GLYCOLYSIS-INDEPENDENT ROLES OF HEXOKINASE 1 IN CANCER

Heneberg P.[1], Šimčíková D.[1], Gardáš D.[1], Žáček P.[2]

[1]*Charles University, Third Faculty of Medicine, Prague, Czech Republic*
[2]*Charles University, Faculty of Science, BIOCEV, Vestec, Czech Republic*

Hexokinases (HKs) have been found amplified in primary cancer cells, with the amplification of HK1 reported commonly in ovarian cancer cells. The function of HKs extends beyond glucose phosphorylation, and HKs serve as regulatory proteins in tumorigenesis. We addressed the non-canonical roles of HK1 in ovarian cancer cells by examining the effects of the deletions of HK1 and HK2 in TOV-112D ovarian adenocarcinoma cells. We reverted these effects by re-introducing wild-type HK1 and HK2, and we compared the HK1 revertant with the knock-in of catalytically-dead HK1 p.D656A. We subjected these cells to a battery of metabolic and proliferation assays and targeted GCxGC-MS metabolomics. When expressed *in vitro*, the p.D656A mutant did not manifest a measurable K_M and exhibited V_{max} lower by almost two orders of magnitude when compared to the wild-type enzyme. Knock-in of HK1 p.D656A into HK1 KO cells reverted the HK1 KO-induced effects in a broad range of assays, including the alamarBlue cell viability assay under low glucose conditions, the NADPH/NADP and NAD/NADH ratios, and glutathione levels in metformin-treated cells. In the presence of 0.4 mM glucose, the phenotype of HK1 p.D656A knock-in cells resembled the phenotype of cells with deleted HK2 but not those with deleted HK1 with regards to the levels of TCA intermediates, aspartate and cysteine, and glutamate. Moreover, HK1 deletion increased the levels of branched amino acids, which was completely eliminated by the expression of catalytically-dead HK1. The HK1 deletion (but not HK2 deletion) suppressed the growth of xenotransplanted ovarian cancer cells, and nearly abolished the tumor growth when the mice were fed the glucose-free diet. In summary, we provided the evidence that HK1 is involved in the so far unknown glycolysis-independent moonlighting function and influences metabolism even in the glucose-free conditions.