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Regulation of Trophoblast Invasion by Pyruvate Kinase Isozyme M2 (PKM2)

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Introduction

Abnormal placentation in pregnancy may result in complications such as preeclampsia (PE). PE is one of the leading causes of maternal and fetal morbidity and mortality in developed countries. This disease is characterized by a decrease in trophoblast invasion due to a hypoxic environment. The pyruvate kinase isozyme M2 (PKM2) is an enzyme exclusively present in highly proliferating cells such as in embryonic tissues, cancer, and placental trophoblast cells. In its active form PKM2 is phosphorylated and has an active role in the conversion of PEP to pyruvate in the cytosol. Inactive PKM2 is present in nuclear tissues where it directs glycolytic intermediates for use in cell proliferation. The role of PKM2 in PE is still unknown. The objective of this study was to determine the cytosolic and nuclear PKM2 expression pattern and determine PKM2 regulation of trophoblast cell invasion in the presence of PKM2 activator and inactivator during normoxia or hypoxia.

Methods

Cell culture and treatments

SW71 invasive trophoblast cells were used for these experiments. Cells were cultured in RPMI medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin and streptomycin. Trophoblast cells were treated with Fructose-1,6-bisphosphate (FBP6; PKM2 activator) or Shikonin (PKM2 activation inhibitor) in normoxic and hypoxic (2% O_2) conditions.

Real time cell invasion

Trophoblast cells were plated at a concentration of 20,000 cells/well in the presence or absence of FBP6 or Shikonin. Cells were placed in the RTCA DP instrument and invasion readings were completed every 5 minutes for 24 hours.

Western blot for PKM2

Placental cells were lysed in protein lysis buffer. Nuclear and cytosolic protein extraction was performed. Membranes were incubated overnight with phosphorylated or total PKM2 (CellSignaling) antibody.

Results

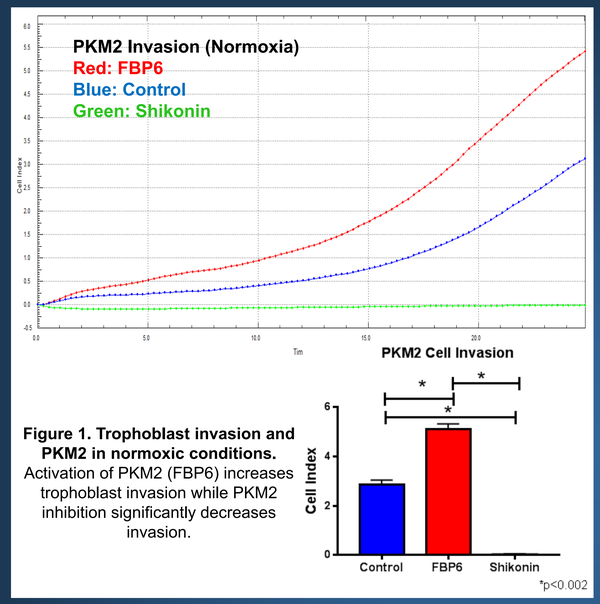

Figure 1. Trophoblast invasion and PKM2 in normoxic conditions. Activation of PKM2 (FBP6) increases trophoblast invasion while PKM2 inhibition significantly decreases invasion.

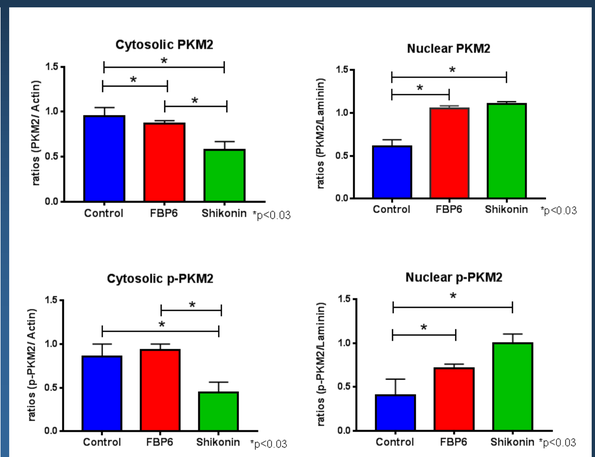

Figure 2. Trophoblast PKM2 protein in normoxia. PKM2 protein was decreased in the cytosol, but increased in the nucleus with both treatments during normoxia. Inactive p-PKM2 was decreased in the cytosol, but increased in the nucleus when PKM2 activation was inhibited.

