Hyperoxia and PARP inhibition differentially modulate transcription profile and inflammation in aggressive melanoma and non-transformed lung epithelial cells

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BACKGROUND: The local conditions of tumor cell growth, known as the tumor microenvironment (TME), are characterized by low oxygen supply (hypoxia) caused by insufficient blood delivery. Hypoxia cancers have a strong invasive potential, metastasis, resistance to therapy and a poor clinical prognosis. The key regulator of adaptation to tumor hypoxia is hypoxia inducible factor 1-α (HIF-1α). Despite its significance, the underlying transformations that cause this highly aggressive behaviour are poorly understood, specific pharmacological inhibition of HIF-1α activation in the tumor are not available. Our group has shown that PARP inhibitors can modulate HIF-1α levels and its activation. On the other hand, hyperoxia could be a treatment of medical interest to fight tumors that present with hypoxia; nevertheless, its use may involve clinically unacceptable lung damage.

AIMS: In this study, we aim to demonstrate that the use of PARP inhibitors **(1)** will interfere with the adaptation of the tumor to the hypoxic microenvironment (which is recreated with the hypoxia-mimetic,$CoCl_2$), in combination with the use with oxygen to induce hyperoxia and **(2)** will decrease the lung damage induced by reactive oxygen species. Therefore, the combined use of oxygen and PARP inhibitors in metastatic melanoma (expressing high levels of HIF-1) could delay metastasis and improve the efficacy of anti-tumor therapy.

Fig 1. Significant decrease ROS levels in normal epithelial pulmonary cells in contrast to tumor cells under PARP inhibition during hyperoxia.

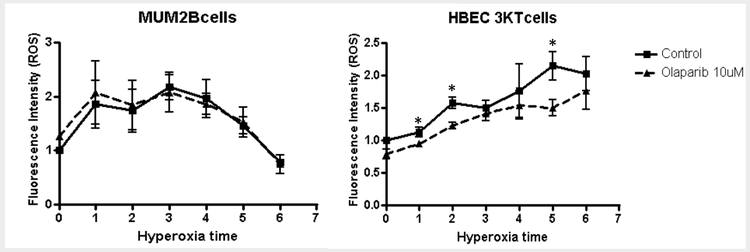

Fig 1. Significant decrease ROS levels in normal epithelial pulmonary cells. Uveal melanoma cells Mum2B and normal epithelial pulmonary cells HBEC3-KT were incubated under hyperoxia conditions (30% p O2) or hyperoxia with PARP inhibitor olaparib (5 μM).

Fig 2. Hyperoxia partially prevents $CoCl_2$-induced HIF-1α accumulation

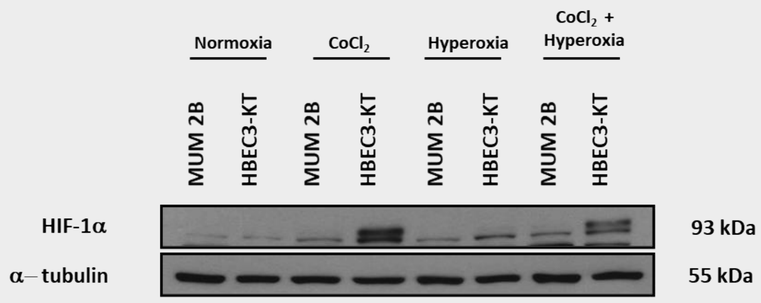

Fig 2. Western blot analysis of HIF-1α expression. Uveal melanoma cells Mum2B and normal epithelial pulmonary cells HBEC3-KT were incubated in normoxia; treated with $CoCl_2$ (100 μM for 24 h); incubated under hyperoxia conditions (30% p O2); or under both of them.

Fig 3. Hyperoxia or PARP inhibition decrease HIF-1α activity on its dependent genes.

