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.

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).

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