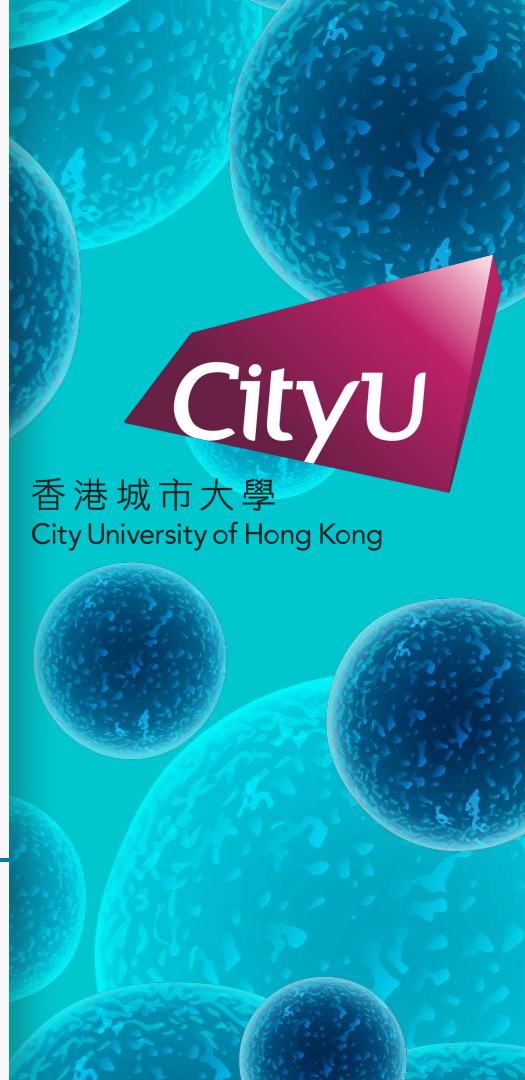
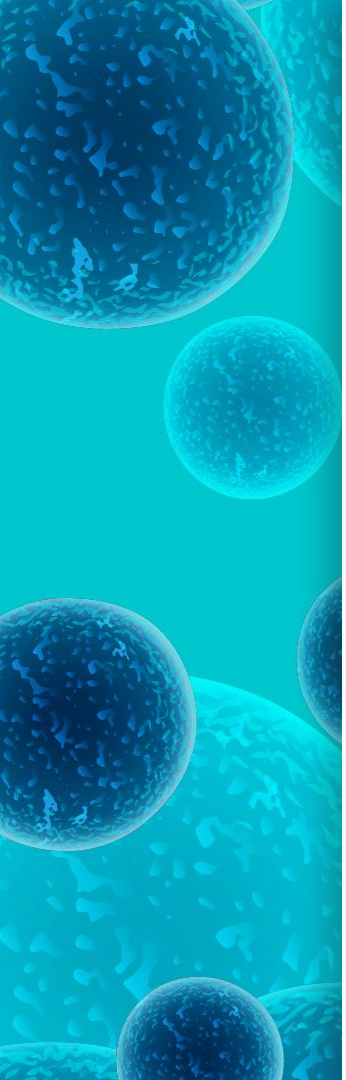
BMS4006 Medical Laboratory Research

Final Year Project 2023-24

Investigation of the Apoptotic Effects of Metformin: Potential for Anti-cancer Drug Development

HUANG Zechao 56642581





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Background & Significance

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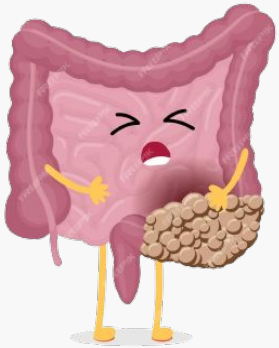
Metformin

- **Antidiabetic drug**, first-line treatment of T2D
- **Mechanism:** alternation of the energy metabolism in cells
- More and more studies illustrate **its anti-cancer effects**



Colorectal Cancer

- highly prevalent, second leading cause of cancer death
- **Chemotherapy** is one of the established first-line treatment
- Chemotherapeutic agents now, like **5-FU**, still have side--effects, including toxic effects, chemoresistance.

Literature Review

- Chen, K., Qian, W., Jiang, Z., Cheng, L., Li, J., Sun, L., … Ma, Q. (2017). Metformin suppresses cancer initiation and progression in genetic mouse models of **pancreatic cancer**. Molecular Cancer, 16(1), 131–131.
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- Zordoky, B. N. ., Bark, D., Soltys, C. L., Sung, M. M., & Dyck, J. R. . (2014). The anti-proliferative effect of metformin in **triple-negative MDA-MB-231 breast cancer** cells is highly dependent on glucose concentration: Implications for cancer therapy and prevention. Biochimica et Biophysica Acta. General Subjects, 1840(6), 1943–1957.

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Objectives

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Objectives

The primary objective of this project is to investigate the **apoptotic effects of metformin** on HT-29 cells so as to assess **its anti-cancer potential**.

MTS assay	→	**Effective time & concentrations**
Cell morphology	→	**Morphological alteration**
Flow cytometry	→	**Cell death**
Synergistic groups	→	**Potential as adjuvant**

03

Methods & Materials

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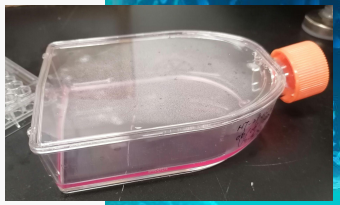
Cell Culture



Cell line:

HT-29 cell line

CAL-1 cell line

Environment:

37˚C, 5% CO2

Drugs:

Metformin

5-FU

Culture medium:

RPMI 1640 medium

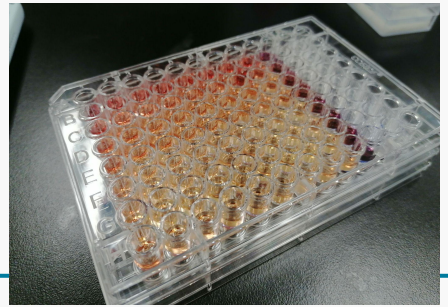
Cell density:

Trypan blue exclusion test

Cell subculture:

Perform regularly

MTS assay



96-well plate; to exam cell viability

Time- & Dose-dependent

Absorbance (492 nm; ref. 620 nm)

Time: 48 & 72 h

Concentrations:

10 mM - metformin,

50 µM - 5-FU

Cell morphology

Inverted Microscope

to exam morphological alteration

Capture the image

Flow cytometry

6-well plate, cell seeding and harvest

Annexin V-FITC & PI Apoptosis Detection Kit

to exam different types of cell death

excited at 488 nm; emitted at 500-560 nm for Annexin V-FITC and at >670 nm for PI