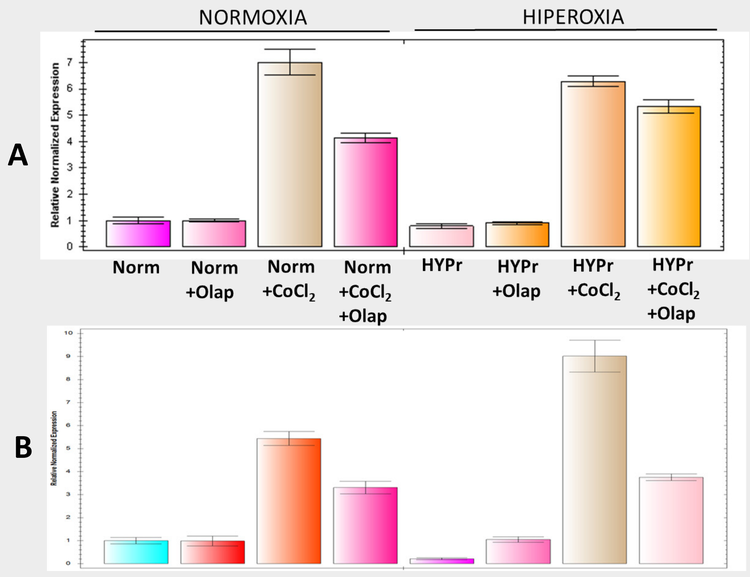

Fig 3. Hyperoxia or PARP inhibitor olaparib downregulate HIF-1α activity on it dependent gene BNIP3 . (A) Uveal melanoma cells Mum2B and **(B)** normal epithelial pulmonary cells HBEC3-KT were incubated in normoxia ; treated with $CoCl_2$ (100 μM for 24 h); incubated under hyperoxia conditions (30% p O2); treated with olaparib; or combination of them. BNIP3 mRNA gene expression was evaluated by qPCR .

CONCLUSIONS: - Olaparib treatment under hyperoxia significantly reduces ROS levels in normal epithelial pulmonary cells HBECK3-KT.
- Global gene expression is modified under hyperoxia and olaparib treatment. This modification is stronger in cancer cells.
- During hyperoxia, olaparib treatment, decreases inflammatory mediators in cancer cells.
- Hyperoxia decreases glycolisis and increases oxygen consumption.

PERSPECTIVES: - Determination of inflammatory mediators by ELISA.
- Toxicity assessment of hyperoxia and olaparib treatment.
- Study the effect of PARP inhibition and hyperoxia on *in vivo* murine model of lung metastasis.

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REFERENCES: - Kumari S et al, *Biomarker insights* , 2018
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Fig 4. Under hyperoxia and PARPi there is a reduction of inflammatory mediators in tumor cells.

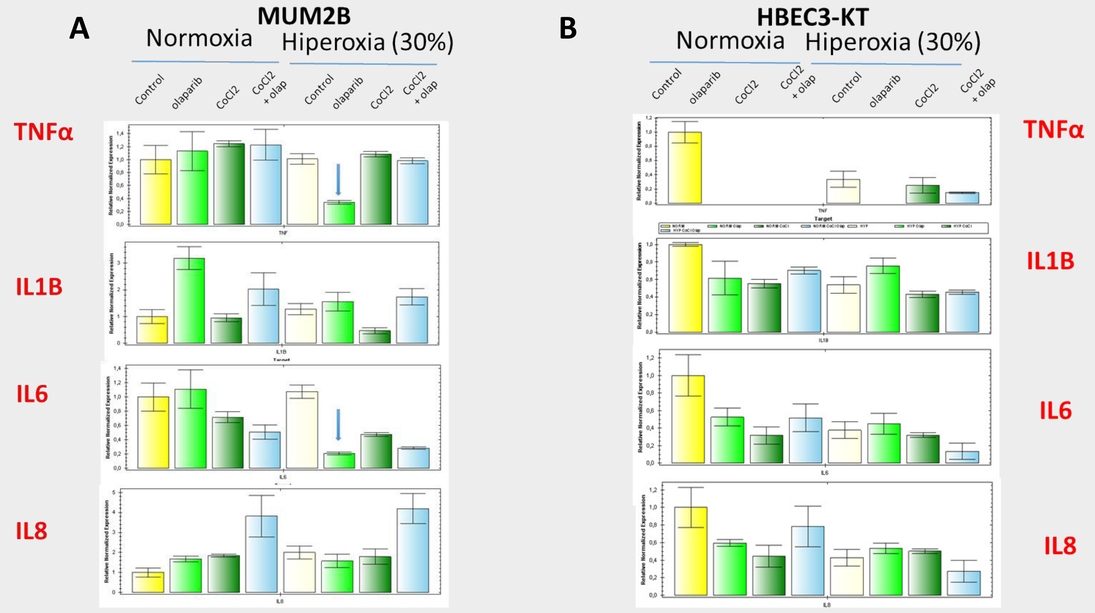

Fig 4. Hyperoxia and PARPi reduce inflammatory mediators in tumor cells. (A) Uveal melanoma cells Mum2B and **(B)** normal epithelial pulmonary cells HBEC3-KT were incubated in normoxia ; treated with $CoCl_2$ (100 μM for 24 h); incubated under hyperoxia conditions (30% p O2); or under both of them. Inflammatory mediators mRNA gene expression was evaluated by qPCR .

Fig 5. Hyperoxia and olaparib modulate the expression of 2762 genes in Mum2B, only 109 RNAs were altered in HBEC3KT.

