LOW GLUCOSE SENSITIZES A431 SKIN CANCER CELLS TO METFORMIN TREATMENTS: A WAY FORWARD TO TARGETING PD- L1

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BACKGROUND

In several drug repurposing trials, metformin has shown promising effects against various cancers. Metformin, a complex I inhibitor, actively regulates the metabolism of tumor cells through LKB/AMPK, Akt and mTOR pathways.

This work aims to investigate the antiproliferative effects of metformin on A431 cells and to evaluate its activities as a possible anti-PD-L1 adjuvant.

MATERIALS AND METHODS

Epidermoid carcinoma cell culture A-431 (ATCC, USA) was incubated with metformin (15 mM) in a high (4.5 g/L) and low (1.0 g/L) glucose growth medium. The antiproliferative activity of metformin was assessed by the MTT test.

Expression of PD-L1 was evaluated quantitatively by immunofluorescent assay and flow cytometry. The primary anti-PD-L1 (ARG65862) and secondary (DyLight650, ab98729) antibodies were used.

Two indexes of PD-L1 expression were calculated:

- **Level is the ratio (%) of specifically fluorescent cells to the control (incubation with the secondary antibody only)**
- **Intensity is the ratio of mean fluorescence intensity in the experimental sample to the control.**

Immunoblotting was used to identify signalling proteins in A431 skin cancer cells.

RESULTS

1. **Metformin has shown significant antiproliferative effects on A431 cells.**
2. **As we see on Fig.1 – high level of the phosphorylated form of AMPK (p-AMPK), one of the metformin targets, was found in A431 cells. Metformin treatment did not increase p-AMPK but significantly reduced the expression of cyclin D1, a key regulator of the cell cycle, and also blocked the expression of glucose transporter GLUT1. Low glucose significantly enhanced metformin effects on GLUT1 expression.**

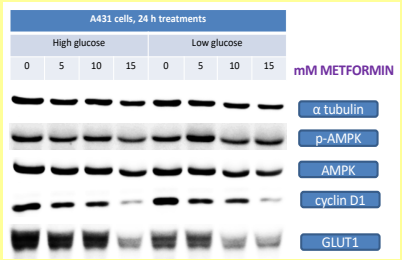

Fig. 1. Western Blot analysis

3. **In untreated A431 cells, there was no difference in both the level and intensity of PD-L1 expression between high vs low glucose growth medium (Fig. 2A vs 2D).**

4. **In the high glucose growth medium, 24 or 72h metformin treatment did not alter PD-L1 expression (Fig. 2A vs 2B; Fig. 2A vs 2C).**

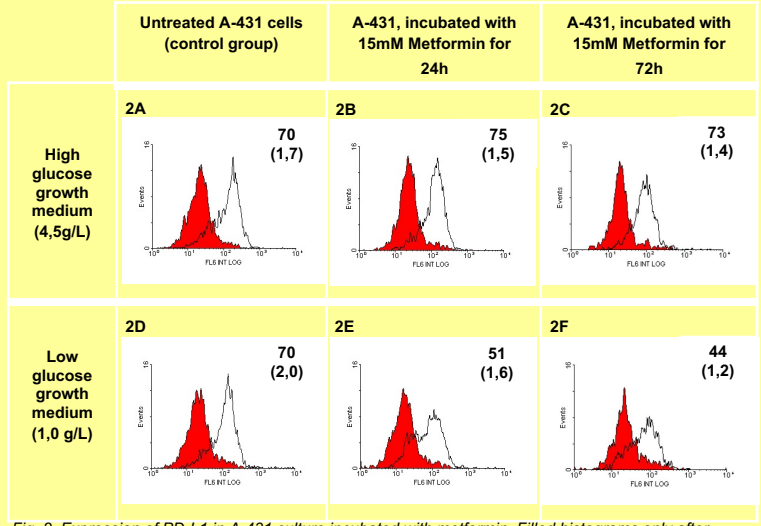

Fig. 2. Expression of PD-L1 in A-431 culture incubated with metformin. Filled histograms only after incubation with secondary antibodies (control); clear histograms after incubation with antibodies to PD-L1. Numbers designate PD-L1 expression level (%) and intensity of PD-L1 expression (arb. units in brackets).

5. **In the low glucose growth medium, metformin treatment of A431 cells for 24 and 72h decreased the PD-L1 expression: the level – in 1.4 and 1.6 times; intensity of the marker expression – in 1.3 and 1.7 times, respectively (Fig. 2D vs 2E; Fig. 2D vs 2F).**

CONCLUSIONS

Metformin exhibits pronounced antiproliferative effects against skin cancer cells, including through blocking the cell cycle and glucose transporter GLUT1. Low glucose enhances the action of metformin as a potential adjuvant to anti-PD- L1 therapy.

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Conflict of interests: nothing to declare