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Results

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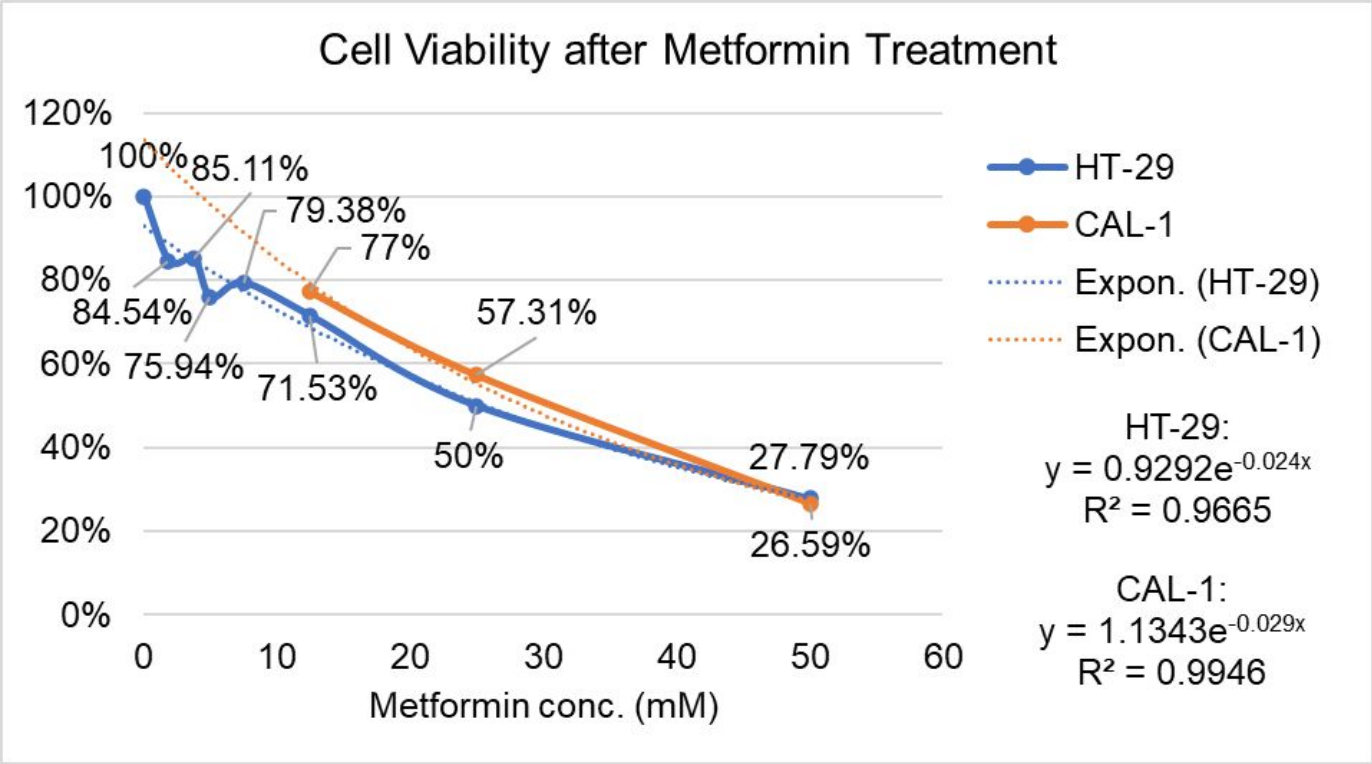
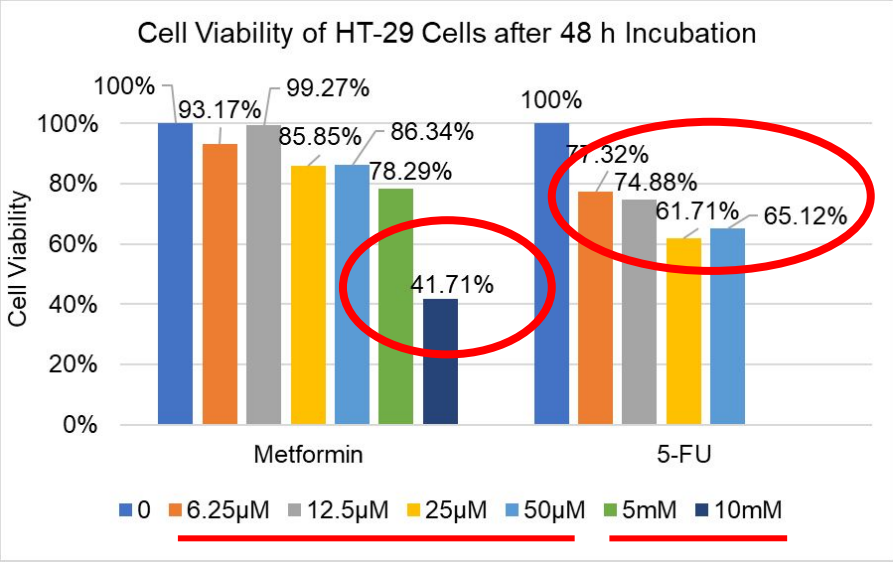
Results of wide range of metformin concentrations, 24 h

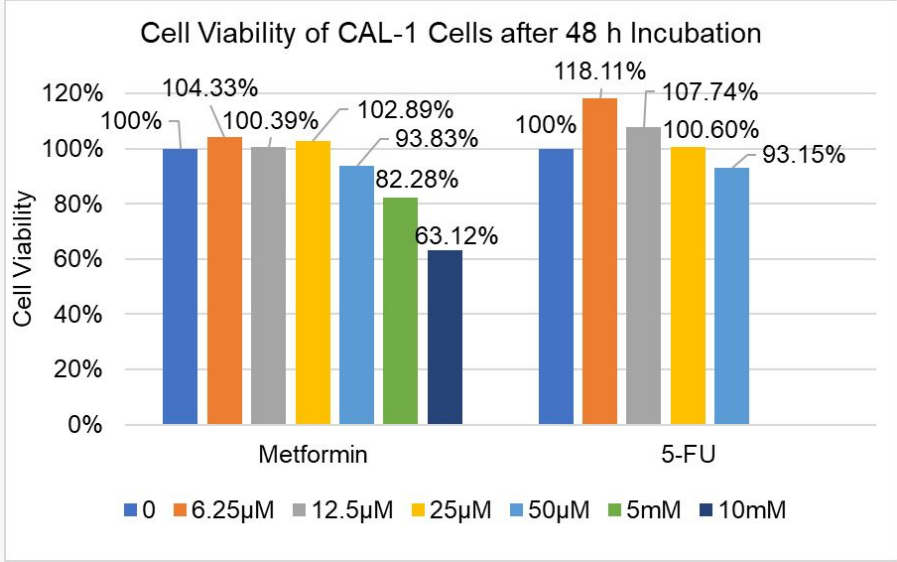


IC50 value of HT-29 cell: **25 mM**

The cytotoxic effect of metformin to normal cell line is too severe when the concentration is larger than 10 mM.

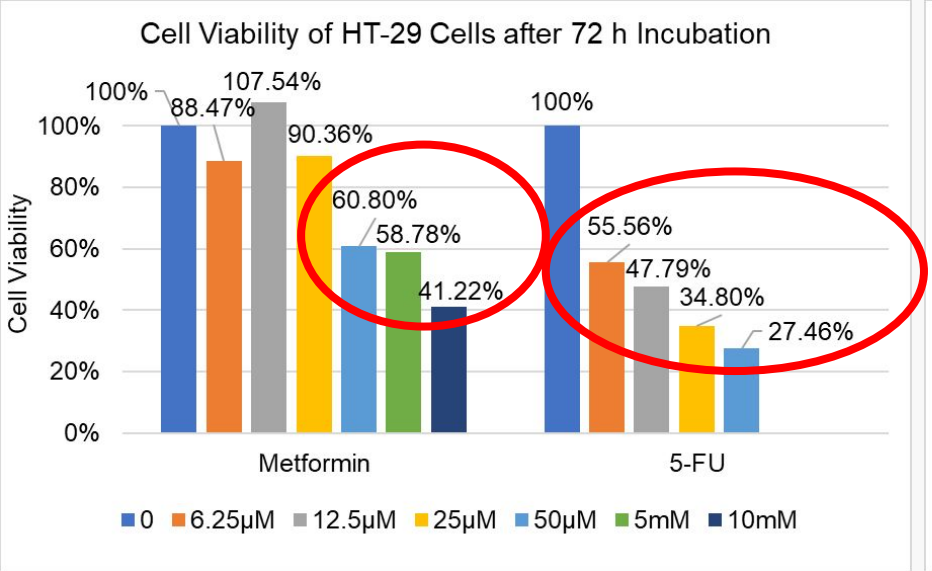
Time- & Dose-Dependent Impact of Metformin on HT-29 Cell Viability

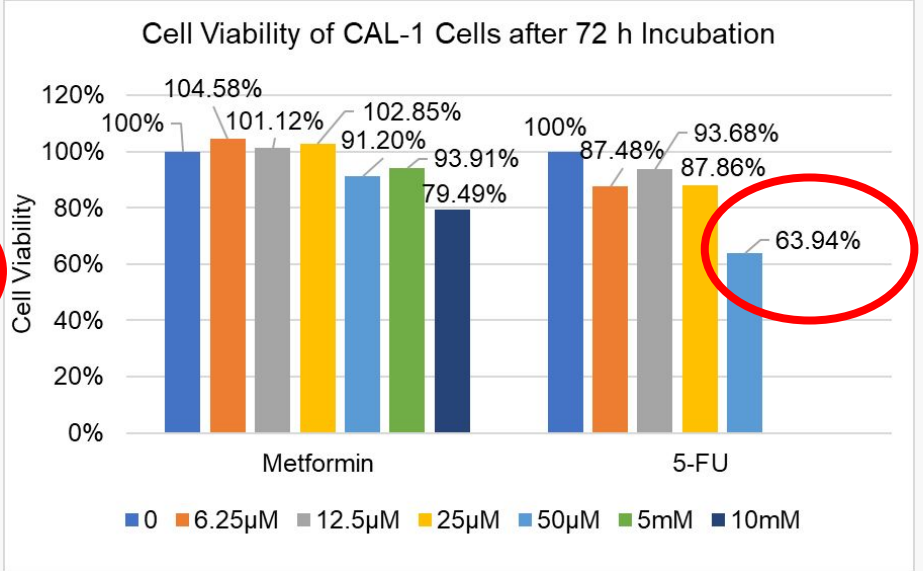




48 h group

Time- & Dose-Dependent Impact of Metformin on HT-29 Cell Viability

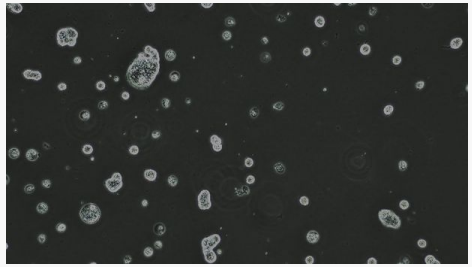


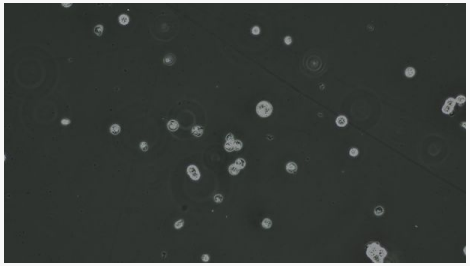


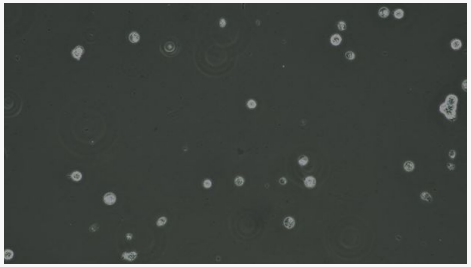
72 h group

Metformin-Induced Morphological Alterations in HT-29 Cells

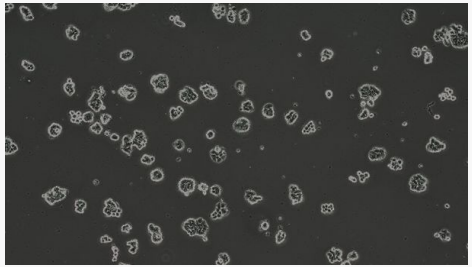
48 h:

