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Metformin upregulates anti-angiogenic thrombospondin-1 in tumour microvascular endothelial cells exposed to 2-deoxyglucose

S. M. Samuel, Y. Majeed, H. Ding, C. R. Triggle. Pharmacology, Weill Cornell Medicine-Qatar, Doha, QATAR.

Introduction: Metformin exhibits anti-cancer properties in different types of cancer. Furthermore, a combination of 2-deoxyglucose (2DG) and metformin inhibits pro-survival autophagy in prostate cancer cells and promotes apoptosis (1). Reports also show that treatment with metformin increases the levels of anti-angiogenic thrombospondin-1 in the serum of women with polycystic ovarian syndrome (2). Preliminary studies from our laboratory indicate that treatment of glucose-starved tumour endothelial cells of mouse pancreatic microvasculature origin (MS1-VEGF cells) with a high concentration of metformin (2mM) resulted in a robust increase in anti-angiogenic thrombospondin-1 levels. Since angiogenesis is an integral part of tumor growth, cancer cell survival and metastasis, we investigated the effects of a combination of metformin and the glycolytic inhibitor, 2DG, on angiogenesis in MS1-VEGF cells.

Method: MS1-VEGF cells were treated with 2DG (5mM) for 48h in the presence or absence of metformin (2mM). Western blot analysis was performed to assess the status of angiogenic and anti-angiogenic marker proteins. Cell proliferation assay and wound healing assay were also performed.

Results: Cell proliferation rate markedly decreased in cells treated with a combination of 2DG and metformin (~75%, n=4) when compared to cells that were treated with either metformin (~14%, n=4) or 2DG (~54%, n=4) alone. We observed robust up-regulation of anti-angiogenic thrombospondin-1 (~9 fold, n=5-6), while the levels of pVEGFR2 (Y1175, ~1.5 fold, n=4-5) markedly decreased, in 2DG-exposed cells treated with metformin when compared to cells that were treated with 2DG alone. Data from the wound healing assay (n=5) revealed a marked reduction in cell proliferation and migration in 2DG exposed cells treated with metformin when compared to cells that were treated with either 2DG or metformin alone. Furthermore, treatment with metformin significantly decreased the levels of pmTOR (S2448, ~1.8 fold, n=5-6) and downstream p4E-BP1 (T36/47, ~1.5 fold, n=5-6), pS6 (S235/236, ~5 fold, n=5-6) and pS6 (240/244, ~3 fold, n=5-6) in 2DG-exposed cells treated with metformin when compared to cells that were treated with 2DG alone. Metformin treatment in 2DG-exposed cells also increased the levels of pRap (S792, ~1.6 fold, n=5-6), which is known to inhibit the mTOR pathway.

Conclusion: Results show that using metformin in combination with 2-DG could prove to be a potential anti-angiogenic cancer therapy owing to the up-regulation of thrombospondin-1.

References: 1) Ben Sahra I *et al*. (2010). *Cancer Research* **70**: 2465-2475. **2)** Tan BK *et al*. (2009). *Cardiovascular Research* **83**: 566-574. *Supported by Qatar National Research Fund grants: NPRP-04-910-3-244 & JSREP-03-016-3-009