SUMMARY REPORT

Cancer cells represent one of the best examples of how cells can modify their metabolism to proliferate and to adapt to stressful environmental conditions. In many cases, cancer cells shift from ATP generation through oxidative phosphorylation to ATP production through glycolysis, a process known as the Warburg effect[1]. In doing so, cancer cells would generate large amounts of metabolites derived from glycolysis, which are essential for building membranes, nucleotides and other components required for cell proliferation. On the contrary, they would maintain mitochondria at rest, preventing them to form deleterious reactive oxygen species. We still don't understand precisely how cells can select aerobic glycolysis to the detriment of oxidative phosphorylation. In this regard, pyruvate is an interesting metabolite as it is at the interface between glycolysis in the cytosol and oxidative phosphorylation in mitochondria. When it is not reduced into lactate by lactate dehydrogenase (LDH), pyruvate is imported into the mitochondrion by a specific carrier of the inner mitochondrial membrane, the mitochondrial pyruvate carrier (MPC), which our group identified in 2012. The MPC is composed of two subunits, MPC1 and MPC2[2] that form an hetero-oligomer in the inner mitochondrial membrane. Once in the mitochondrial matrix, pyruvate is further oxidized to provide electrons and protons for the respiratory chain of mitochondria.

We hypothesize that an abnormal activity of the MPC could explain, at least in part, the Warburg effect. **The objectives of Vincent Compan's work have been to investigate the functional properties of the MPC in normal and cancer cells using a biosensor that allows to monitor the activity of the carrier in real time.**

We engineered a genetically encoded biosensor based on bioluminescence resonance energy transfer (BRET) to monitor the activity of the MPC in real time. It is assumed that reversible conformational changes in a carrier is required for the transport of a solute. Therefore, we constructed a BRET based sensor in which the two constituents of the carrier, MPC1 and MPC2, are fused at their C terminals to either the acceptor Venus (a variant of yellow fluorescent protein) or the donor RLuc8 (a variant of Renilla luciferase) (Figure 1A). We hypothesized that conformational changes during pyruvate transport would cause changes in the energy transfer between MPC1 and MPC2 and would allow to monitor MPC activity in real time by measuring BRET variations. We first confirmed that the functional features of the tagged proteins were similar to those of endogenous MPC. We next recorded BRET signals. When expressed in HEK293 cells, in the presence of the luciferase substrate coelenterazine h, MPC1-Venus and MPC2-RLuc8 displayed a strong BRET signal of 738±11 mBU indicating that the conformation of the two proteins allowed energy transfer between the donor and the acceptor. Incubation of the cells with pyruvate (5mM) increased rapidly the BRET by ≈ 20 % compared with the basal BRET signal (Figure 1B). The BRET changes remained stable for up to 30 min in the presence of pyruvate. Dose response curves on plasma membrane permeabilised HEK cells showed that the BRET sensor had an EC50 of ≈ 250 µM. To demonstrate that BRET variations are a direct measure of MPC activity and pyruvate import into the mitochondria, we incubated the cells with AR-C155858, an inhibitor of the monocarboxylase transporter MCT, the pyruvate carrier of the plasma membrane. AR-C155858 (1 µM) progressively and

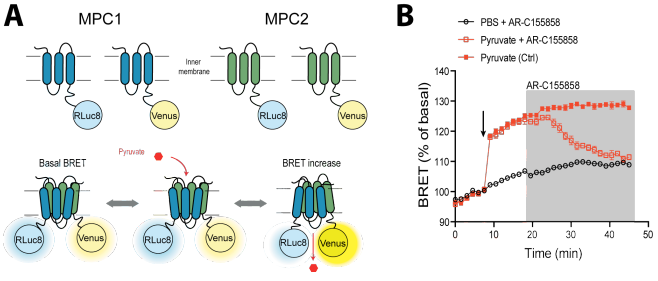

Figure 1 : A genetically encoded BRET sensor to monitor MPC activity in living cells. A/ Top panel: Schematic representation of MPC subunits tagged with either Venus or RLuc8. Bottom panel: Illustration of BRET changes induced by pyruvate. **B/** BRET variation monitored in living HEK cells. Basal BRET signal was recorded in cells transfected with MPC1-Venus and MPC2-RLuc8. Cells were next incubated (arrow) with either PBS (open black circle) or pyruvate (open red square) before inhibition of MCT with AR-C155858 (gray font). Note that BRET remains high when pyruvate is applied alone (solid red square).

completely reversed the BRET changes of the MPC sensor (Figure 1B).