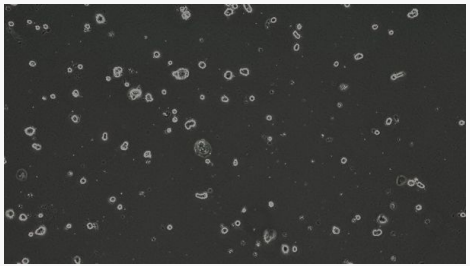


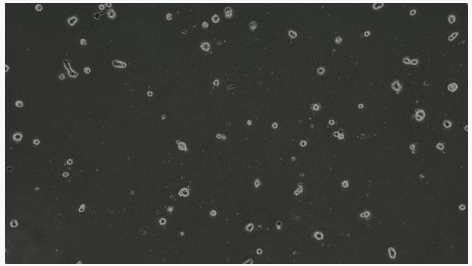




72 h: Blank Metformin (10mM) 5-FU (50μM)

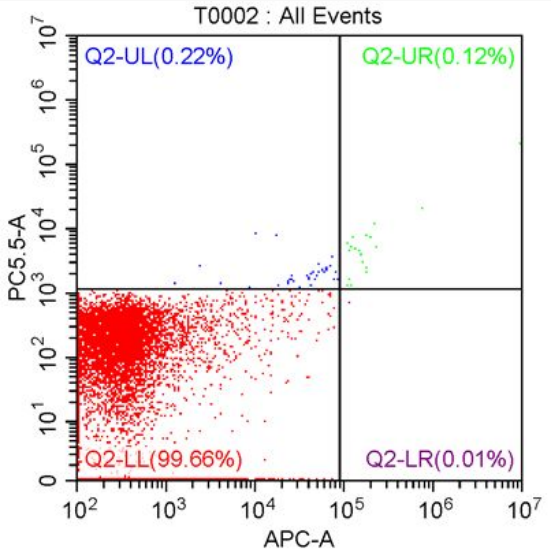


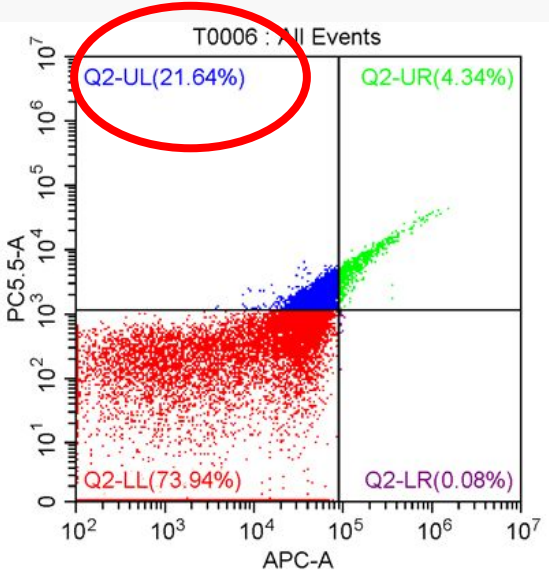


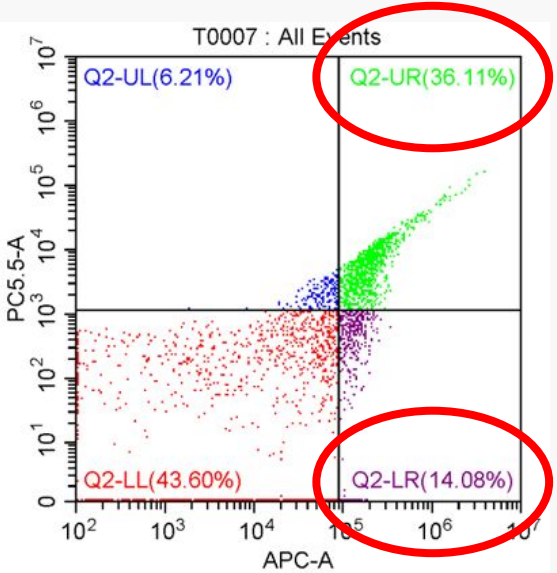


Metformin-Induced Cell Death in HT-29 Cells

HT-29 cell, 72 h incubation:



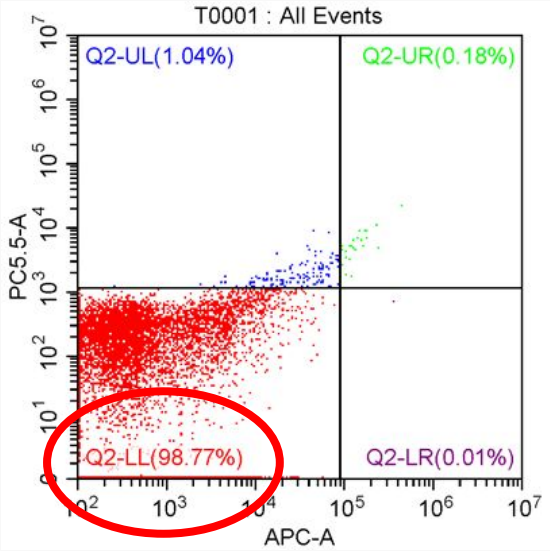


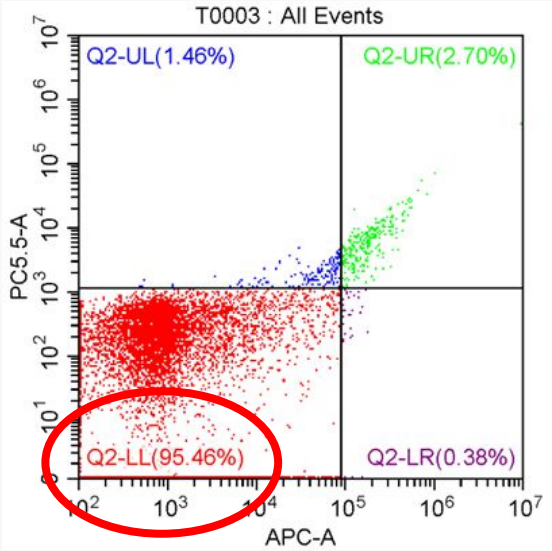


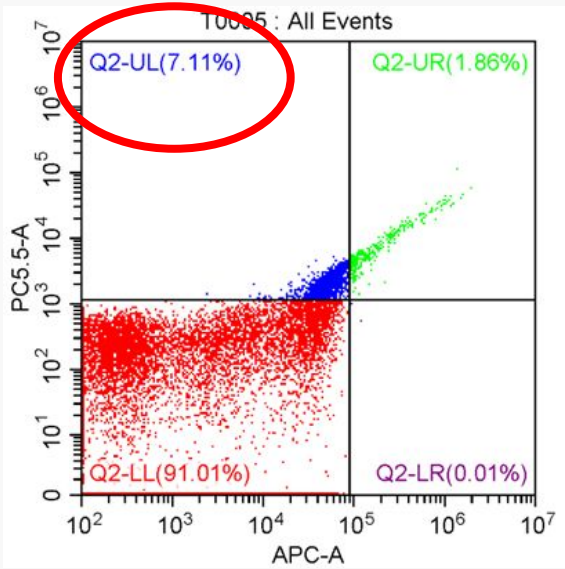
Blank | Metformin (10mM) | 5-FU (50μM)

Metformin-Induced Cell Death in HT-29 Cells

CAL-1 cell, 72 h incubation:

