

Cytotoxic effect of Metformin on Breast Cancer Cell lines



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Background

Breast cancer is still one of the most common tumours, and the primary leading cause of cancer deaths in women worldwide⁽¹⁾⁽²⁾. Emerging evidence suggests that metformin, the most widely used antidiabetic drug, shows antitumor effect in various cancer models⁽³⁾⁽⁴⁾, however the exact mechanism of action remains to be elusive. we aim to investigate the anti-tumour effect of metformin on breast cancer cell lines

Methods

The effect of metformin on MCF-7 and MDA-MB-231 was evaluated on:

- Cytotoxic effect using CCK-8 kit.
- Apoptotic effect using Annexin-V staining and analysed by flowcytometry.
- Cancer stem cells characterization using CD44 and CD24 stain.

Objectives

- To investigate the cytotoxic effect of metformin on breast cancer cells (MCF-7, MDA-MB-231), and on cancer stem cells in both MCF-7 and MDA-MB-231.
- To identify the effect of metformin on tumour metabolism in MCF-7 and MDA-MB-231

Results

- MCF-7 and MDA-MB-231 breast cancer cell lines were exposed to serial dilution of metformin for 72h and IC50 were determined.
- Metformin induced 2.1±0.5% and 22.1±2.8% apoptosis in MCF-7 and MDA-MB-231 cells respectively.
- Currently no change was observed in (CD44+ CD24-) population (CSC)

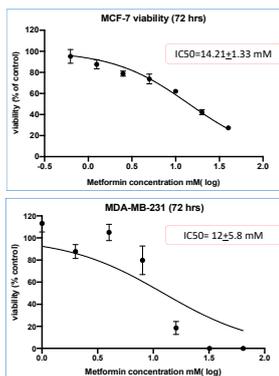


Figure 1. Cell viability was determined using CCK8. Data is expressed as mean ± SD (n=3).

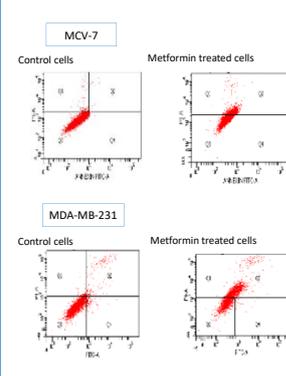


Figure2: Effect of metformin on Apoptosis/necrosis. Cells were exposed to 14 mM and 12 mM for 48 h. Cells were stained with annexin V-FITC/PI. Different cell populations were plotted as percentage of total events. Data is presented as mean ± SD; n=3, significantly different from control group. (***)P<0.0002; (****)P<0.0001.

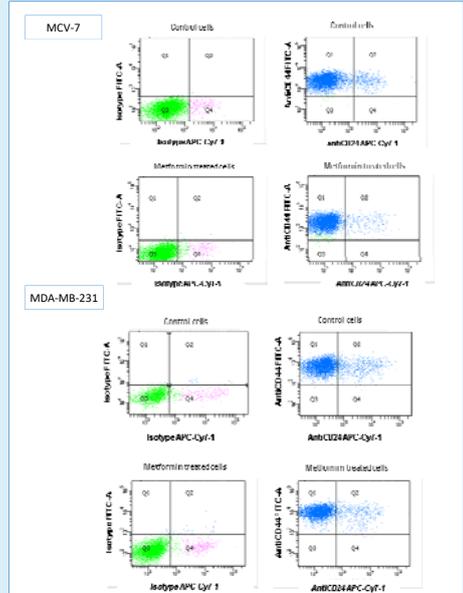


Figure3:Figure 3:Effect of metformin on the expression of CD44 and CD24 stem cell markers Cells were exposed to metformin for 24 h. Expression levels of CD44 and CD24 were assessed using flow cytometry plotted as percentage of total events.

Discussion

- We evaluated the effect of metformin on cell viability, which are the MCF-7 and MDA-MB-231 cell lines (which are ER+ and triple negative, respectively). The cytotoxic effect of metformin was dose dependent on both cell lines
- The effect was observed in the TNBC cell subtype MDA-MB-231 as well as in ER+ and that mean the mechanism may be based on something rather than the receptor.
- Metformin has an anti-cancer activity which inhibited the growth of two breast cancer cell lines in addition to the induction of apoptosis.
- To confirm the exact mechanism by which metformin causes apoptosis it is highly recommended to study the expression of the anti-apoptotic proteins B-cell lymphoma 2 (BCL-2) and myeloid cell leukemia-1, and the expression of the pro-apoptotic BCL-2-associated X protein (BAX) in the future⁽⁵⁾⁽⁶⁾.
- Currently no change was observed in (CD44+ CD24-) population which treated 24 hours only before detection of CSC characterization.
- study its effect on stemness related transcription factors including SOX2⁽⁷⁾, OCT4, NANOG, c-Myc and KLF4⁽⁸⁾⁽⁹⁾. will confirm the effect of metformin on CSC.

How to improve ?

- To confirm apoptosis mechanism further study needed such as expression of the anti-apoptotic and pro-apoptotic protein.
- study its effect on stemness related transcription factors is highly recommended.
- Our next step is study its effect on metabolism.

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