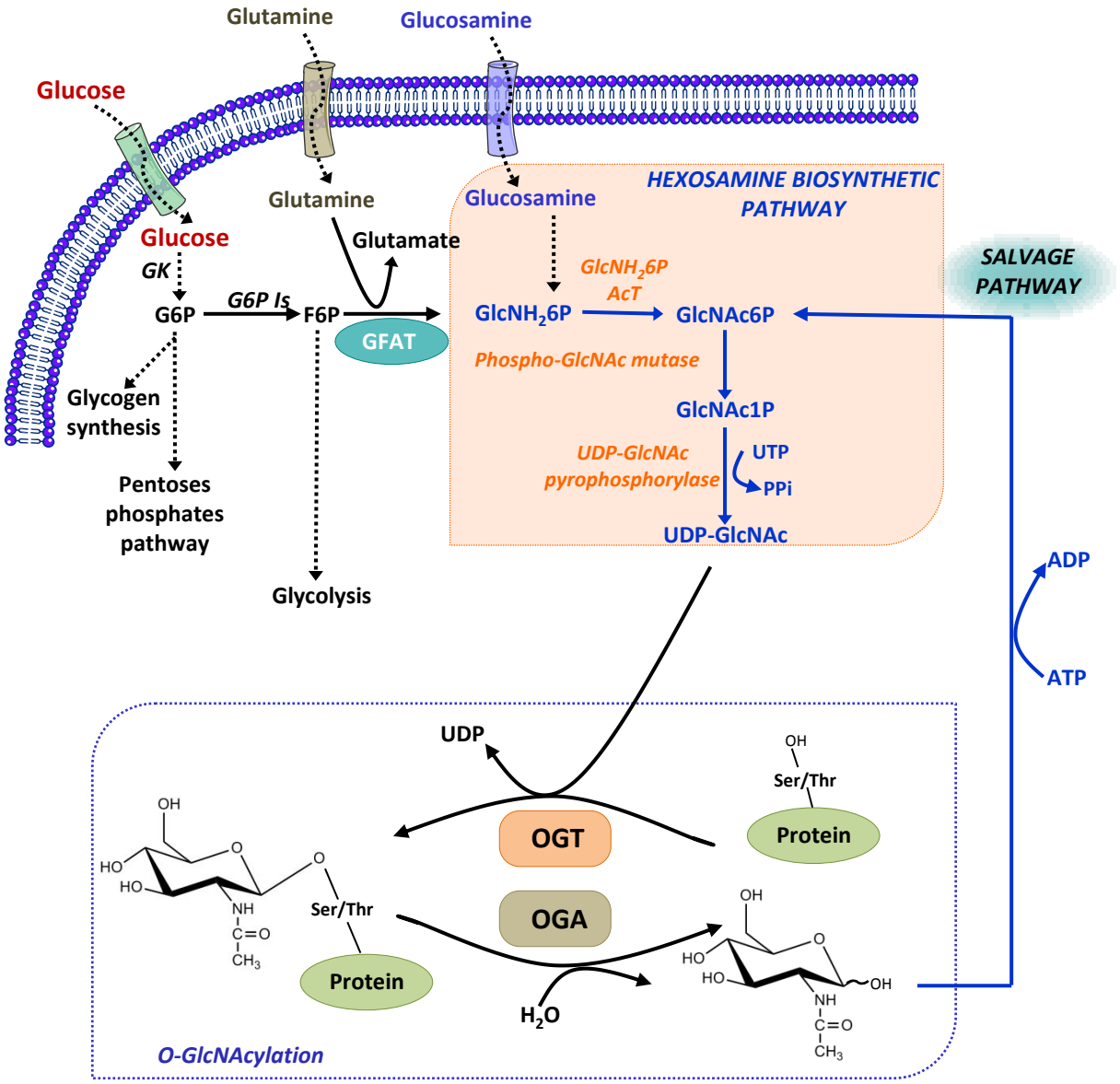
Christian Slomianny and Elodie Richard

Thank you for your attention !

Previously :



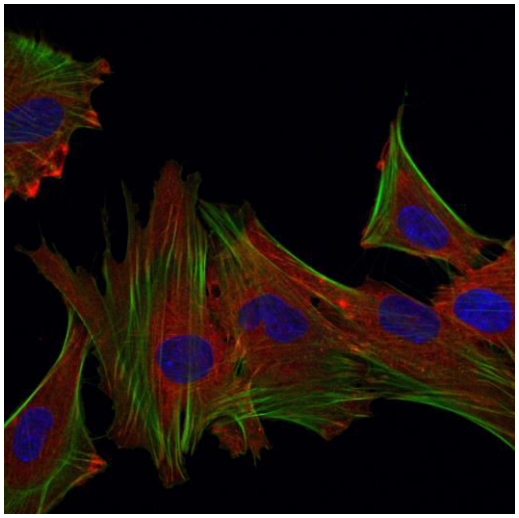
Glucose ↑ - increased intracellular protein O-GlcNAcylation