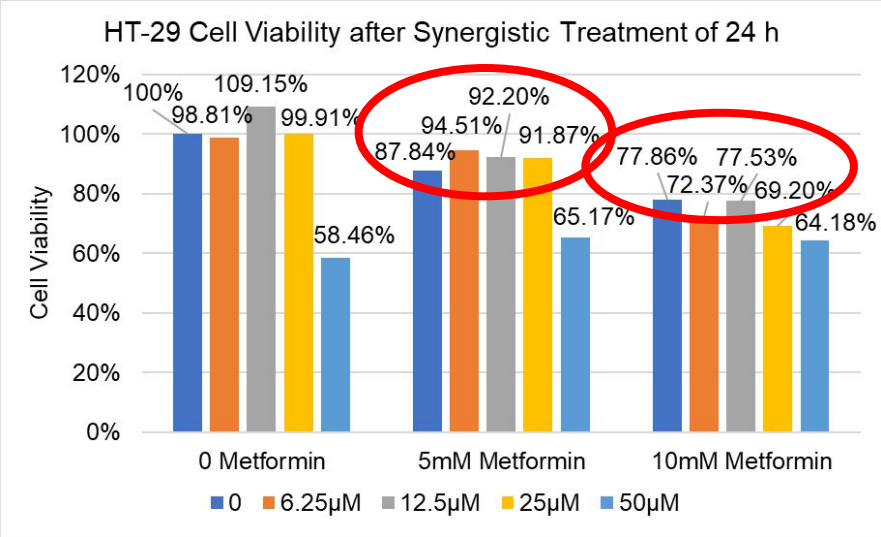






Blank | Metformin (10mM) | 5-FU (50μM)

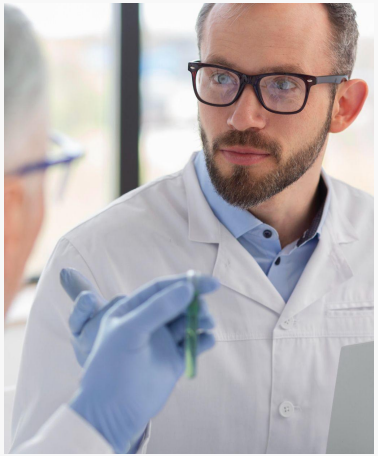
Synergistic Anti-Cancer Effects of Metformin and 5-FU in HT-29 Cells



Metformin has a **significant synergistic effect** with low concentrations of 5-FU (0 - 25 μM) on HT-29 cells ($p < 0.05$).

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.153763	1	0.153763	75.31889	0.000129	5.987378
Within Groups	0.012249	6	0.002041			
Total	0.166012	7				



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Discussion

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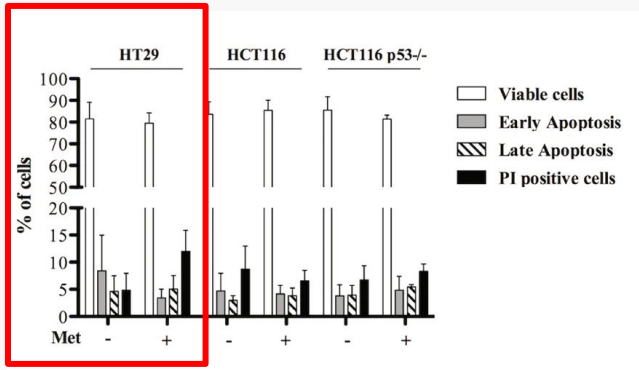
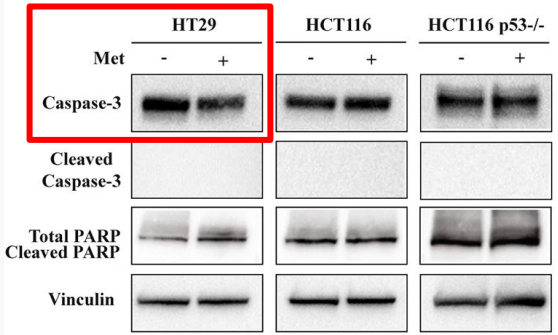
Metformin **does not have a significantly the apoptotic effects** on HT-29 cells



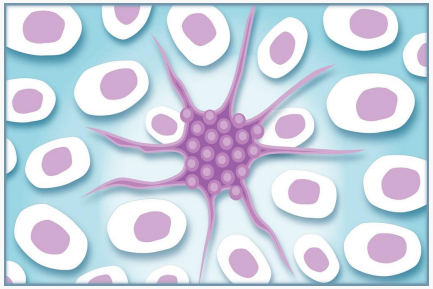
Activate **AMPK pathway** → suppress cancer cell, inhibit cancer cell proliferation (e.g. cyclin D1), <u>decrease cell viability</u> observed



Increase ROS, suppress **mTOR pathway**→ oxidative stress and mitochondrial depolarization, may cause <u>necrosis</u>

Metformin still possesses excellent **anti-cancer effects.**

Limitation



- May not accord with the complex **in vivo** environment
- Other cancer and normal **cell lines** are required to get a full image
- **Apoptosis assay** needs to be performed for synergetic group
- Further **molecular mechanism** study is required

Some **technical issues** are also encountered during experiment.

Good time management and experiment design are crucial for lab research!

06

Conclusion

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Conclusion

- Metformin **does not significantly induce the apoptosis** of HT-29 cells
- Metformin still possesses excellent **anti-cancer effects**
  - limit cell viability
  - cause morphological changes
  - induce cancer cell necrosis
  - strong synergy with 5-FU
  - less cytotoxic to normal cells
- Further study can focus on the mechanisms, adjuvant drug, and clinical trials in vivo.

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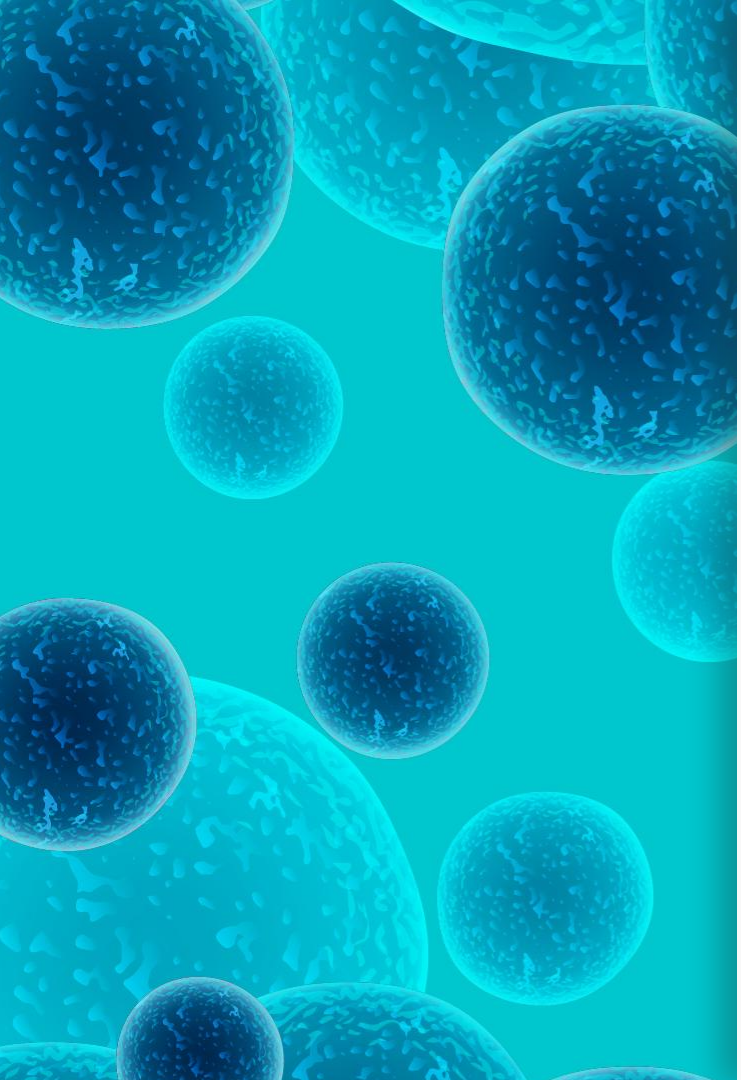


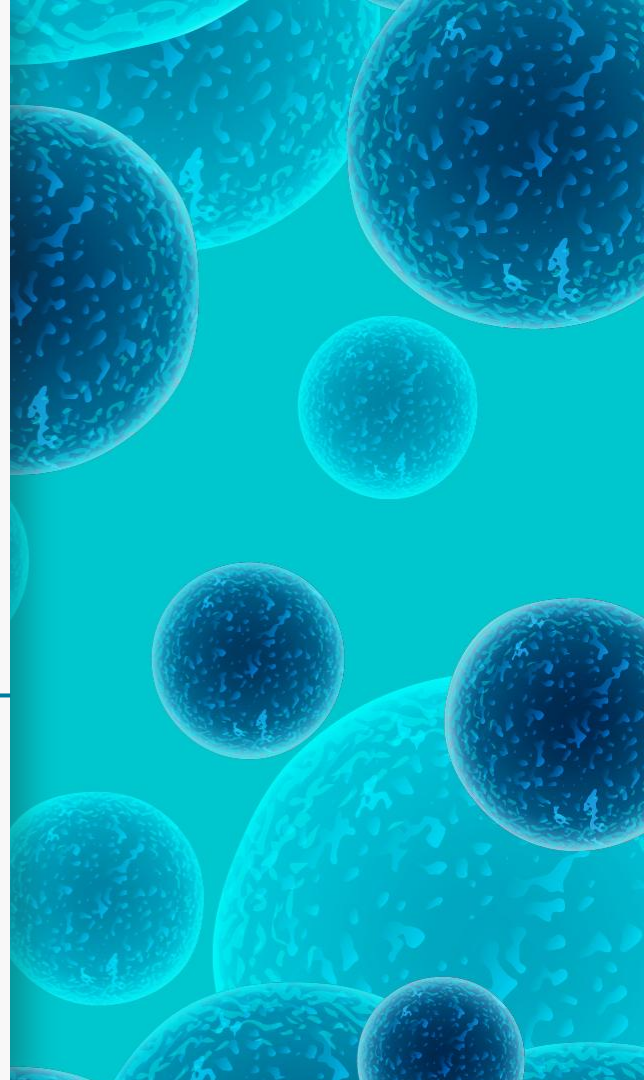
Thank You!

