GLIOBLASTOMA OXAPHOSPHINANES OXYSTEROLS

G_2O

ANR PCV 2007

Norbert BAKALARA (INM INSERM U61051, Jean-Luc PIRAT (ICG UMR-5253), Emmanuelle URO-COSTE (I2MR INSERM U-858), Jacques ROBERT (INSERM U-916)

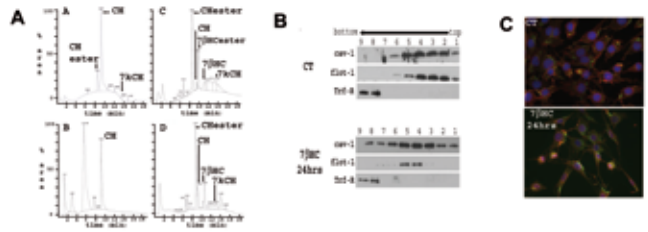
OXAPHOSPHINANES: A THERAPEUTIC OPPORTUNITY FOR GLIOBLASTOMA (J. Med Chem, submitted)
SEQUENTIAL OPPOSING SIGNALING RESPONSES TO 7β-HYDROXYCHOLESTEROL-INDUCED ENERGY STRESS LEADS TO DEATH OF C6 GLIOBLASTOMA CELLS (Biochem .Pharmacol. Submitted)
REVISITED SYNTHESIS OF ARYL-H-PHOSPHINATES (Synthesis, accepted)
NEW PHOSPHORUS CONTAINING HETEROCYCLIC COMPOUNDS, SUGAR ANALOGUES, AND COMPOSITIONS HAVING ANTI-CANCER ACTIVITY CONTAINING THE SAME (CNRS-ENSCM, PCT/EP2008/058788)

INTRODUCTION

Gliomas are **the most frequent brain tumor** with an incidence of 5/100 000 inhabitants. These tumors are divided in two main categories: primary high grade gliomas (WHO grade IV), with a **poor prognosis** (median survival around 1 year), and "low grade gliomas" (WHO grade II glioma), with a constant growth and which ineluctably evolves to anaplasia (WHO grade III and then IV). Therefore effective drugs to treat gliomas still need to be found.
In this context we firstly focused on the mode of action **7β-hydroxycholesterol (7βHC)-ester** showing *in vivo* no toxicity and inhibiting growth of experimental rat C6 glioblastoma.
Secondly we synthesized a new set of compounds, the **phostines** which belongs to the 1,2-oxaphosphinane heterocyclic core. Their chemical characteristics led us to consider them as biological mime of glycosides with unknown biological properties.

MODE OF ACTION OF THE 7β-HYDROXYCHOLESTEROL

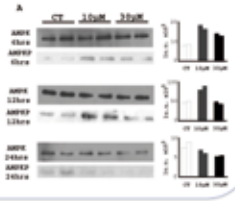
Alteration of sterols and flotillin contents in lipid rafts after 24 h of 7βHC treatment



A - Sterols from lipid-raft fractions of non treated (right) and treated cells (left) were analyzed by LCMS before (top) and after saponification (bottom). **B** - C6 cell lysates from non treated (CT) and treated cells were separated on an OptiPrep™ step gradient and analyzed by WB with caveolin-1, flotillin-1 and transferin antibodies. **C** - IF analysis : cav-1 green, flot-1 red

Lipid raft sterol and flot-1 redistributions are associated with energy stress and cell death

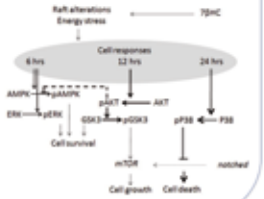
Transient increase of AMPK phosphorylation during the first 6 of 7βHC treatment indicates that C6 cells undergo energy stress.



Under 7βHC treatment cells alternate between cell survival with an active mitochondria and cell proliferation associated with glycolysis

The energy stress is followed by a transient mitochondrial activation at 7h maintaining the ATP level during that period and indicating **a shift from glycolysis to glucose oxydation.**

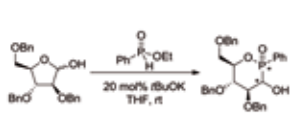
Under the energetic stress signaling pathways are sequentially and transiently activated : at 6h (ERK, AMPK) at 12h (Akt, GSK3β) and if the 7βHC induced stress persists P38 at 24h. P38 activation precedes cell death. These pathways activations are **dependent upon 7βHC in situ esterification.**



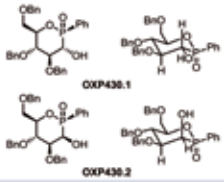
Conclusion: We see the relevance of developing drugs, such as 7βHC, to exploit the addiction of tumor cells to glycolysis. We are currently running to validate the proof of concept with an industrial partnership.

THE PHOSTINES

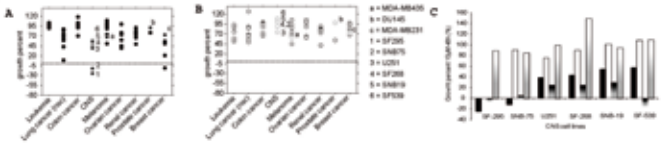
More than eighty phostines bearing various substituents on the phosphorus atom and carbon in position 2 have been synthesized



General synthetic pathway



Antiproliferative activity of the two diastereoisomers OXP430.1 and OXO430.2 against a panel of NCI human cancer cell lines(AB). Comparaison with paclitaxel profile (C)



Based on gene expression, cluster trees of the 57 NCI cell lines indicate that the breast cancer HS578T and the non-small cell cancer lung HOP-62 cell lines clustered with the CNS cell line.

Conclusion : By the fact that the structurally original phostines exhibit a new mode of action *in vivo*, this project is currently under an industrial valorization process (ANR Emergence Bio 2008 IDPHOST – Industrial contacts).

CONTACT :
Norbert.bakalara@enscm.fr
Institut des Neurosciences de Montpellier
INM
INSERM U-1051