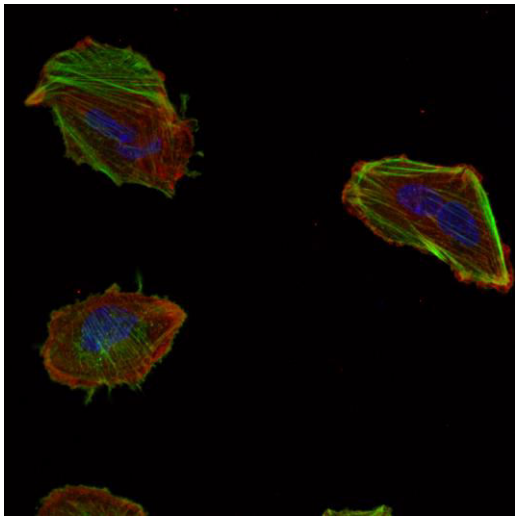

Glutamine
Glucosamine
Glucose
Glutamine
Glucose
Glutamate
GK
G6P
G6P Is
F6P
GFAT
Glycogen synthesis
Pentoses phosphates pathway
Glycolysis
Glucosamine
HEXOSAMINE BIOSYNTHETIC PATHWAY
GlcNH2 6P AcT
GlcNH2 6P
GlcNAc6P
Phospho-GlcNAc mutase
GlcNAc1P
UDP-GlcNAc pyrophosphorylase
UTP
PPi
UDP-GlcNAc
SALVAGE PATHWAY
ADP
ATP
UDP
OGT
Ser/Thr
Protein
OGA
H2O
Ser/Thr
Protein
O-GlcNAcylation

siCrl

siOGT

CCD 841CoN



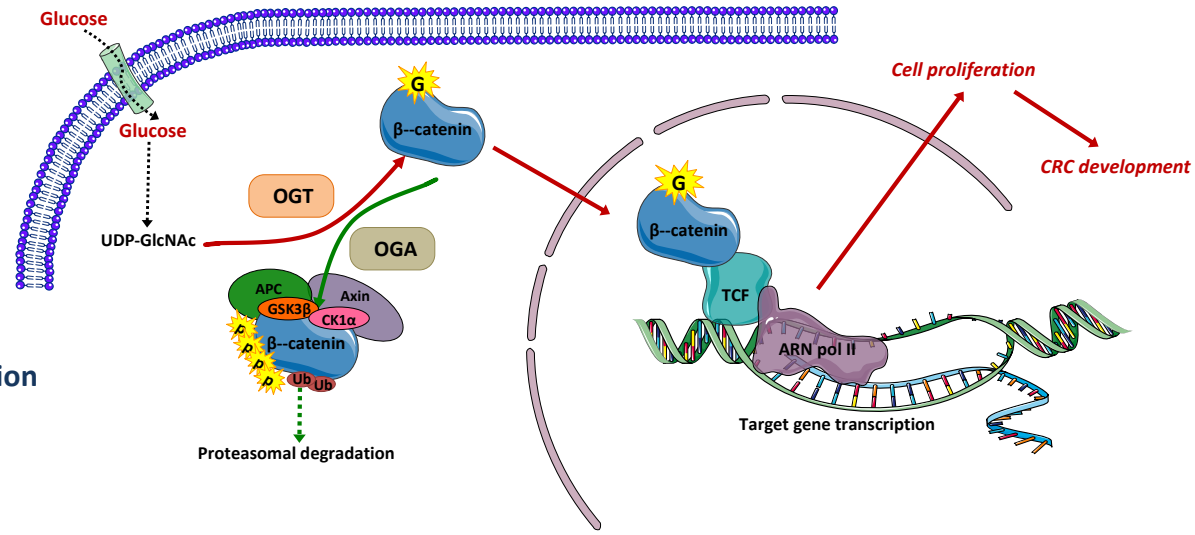


O-GlcNAcylation and β-catenin stability

- Wnt/β-catenin pathway is modified in 90% of cases of colorectal cancers by genetic alteration of **β-catenin** or one member of the destruction complex: APC, axin, GSK3β or CK1α

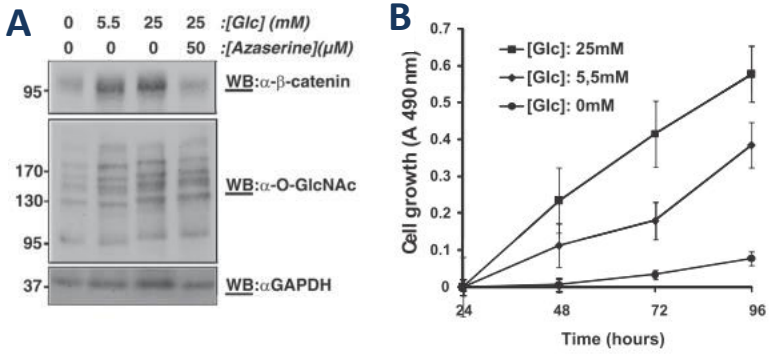
Consequence

- β-catenin stability→ uncontrolled cell proliferation



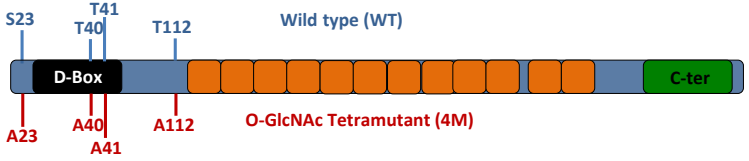
Previously

Cell culture: high glucose ↗ β-catenin expression (A) and stimulation of cell proliferation (B)