Q & A

07

Supplementary Materials

HUANG Zechao 56642581



Information of HT-29 cell line

HT-29

HTB-38 ™

HT-29 is a cell line with epithelial morphology that was isolated in 1964 from a primary tumor obtained from a 44-year-old, White, female patient with colorectal adenocarcinoma. This cell line is a suitable transfection host and has applications in cancer and toxicology research.

99/100 Bioz Stars [5,136 Product Citations]

Product category	Human cells
Organism	*Homo sapiens*, human
Morphology	epithelial
Tissue	Colon
Disease	Adenocarcinoma; Colorectal
Applications	3D cell culture Cancer research High-throughput screening Toxicology

MTS assay

Product name

MTS Assay Kit (Cell Proliferation) (Colorimetric)

See all Cell viability/proliferation kits

Detection method

Colorimetric

Sample type

Adherent cells, Suspension cells

Assay type

Quantitative

Assay time

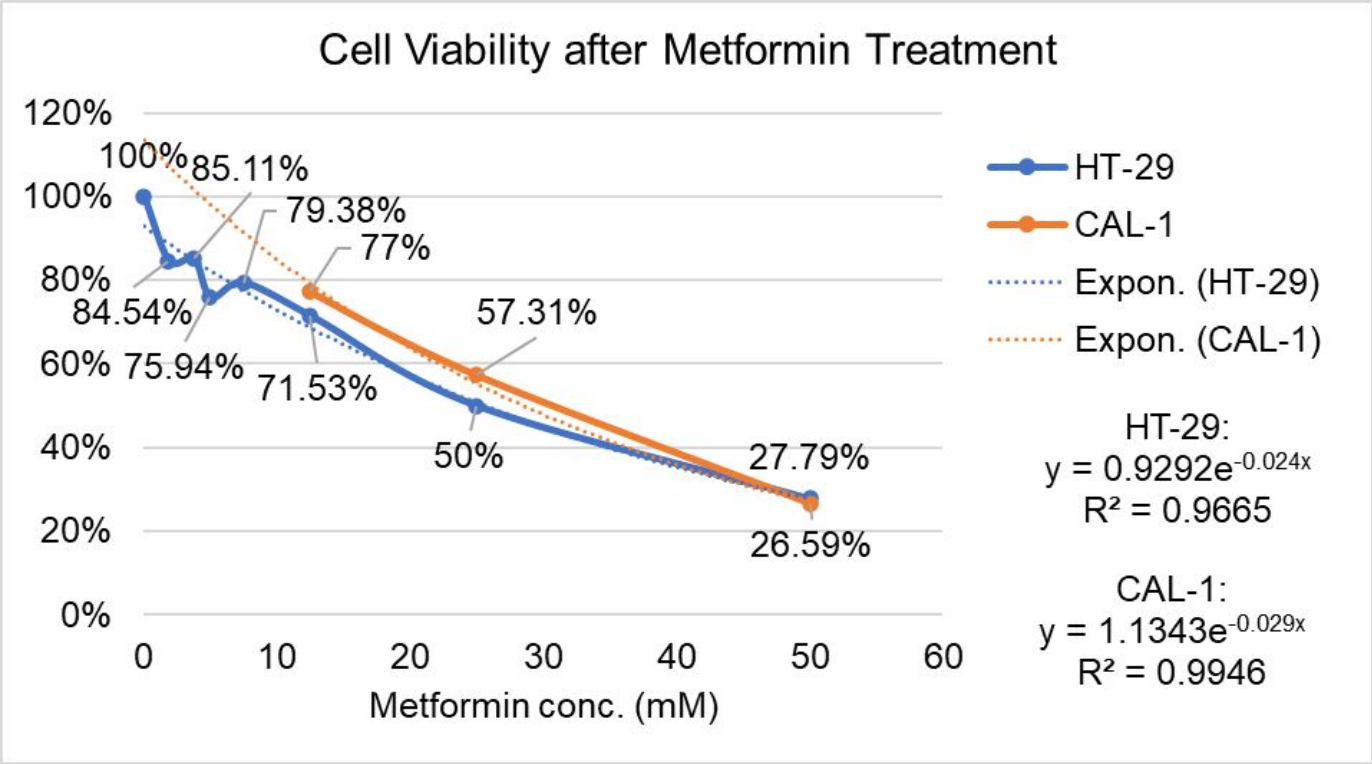
4h 00m

Product overview

MTS Assay Kit ab197010 uses a colorimetric method for the sensitive quantification of viable cells. It is based on a single ready-to-use reagent. The MTS assay is used to assess cell proliferation, cell viability and cytotoxicity.

The MTS assay protocol is based on the reduction of the MTS tetrazolium compound by viable mammalian cells (and cells from other species) to generate a colored formazan dye that is soluble in cell culture media. This conversion is thought to be carried out by NAD(P)H-dependent dehydrogenase enzymes in metabolically active cells. The formazan dye is quantified by measuring the absorbance at 490-500 nm. NB: MTS is also available as free molecule as **ab223881** (Tetrazolium inner salt cell proliferation reagent).

Results of wide range of metformin concentrations, 24 h



IC50 value of HT-29 cell: **25 mM**

The cytotoxic effect of metformin to normal cell line is too severe when the concentration is larger than 10 mM.

Determination of effective cell density

	7.5 mM	3.75 mM	1.875 mM	0
1 x 10^4 cells/well	0.5545	0.5945	0.5905	0.6985
	79.38%	85.11%	84.54%	100%
5 x 10^3 cells/well	0.456	0.576	0.5257	0.769
	59.30%	74.90%	68.60%	100%
2.5 x 10^3 cells/ well	0.2725	0.155	0.342	0.322
	84.63%	48.14%	106.21%	100%
1.25 x 10^3 cells/well	0.1455	0.116	0.154	0.159
	91.51%	72.96%	96.86%	100%

Results of wide range of metformin concentrations, 24 h

Cancer cell:

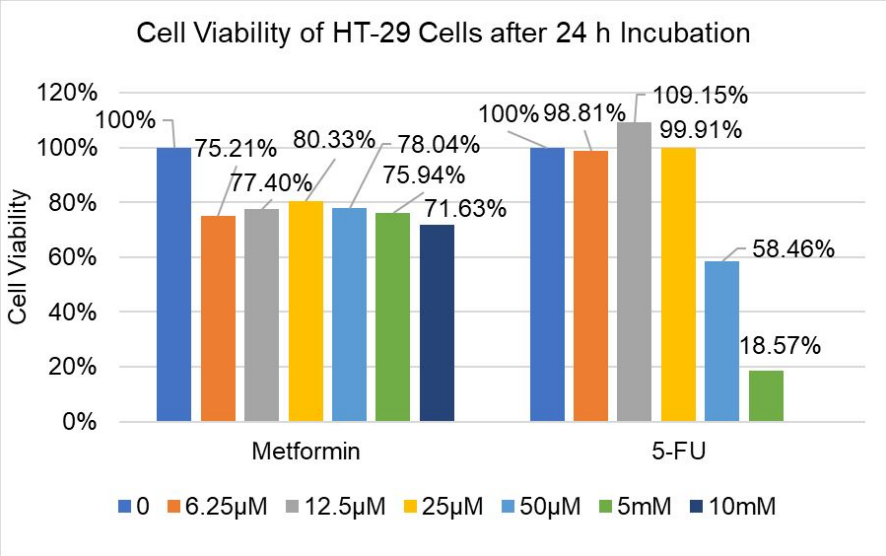
Drug	5-FU					Blank
Concentration	50mM	25mM	12.5mM	20μM	10μM	0
Absorbance	0.137	0.11	0.179	0.404	0.45	0.555
	0.171	0.111	0.187	0.507	0.52	0.527
Average	0.154	0.1105	0.183	0.4555	0.485	0.541
Viability Rate	28.47%	20.43%	33.83%	84.20%	89.65%	100%

