# Fructose-1, 6-bisphosphatase opposes renal carcinoma progression

Bo Li, Bo Qiu, David S.M. Lee, Zandra E. Walton, Joshua D. Ochocki, Lijoy K. Mathew, Anthony Mancuso, Terence P.F. Gade, Brian Keith, Itzhak Nissim, M. Celeste Simon

## Supplementary Discussion

In non-transformed renal tubule cells, FBP1 inhibition increases cell proliferation, glycolysis, and pentose phosphate pathway flux, likely through its enzymatic activity. These metabolic shifts are consistent with metabolic gene alterations correlating with worse prognosis in ccRCC patients[7]. Nevertheless, the metabolic role of FBP1 may not be sufficient to explain its tumor-suppressive function. In ccRCC tumor cells, FBP1 impacts cell growth and glucose metabolism independent of its catalytic activity, by inhibiting HIFα through its regulatory domain. Therefore, FBP1 protein has two separate domains mediating two anti-tumor functions (gluconeogenesis and HIF inhibition), both of which need to be attenuated during ccRCC progression. This is consistent with the fact that no FBP1 intragenic mutations were identified in ccRCC based on our evaluation of exome sequencing data[7], indicating that these tumors prefer to suppress FBP1 expression rather than selecting for FBP1 mutations. However, how FBP1 expression is ubiquitously suppressed in ccRCC is not fully understood, since genomic deletions at the *FBP1* locus occur in ~25% of screened tumors[27]. Recently, a Snail-G9a-Dnmt1 complex was reported to mediate *FBP1* promoter methylation and reduce FBP1 levels in basal-like breast cancer cells, suggesting a potential mechanism to further inhibit FBP1 expression[31].

In addition to FBP1, PKM2 (pyruvate kinase, M2 isoform) has been reported to regulate HIF activity[32]. PKM2 can function as a HIF coactivator by increasing HIF binding affinity to its target promoters in hypoxic cancer cells[32]. Although PKM2 is upregulated in multiple tumor types[33] and critical for cancer cell growth *in vitro*[34,35], *in vivo* function(s) of PKM2 have been challenged by recent studies[36,37]. In a breast cancer mouse model, *PKM2* deletion promoted tumor growth and distant metastasis, suggesting that PKM2 is not required in all human cancers[37]. Therefore, whether PKM2 contributes to tumor progression by potentiating HIF activation awaits further evaluation. In contrast, we demonstrate here that the gluconeogenic enzyme FBP1 is uniformly suppressed in a large number of primary ccRCC tumors, and its loss correlates with increased HIF activity *in vivo*, advanced tumor stage, and

worse patient prognosis. Whether FBP1 inactivation in renal tubule cells is sufficient to induce ccRCC in a *VHL*-deficient background is under current investigation.

In healthy liver, differentially oxygenated blood flow creates a natural oxygen gradient[38]. It has been reported that hepatocytes proximal to periportal blood (higher oxygen tension, 60-65 mmHg) are specialized in gluconeogenesis, while hepatocytes located at perivenous sites (lower oxygen tension, 30-35 mmHg) mainly consume glucose and execute glycolysis[39,40]. Similarly, lower oxygen tension (5 mmHg) is detected in the renal medulla, while higher oxygen levels (up to 50 mmHg) are observed in the renal cortex where gluconeogenesis occurs[41,42]. This phenotype, previously described as "metabolic zonation"[38], reveals a mutually exclusive pattern between hypoxic and gluconeogenic regions within liver and kidney, which is consistent with our finding that FBP1 inhibits HIF activity. As a typical metabolic sensor, HIF accumulates in areas experiencing depleted oxygen and nutrient supply, and antagonizes gluconeogenesis by promoting glycolysis and suppressing oxidative phosphorylation[3,20]. Conversely, FBP1-mediated HIF inhibition in gluconeogenic regions may act as a safeguard to help maintain glucose production. Therefore, our data highlight novel crosstalk between oxygen gradients and glucose metabolism, which may have broad impact on the metabolic homeostasis of gluconeogenic organs.


31. Dong, C. *et al.* Loss of FBP1 by Snail-mediated repression provides metabolic advantages in basal-like breast cancer. *Cancer Cell* **23**, 316-331 (2013).
32. Luo, W. *et al.* Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. *Cell* **145**, 732-744 (2011).
33. Cairns, R. A., Harris, I. S. & Mak, T. W. Regulation of cancer cell metabolism. *Nat. Rev. Cancer* **11**, 85-95 (2011).
34. Christofk, H. R. *et al.* The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature* **452**, 230-233 (2008).
35. Anastasiou, D. *et al.* Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. *Science* **334**, 1278-1283 (2011).
36. Cortes-Cros, M. *et al.* M2 isoform of pyruvate kinase is dispensable for tumor maintenance and growth. *Proc Natl Acad Sci U S A* **110**, 489-494 (2013).
37. Israelsen, W. J. *et al.* PKM2 Isoform-Specific Deletion Reveals a Differential Requirement for Pyruvate Kinase in Tumor Cells. *Cell* **155**, 397-409 (2013).
38. Jungermann, K. & Kietzmann, T. Zonation of parenchymal and nonparenchymal metabolism in liver. *Annu. Rev. Nutr.* **16**, 179-203 (1996).
39. Nath, B. & Szabo, G. Hypoxia and hypoxia inducible factors: diverse roles in liver diseases. *Hepatology* **55**, 622-633 (2012).
40. Jungermann, K. & Kietzmann, T. Oxygen: modulator of metabolic zonation and disease of the liver. *Hepatology* **31**, 255-260 (2000).
41. Epstein, F. H. Oxygen and renal metabolism. *Kidney Int* **51**, 381-385 (1997).
42. Haase, V. H. Mechanisms of hypoxia responses in renal tissue. *J Am Soc Nephrol* **24**, 537-541 (2013).