Non-metabolic functions of Hexokinase 2 promote cancer stemness and oncogenicity by regulating an EMT-like phenotype and cell-extracellular communications

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INTRODUCTION

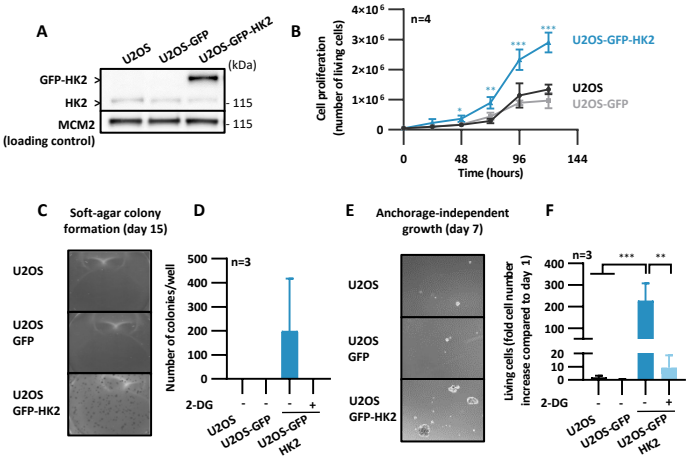
Increased glucose consumption is one of the main hallmark of cancer cells. This metabolic reprogramming is partly due to the increased expression and activity of key glycolytic enzymes, such as Hexokinase 2 (HK2). Overexpression of HK2 has been reported in many solid tumours as well as in leukaemia and it is often correlated with poor prognosis and/or chemotherapy resistance[1,2]. It was shown that HK2 is essential for cancer cell proliferation[3] and that its genetic deletion can impair oncogene-induced tumorigenesis in mice[4], which makes it an interesting target. However, HK2 inhibitors are not fully exploited in clinics due to off-targets and adverse effects that occurred during clinical trials.

In addition to its well-established role in glycolysis, HK2 can have non-glycolytic functions. For instance, as the yeast Hxk2, several studies indicate that human HK2 may have nuclear functions which could be mediated by post-translational modifications or specific domain functions[5,6]. A better understanding of the cellular and molecular functions of HK2 in cancer cells may thus help to find new ways to target this protein.

Then, our project aims to describe the consequences of HK2 overexpression and the pathways regulated by the enzyme, to identify potential non-glycolytic functions.

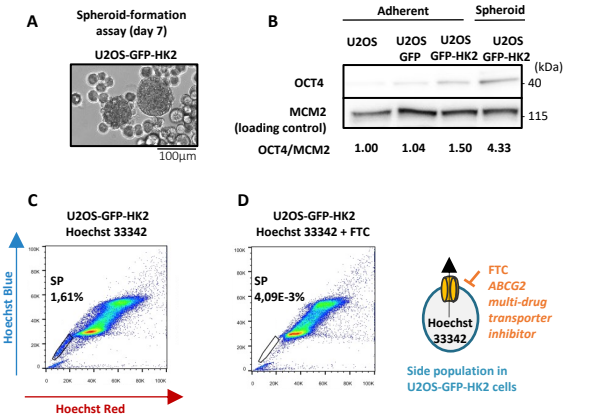
RESULTS

1: HK2 overexpression promotes oncogenicity in osteosarcoma cells



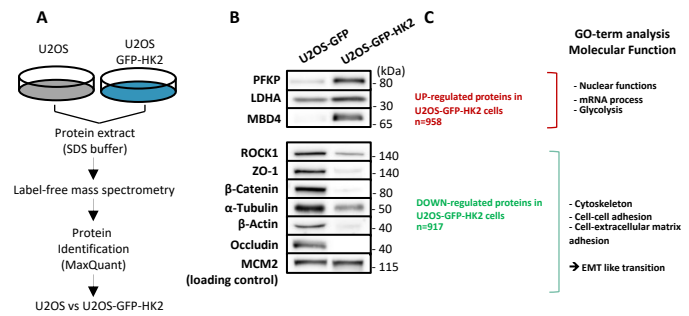
HK2 overexpression increases U2OS cell proliferation and promotes colony formation and anchorage-independent growth. (A) Western blot analysis of HK2 expression after U2OS electroporation with GFP and GFP-HK2 expressing plasmids and selection of stable GFP and GFP-HK2 expressing cell lines. **(B)** Follow-up of cell proliferation of U2OS, U2OS-GFP and U2OS-GFP-HK2 cells. (n=4) **(C-D)** Representative images of soft-agar colony formation assay of U2OS, U2OS-GFP and U2OS-GFP-HK2 cells and the quantification of the number of colonies/well showing the mean +/- SD. 2-DG: treatment with 2g/L of 2-deoxyglucose, a glycolytic inhibitor. **(E-F)** Representative images of U2OS, U2OS-GFP and U2OS-GFP-HK2 cells cultured in low-adherent plates and the quantification of the living cells showing the mean +/- SD. Asterisks indicate statistical significances (*p<0,05 **p<0,01 ***p<0,005).