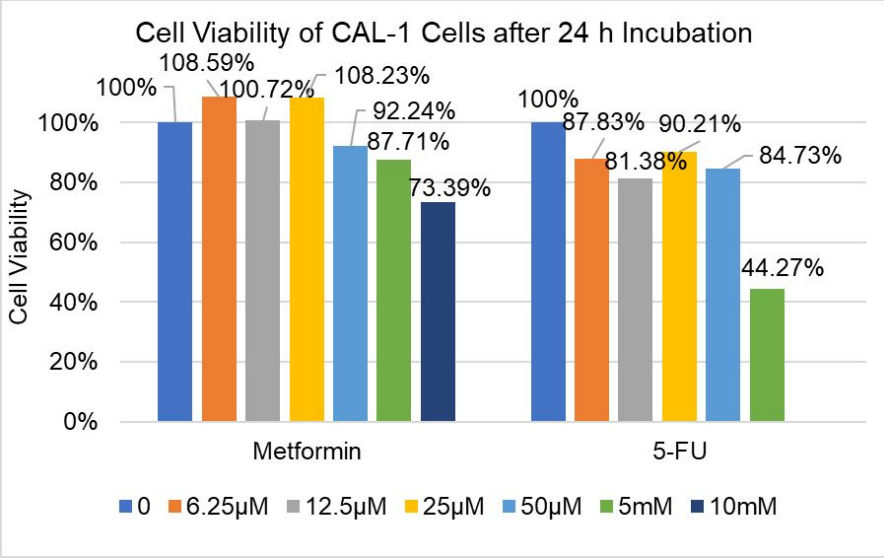
Drug	Metformin					Blank
Concentration	50mM	25mM	12.5mM	20μM	10μM	0
Absorbance	0.098	0.16	0.223	0.439	0.386	0.39
	0.106	0.207	0.302	0.338	0.434	0.344
Average	0.102	0.1835	0.2625	0.4135	0.41	0.367
Viability Rate	27.79%	50%	71.53%	112.67%	111.72%	100%

Normal cell:

Drug	Metformin					Blank
Concentration	50mM	25mM	12.5mM	20μM	10μM	0
Absorbance	0.091	0.21	0.312	0.276	0.322	0.453
	0.109	0.221	0.269	0.336	0.365	0.299
Average	0.1	0.2155	0.2905	0.306	0.3435	0.376
Viability Rate	26.59%	57.31%	77.26%	81.38%	91.36%	100%

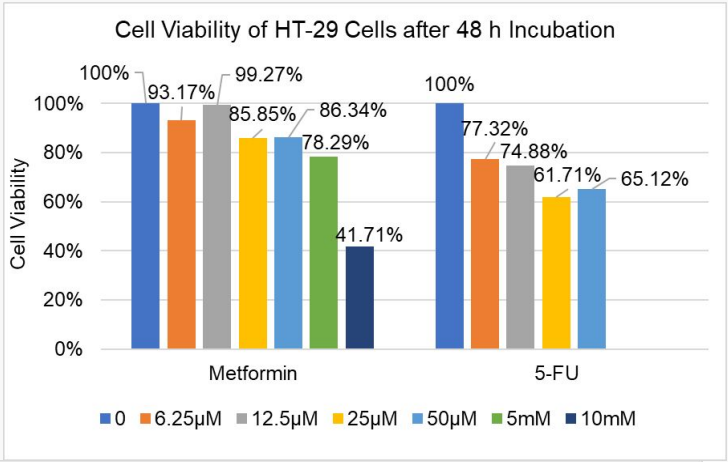
Time- & Dose-Dependent Impact of Metformin on HT-29 Cell Viability