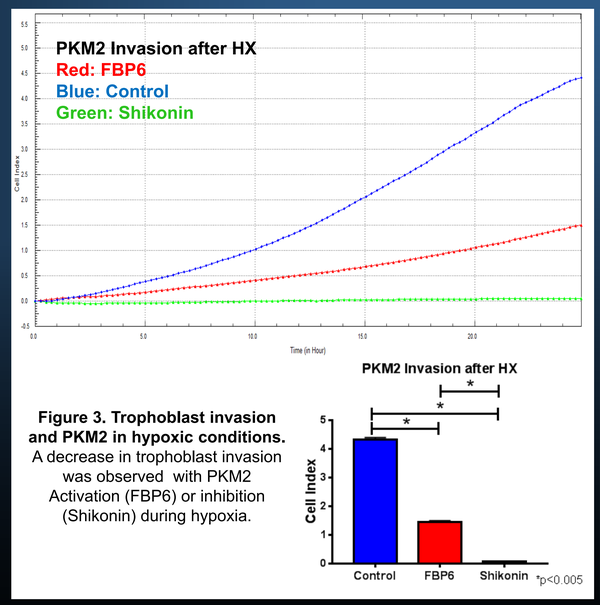

Figure 3. Trophoblast invasion and PKM2 in hypoxic conditions. A decrease in trophoblast invasion was observed with PKM2 Activation (FBP6) or inhibition (Shikonin) during hypoxia.

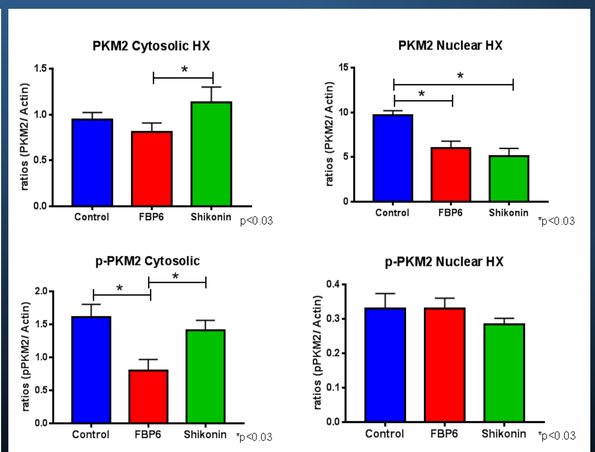

Figure 4. Trophoblast PKM2 protein and hypoxia. PKM2 protein was increased in the cytosol when PKM2 activation was inhibited by Shikonin. A decrease in PKM2 was observed in nuclear extract for both treatments. p-PKM2 was decreased in the cytosol when PKM2 was activated, and increased with PKM2 inactivation.

Summary

<u>During normoxia we observed:</u>

1. 2-fold increase ($p<0.002$) in trophoblast invasion when PKM2 was activated by FBP6.
2. 68-fold reduction ($p<0.002$) in trophoblast invasion when PKM2 activation was inhibited by Shikonin.
3. 1.4-fold induction ($p<0.03$) of nuclear PKM2 in the trophoblast when PKM2 was activated.
4. 1.6-fold induction ($p<0.04$) of nuclear PKM2 after PKM2 inactivation.
5. 1.4-fold decrease ($p<0.03$) in cytosolic PKM2 after PKM2 inactivation.

<u>During hypoxia we observed:</u>

1. 3-fold decrease ($p<0.005$) in cell invasion in treated cells .
2. 1.6-fold decrease ($p<0.03$) in cytosolic PKM2 expression in treated cells.
3. No significant differences in the expression of cytosolic PKM2 with treatment.

Statistical analysis with Mann-Whitney testing was performed. Data is shown as mean ± SE. $P<0.05$ was considered significant.

Conclusions

We conclude that PKM2 regulates trophoblast cell invasion through localized allosteric activation and inhibition. Our results suggest that phosphorylation of PKM2 is affected by hypoxia. Furthermore, these results suggest that PKM2 could be a mediator of trophoblast cell invasion, and its abundance influences the development of complicated pregnancies like PE.