Finally, MPC activity was measured in normal cells and cancer cells. We generated different cell lines stably expressing the MPC BRET sensor. This system of expression was chosen in order to get a low level of expression of the BRET sensor subunits, close to the endogenous level of MPC. We found that in the seven different cancer cell lines generated (143B, MCF7, HCT-116, Hela, A549, MDA-MB231), the BRET signal increased in the presence of pyruvate (Figure 2A). These results show that the overexpressed MPC were functional in cancer cells and suggest that post-translational modifications of the MPC that might affect the activity of the transporter are not the primary cause of the low mitochondrial respiration observed in these cancer cells. However, in contrast to what was observed with pyruvate, little or no BRET changes were observed in the presence of glucose, even at high concentration (20mM, Figure 2A). As a control, similar experiments were performed on rat pancreatic β cells (INS-1E) as a model of cells producing ATP through oxidative phosphorylation (Figure 2B). After transfection with the BRET sensor, BRET signals increased after incubation with either pyruvate (5 mM) or glucose (20mM). In the latter case, the BRET signal reached progressively a steady state after 5 min of stimulation. The BRET changes observed with glucose were completely reversed when cells were incubated with 2 deoxyglucose (2-DG, 50mM), a glycolysis inhibitor that abolishes pyruvate production. In contrast, BRET changes induced by direct pyruvate stimulation were not affected by 2-DG. These experiments allowed us to validate the functionality of the BRET sensor.

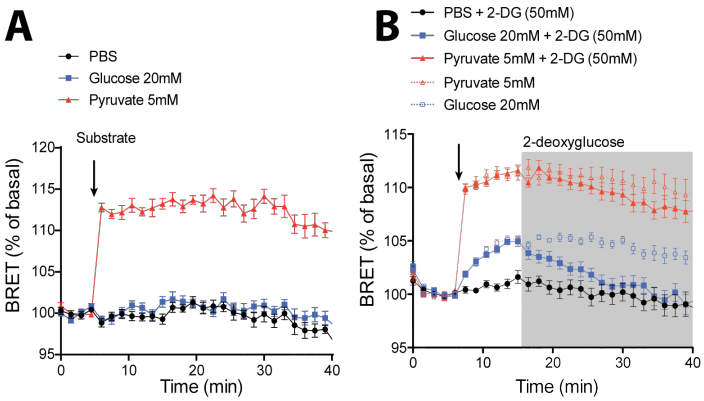

Figure 2 : MPC activity in normal cells and cancer cells. MCF7 cells (**A**) or INS-1E cells **(B)** expressing MPC1-Venus and MPC2-Rluc8 were stimulated (arrow) with either PBS, glucose (20mM) or pyruvate (5mM). Where indicated (gray font), INS-1E were incubated with 2-deoxyglucose (2-DG, 50mM).

Altogether, we have developed a BRET sensor to monitor the activity of the MPC in living cells. In response to pyruvate, we observed an increase in MPC activity in normal cells and cancer cells. However, such changes were not observed in cancer cells when glucose was provided as the only carbon source. These results suggest that changes in pathways upstream of pyruvate import in the mitochondria mostly account for the Warburg effect observed in different tumors. The BRET assay should allow us to identify the mechanisms that prevent pyruvate to be imported into mitochondria. Proteins involved in this process could represent interesting therapeutic targets for cancer.

1 Vander Heiden, M. G., Cantley, L. C. & Thompson, C. B. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* **324**, 1029-1033, doi:10.1126/science.1160809 (2009).

2 Herzig, S. *et al.* Identification and functional expression of the mitochondrial pyruvate carrier. *Science* **337**, 93-96, doi:10.1126/science.1218530 (2012).

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