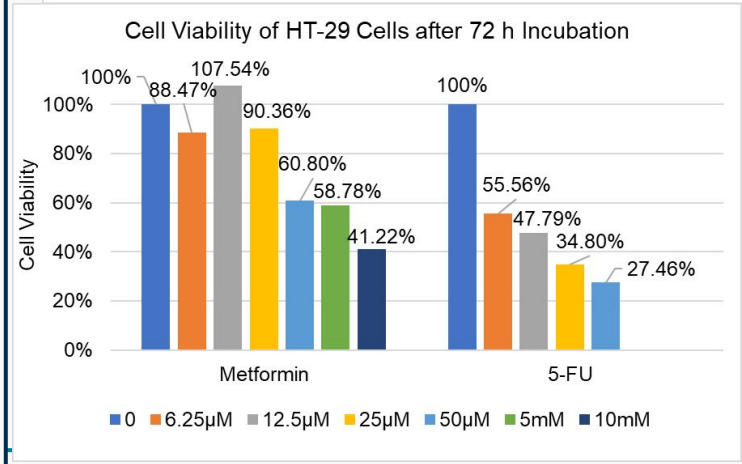


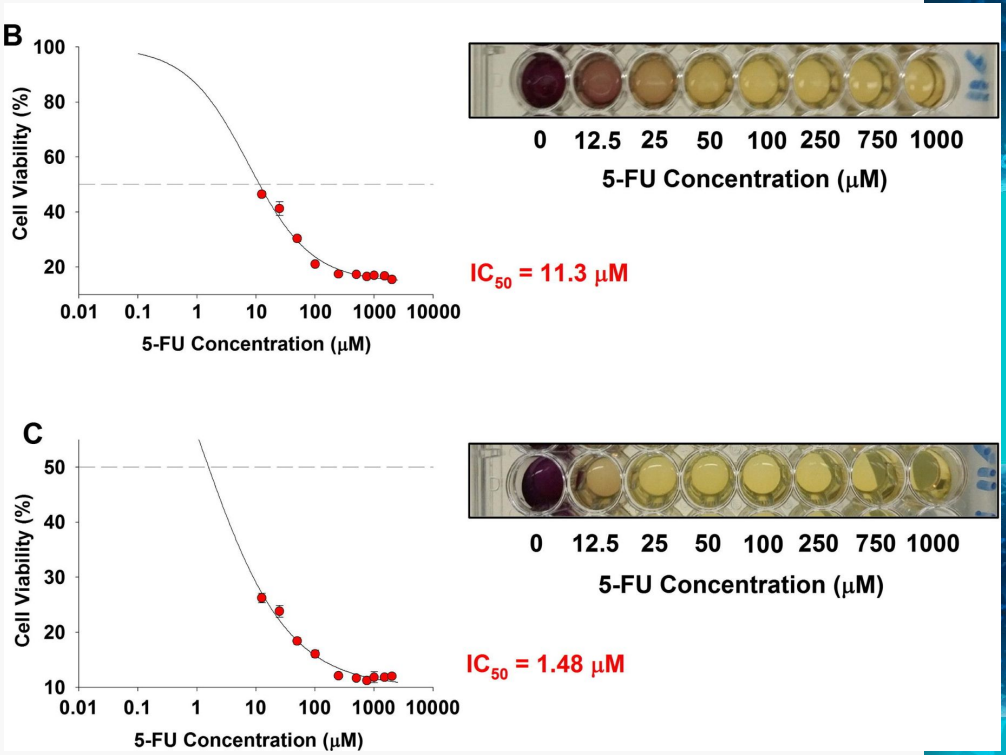


24 h group

Consistency of 5-FU treatment with previous studies

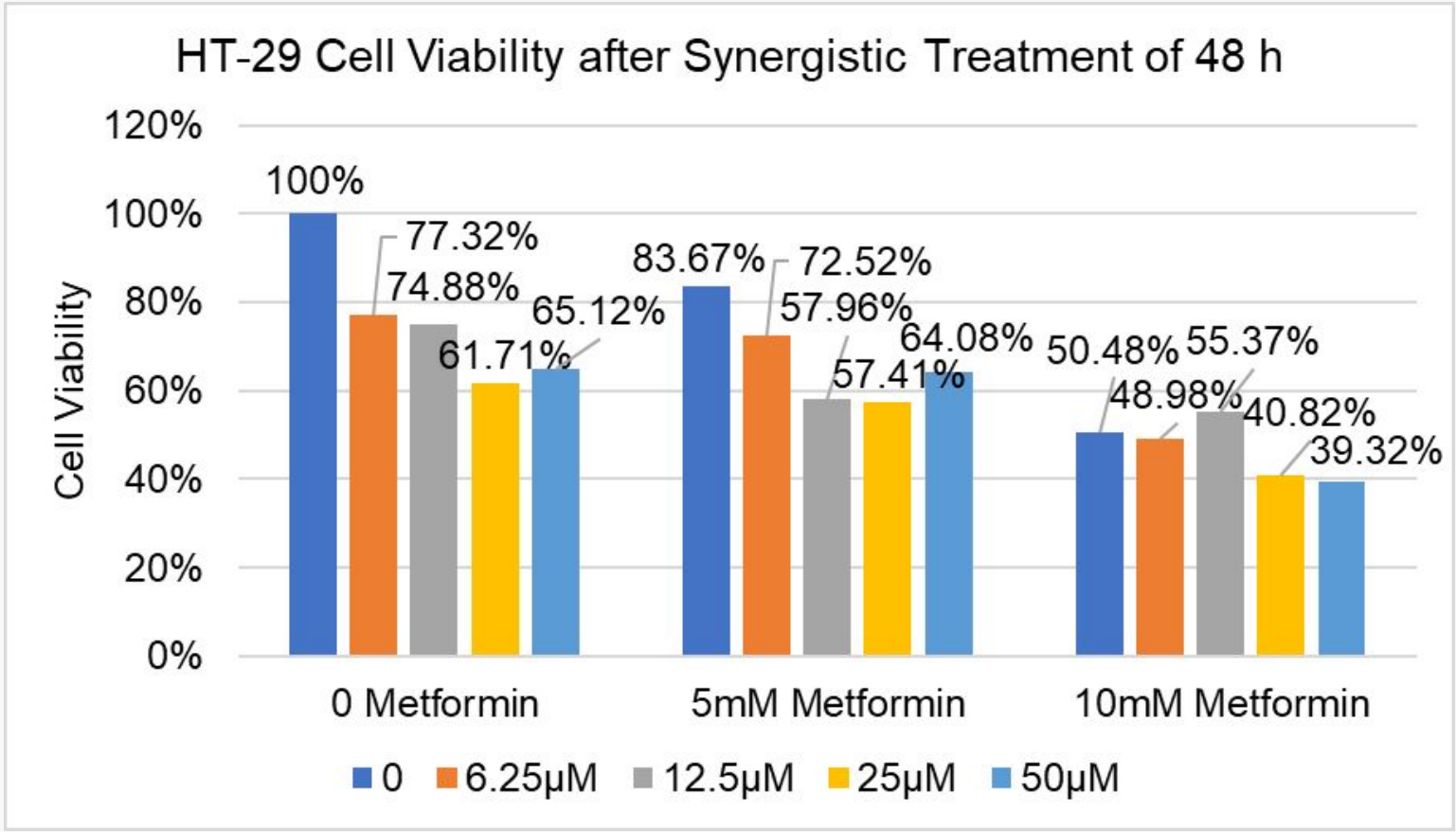






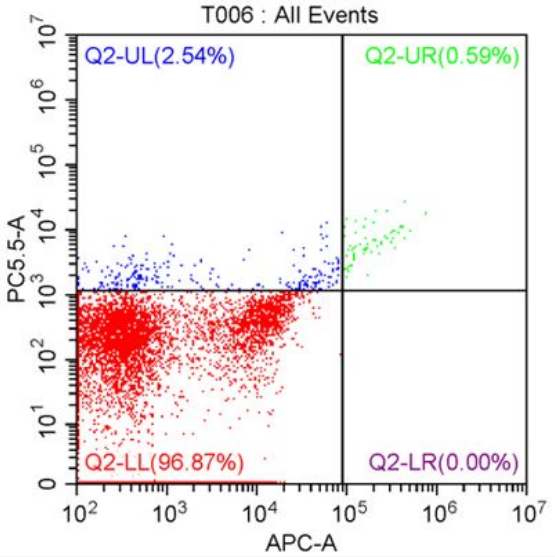
Tawfik et al. (2017)

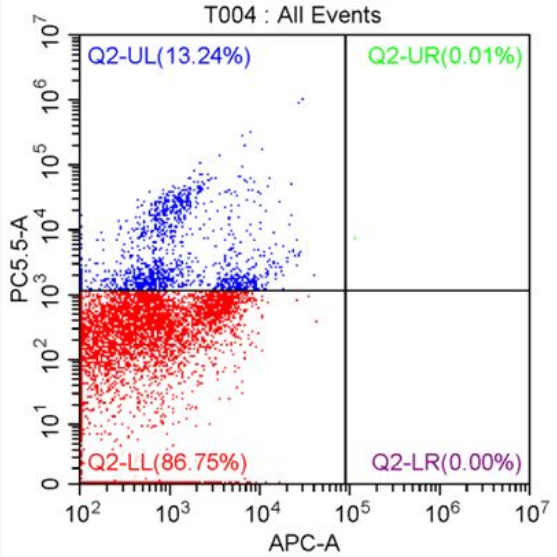
Synergistic group, 48 h

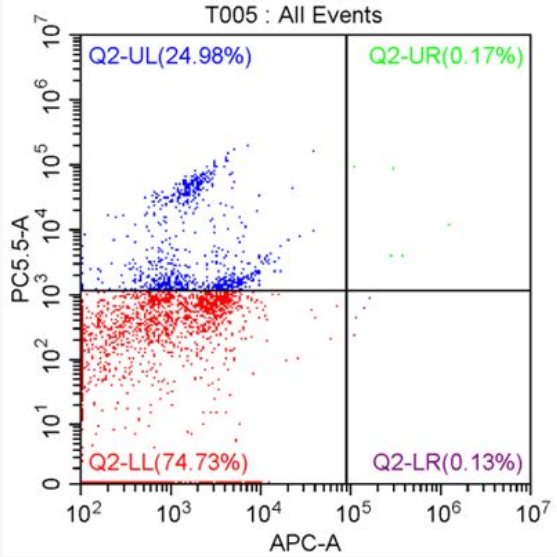


Metformin-Induced Cell Death in HT-29 Cells

HT-29 cell, 48 h incubation:



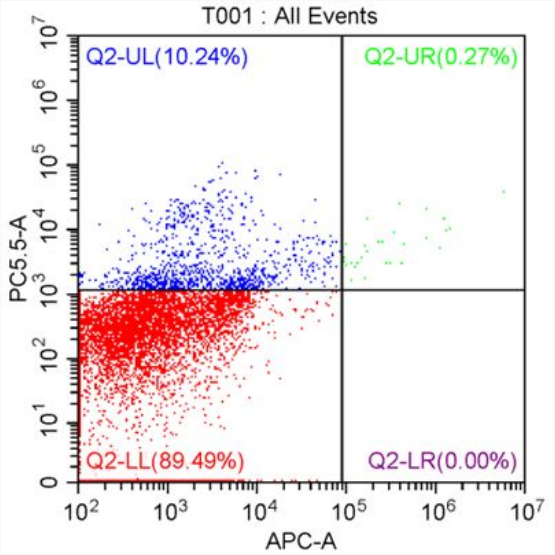


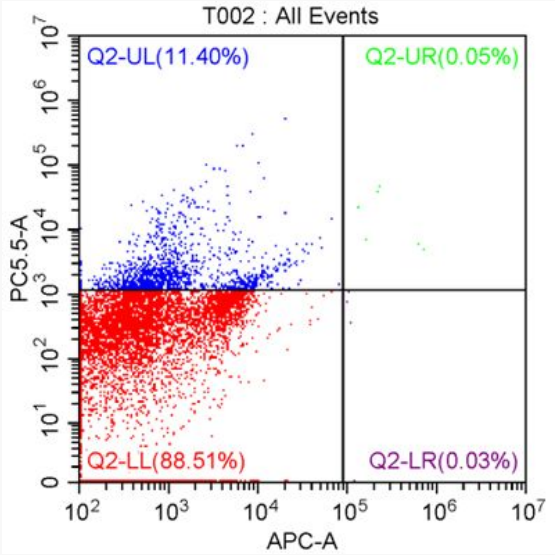


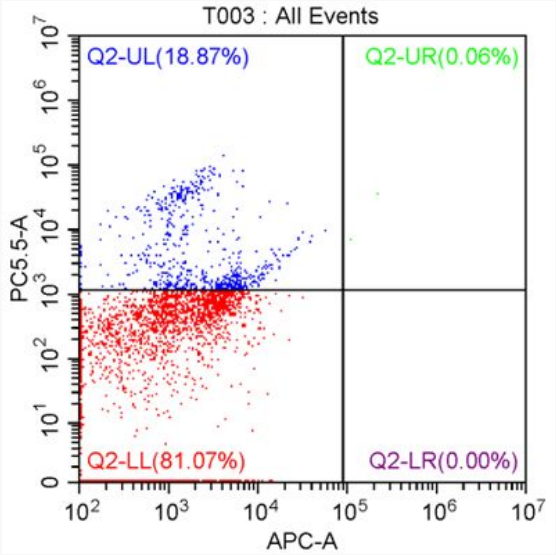
Blank | Metformin (10mM) | 5-FU (50μM)

Metformin-Induced Cell Death in HT-29 Cells

CAL-1 cell, 48 h incubation:



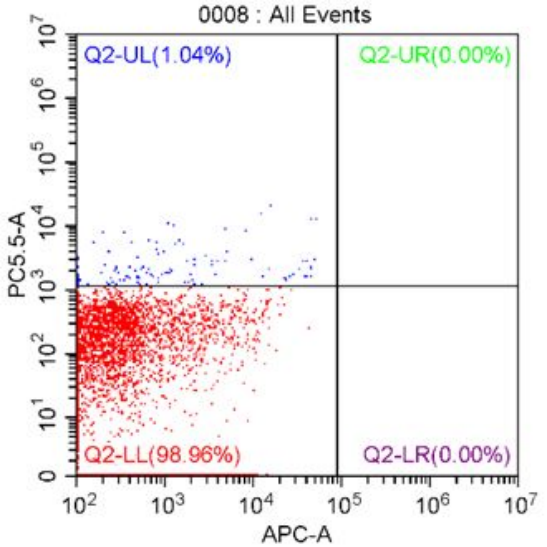


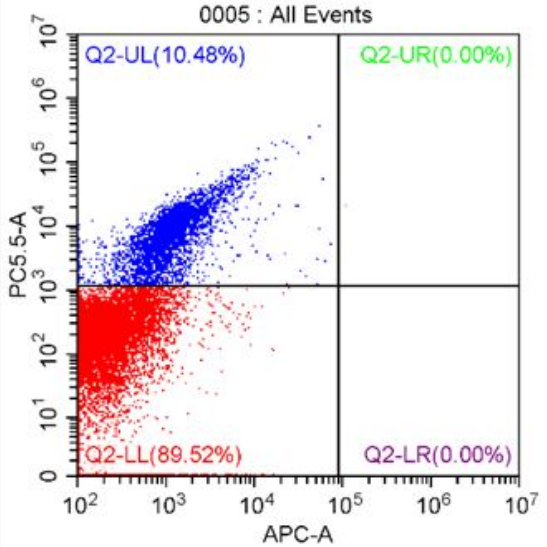


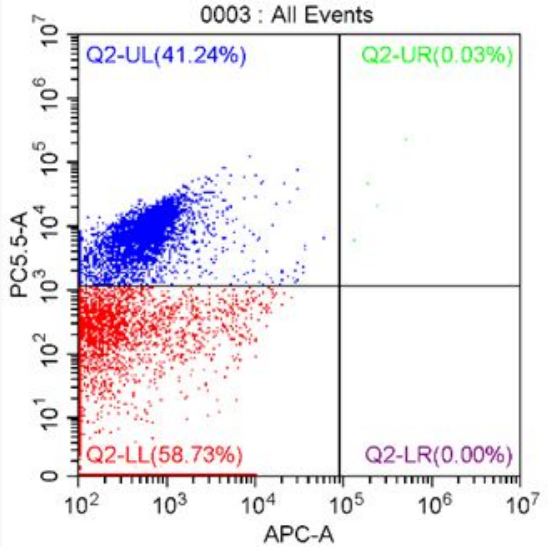
Blank | Metformin (10mM) | 5-FU (50μM)

Metformin-Induced Cell Death in HT-29 Cells

HT-29 cell, 48 h incubation:



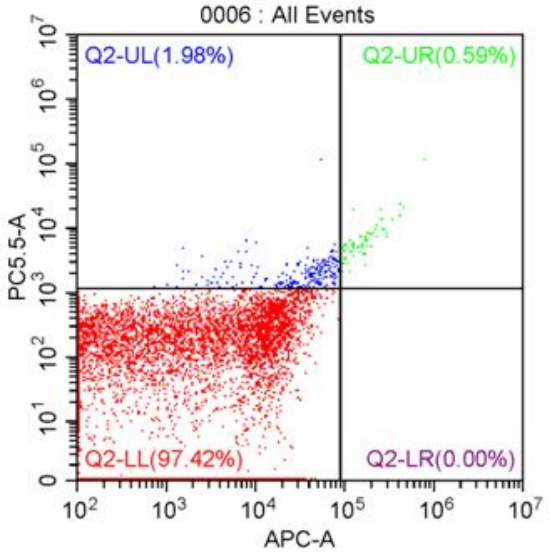


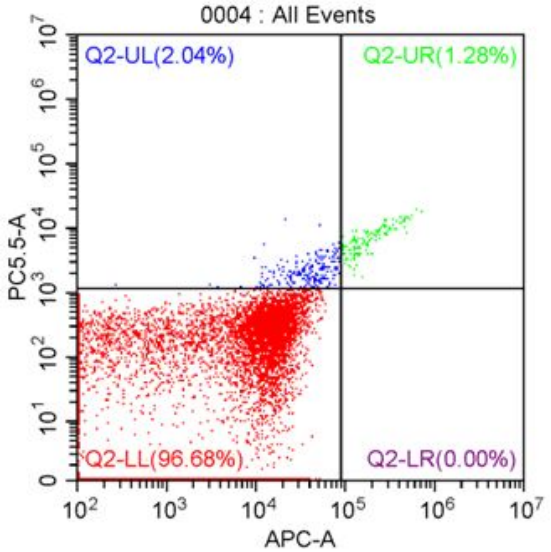


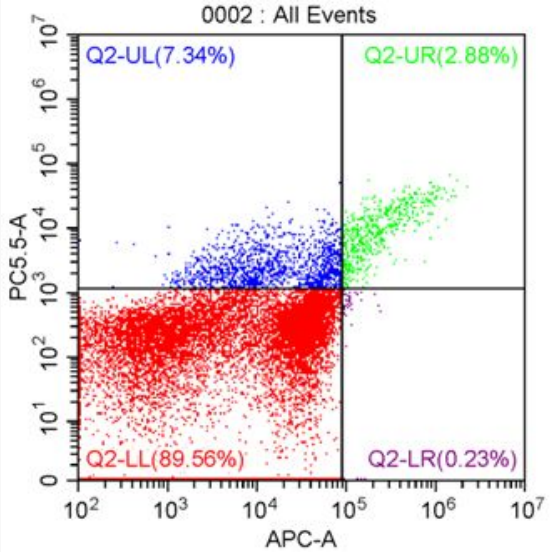
Blank | Metformin (10mM) | 5-FU (50μM)

Metformin-Induced Cell Death in HT-29 Cells

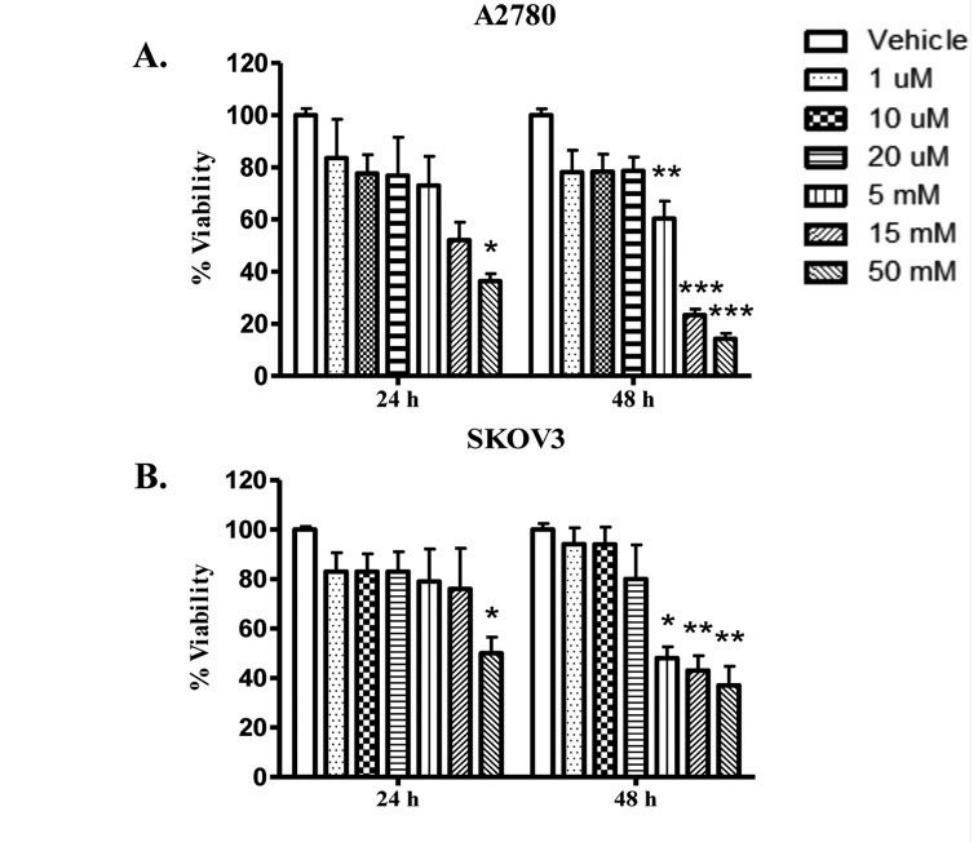
CAL-1 cell, 48 h incubation:







Blank | Metformin (10mM) | 5-FU (50μM)


A2780
A.
% Viability
120
100
80
60
40
20
0
24 h
48 h
*
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***
***
Vehicle
1 uM
10 uM
20 uM
5 mM
15 mM
50 mM
SKOV3
B.
% Viability
120
100
80
60
40
20
0
24 h
48 h
*
*
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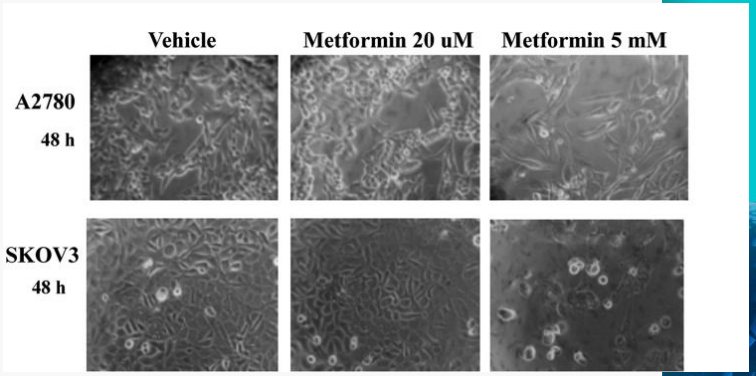
Vehicle
Metformin 20 uM
Metformin 5 mM
A2780
48 h
SKOV3
48 h