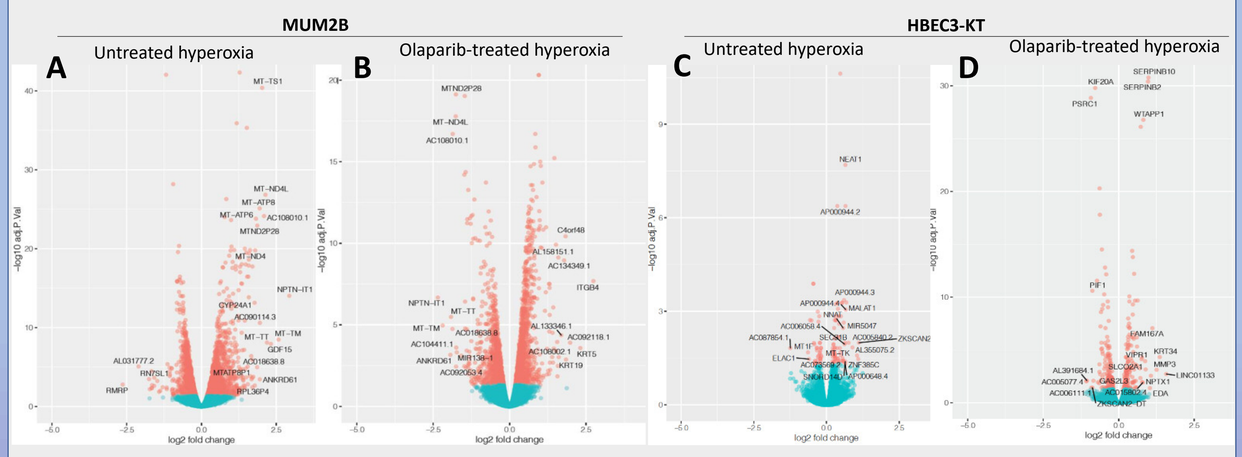

Fig 5. Hyperoxia and PARPi modulate gene expression patterns. (A) Uveal melanoma cells Mum2B and **(B)** normal epithelial pulmonary cells HBEC3-KT were incubated under hyperoxia conditions (30% p O2). RNA was extracted and subjected to RNAseq. When treated with olaparib, we found an upregulation of SOCS family expression, which are inhibitors of cytokine signalling pathways.

Fig 6. Hyperoxia reduces the glycolytic activity and increases oxygen consumption.

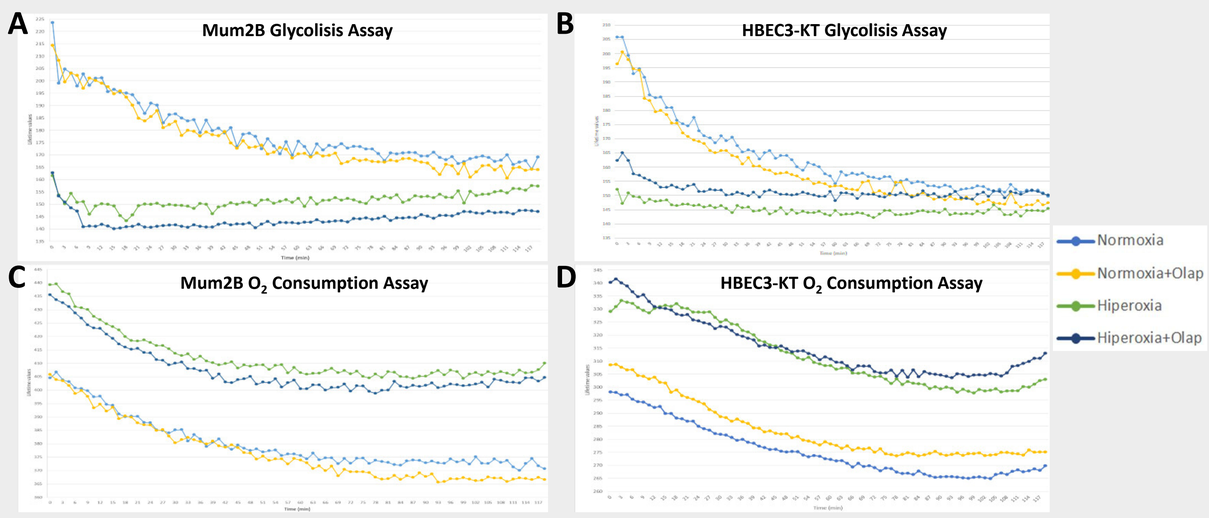

Fig 6. Hyperoxia reduces the glycolytic activity and increases oxygen consumption. (A and C) Uveal melanoma cells Mum2B and **(B and D)** normal epithelial pulmonary cells HBEC3-KT were incubated in normoxia or under hyperoxia conditions (30% p O2), with or without olaparib treatment (5 μM). Measurement of glycolisis **(A and B)** and mitochondrial respiration **(C and D)** was performed using Abcam Assay kits following manufacturer's instructions.