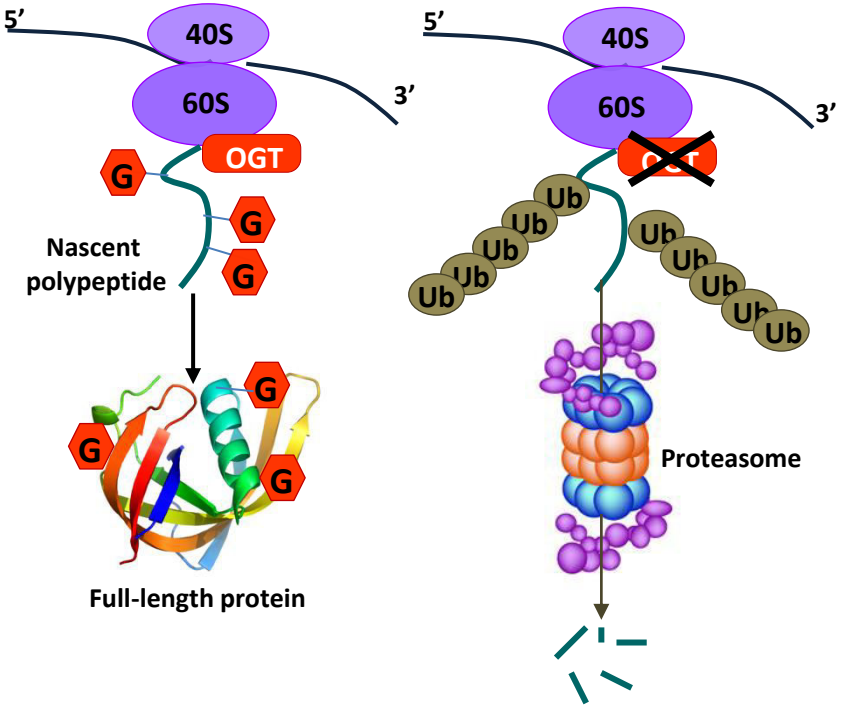


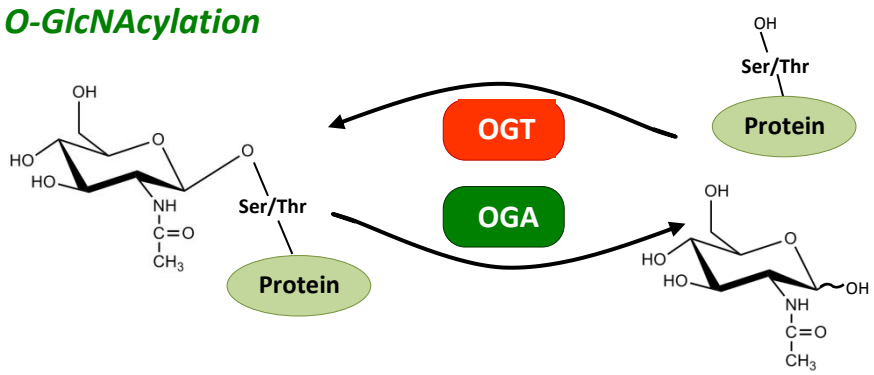
(Olivier Van-Stichelen *et al.*, 2012)

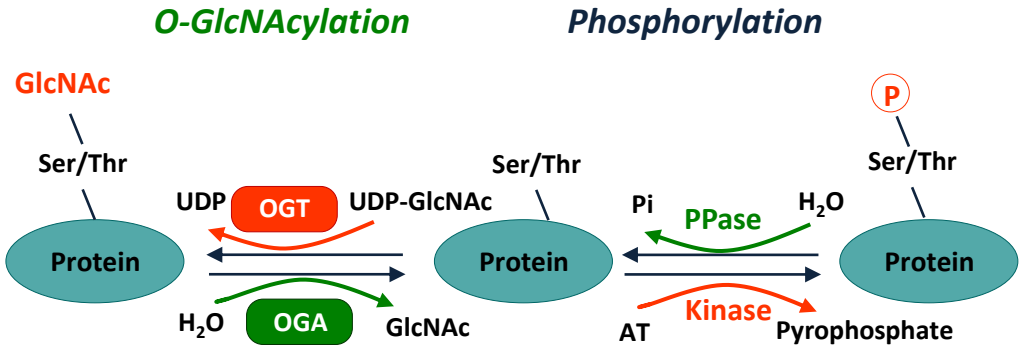
- 4 *O*-GlcNAc sites at the N-term of β-catenin: S23/T40/T41/T112
- 2 of those in the D-box: T40/T41
- T41 is key residue for β-catenin degradation



(Olivier Van-Stichelen *et al.*, 2014)







- *OGT* (*O*-GlcNAc transferase), key and unique regulator of PTM