2: HK2 overexpressing cells exhibit cancer-stem cells features



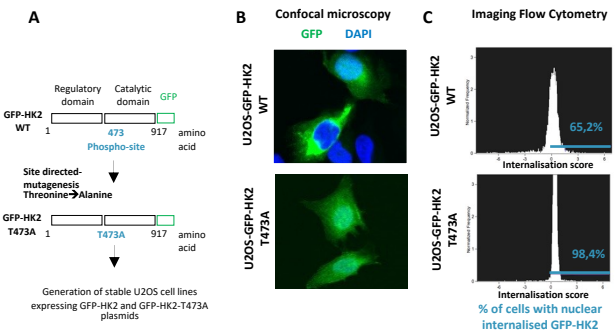
HK2 overexpression promotes tumour-spheroid formation, defined by OCT4 expression, and the emergence of a side population exhibiting drug efflux ability. (A) Representative image of spheroids formed by U2OS-GFP-HK2 cells cultured for 7-days in ultra-low attachment plates and media supplemented for stem-cell growth factors (EVOS microscope, objective 20X). Of note, U2OS and U2OS-GFP cells do not survive upon induction of spheroid formation. **(B)** Western blot analysis of pluripotency factor OCT4 expression in U2OS, U2OS-GFP and U2OS-GFP-HK2 cells cultured in adherent conditions and U2OS-GFP-HK2 cultured in spheroid condition. **(C-D)** Flow cytometry side-population (SP) analysis with Hoechst 33342 (4µg/mL) efflux assay in U2OS-GFP-HK2 cells. This sub-population of cells disappears with 2µM fumitremorgin C (FTC) treatment, a specific ABCG2 transporter inhibitor. The SP was not identified in U2OS and U2OS-GFP cells.

3: HK2 overexpression causes proteomic changes suggesting an EMT-like transition



A proteomic approach revealed nuclear factors and proteins involved in cytoskeleton, cell-cell and cell-extracellular matrix interactions as proteins regulated by HK2. (A) Schematic representation of the protocol used to describe U2OS and U2OS-GFP-HK2 proteome, and identify proteins significantly regulated by HK2 (q-value < 0.001, Benjamini-Hochberg). Four samples of each cell lines were analysed by mass spectrometry (3P5 facility, Institut Cochin). **(B)** Western blot validation of HK2's regulated proteins identified in proteomic analysis. **(C)** Functional classification of HK2's regulated proteins using the PANTHER GO Molecular Function analysis (p-value < 1.10^{-5}).

4: HK2 is a cytoplasmic-nuclear shuttling protein : role of Threonine 473



HK2 can shuttle from the cytoplasm to the nucleus and this shuttling involves Threonine 473 residue. (A) Schematic representation of GFP-HK2 wild-type (WT) and GFP-HK2-T473A mutant introduced in U2OS cells. **(B)** Confocal microscopy analysis (Spinning disk Ixplore Olympus, objective 30X) of GFP-HK2 WT and GFP-HK2 T473A mutant localisation. The nucleus is counterstained with DAPI. **(C)** ImageStream imaging flow cytometer analysis to determine the nuclear internalisation score (IS) of GFP-HK2 WT and GFP-HK2-T473A. IS of 5000 cells was calculated by Amnis IDEAS software. IS>0: more than 50% of the GFP staining colocalises with the nuclear staining.

CONCLUSIONS & PERSPECTIVES

Conclusions

Using the low-tumorigenic U2OS cell line as a model, **we demonstrated that :**

- HK2 overexpression is sufficient to promote cell proliferation, colony formation and anchorage-independent growth; and that 2-DG treatment reduces these features.
- HK2 overexpression promotes cancer-stem cells features including spheroid formation and OCT4 protein expression.
- HK2 regulates the abundance of proteins involved in cell architecture, cell-cell and cell-extracellular matrix communications as well as nuclear processes.
- HK2 localises into the nucleus and Threonine 473 is important for the regulation of HK2 localisation.

Perspectives

- Study the behaviour of U2OS, U2OS-GFP and U2OS-GFP-HK2 cells upon injection in the animal.
- Study the relevance of our findings in cohorts of cancer patients, including patients with osteosarcoma (U2OS cell model).
- Investigate HK2 molecular functions in cell architecture, cell-cell and cell-extracellular matrix communications as well as nuclear processes.
- Analyse the role of Threonine 473 on the functions of HK2 revealed in this work.

References: 1. Wu et al, Oncotarget, 2017 ; 2. Ho and Coomber, Cancer Treat Commun., 2016 ; 3. Ahn et al., J Nucl Med., 2009 ; 4. Patra et al., Cancer Cell, 2013 ; 5. Ghosh et al., Exp Cell Res., 2016 ; 6. Sheikh et al., J Biol Chem., 2018