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Transient Glycolysis Enables Mitochondrial Fusion and Stimulates S Phase Entry: The Role of FoxO3a

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We recently demonstrated that cells arrested by glucose deprivation proceed into S phase when glucose is replenished only in the presence of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase (PFKFB3) – an allosteric activator of phosphofructokinase 1 (PFK1), indicating the necessity to upregulate glycolysis at this stage of the cell cycle. PFKFB3 is expressed only transiently in G1, following a decrease in the activity of the ubiquitin ligase APC/C-Cdh1, implicated in PFKFB3 degradation. In the present study we investigate the functional significance of glycolysis in G1 to S transition. First, we established that PFKFB3 and glycolysis are necessary for the G1 to S phase transition in the cell cycle, since RNAi-mediated silencing of PFKFB3 or inhibition of glucose uptake by 2-deoxyglucose (2-DOG) induced accumulation of cells with DNA content indicative of G1. Next, we observed a strong correlation between PFKFB3 expression, the peak of lactate formation and the appearance of predominantly fused mitochondria at G1 to S transition in synchronized primary human fibroblasts (IMR90) or cancer (HCT 116) cells. The mitochondrial fusion at G1/S was dependent on PFKFB3 and glycolysis since RNAi-mediated silencing of PFKFB3 or inhibition of glucose uptake by 2-DOG abolished mitochondrial fusion, leading to fragmentation of mitochondria. Mdivi-1 prevents mitochondrial fission by inhibiting dynamin-related protein (Drp1). We have observed that this compound induces PFKFB3 expression, mitochondrial fusion and S phase entry (as judged by increased cellular DNA content, the expression of cyclin E and cyclin A and the accumulation of geminin). PFKFB3-silencing or 2-DOG treatment abolished the lactate generation and glycolysis and prevented the mitochondrial fusion and S phase entry induced by Mdivi-1. This could be reversed by overexpression of the glycolytic enzyme phosphofructokinase 1 (PFK1). We have found that increased glycolysis, rather than mitochondrial fusion, is important for initiating S phase, since ectopic expression of PFK1 stimulated starved G0 accumulated cells to enter S phase. S phase entry following the enhancement of glycolysis was accompanied by nuclear exclusion of Foxo3a. This was in turn followed by downregulation of its transcriptional target p27, an inhibitor of CDK2. The reduction in the nuclear levels of Foxo3a was associated with the activation of the IGF-R/Akt axis as shown by the phosphorylation of these proteins upstream of Foxo3a. These results suggest that transient glycolysis is important in the initiation of the S phase through the IGF-1/Akt-dependent nuclear exclusion of Foxo3a, an event which occurs concomitantly with the fusion of mitochondria, both of which are needed for cell cycle progression beyond the G1 phase. Upregulation of glycolytic enzymes was demonstrated in the blood of diabetic patients and shift to glycolysis was demonstrated in diabetes in some organs like heart and pancreas with recent evidence of glucose stimulating β cell proliferation *in vivo.* In the light of this evidence the novel functional link of transient glycolysis and S phase initiation in IGF-1-dependent fashion becomes interesting to discuss as a possible cancer risk mechanism in diabetes.