Effect of curcumin and nutrient deprivation on breast cancer cell viability and proliferation

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INTRODUCTION

- Nutritional interventions often aim to enhance cancer treatment therapies. Extensive research over the last decades suggests potential role of polyphenol curcumin (derived from the rhizome of *Curcuma longa* L.) in modulating cancer development and progression. The aim of this study was to determine whether nutritional deprivation combined with curcumin application can suppress cancer cell viability and proliferation.

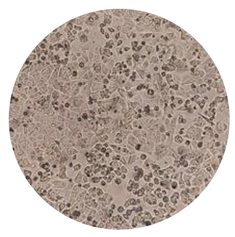
METHODOLOGY and RESULTS

Influence of cell culture media (nutrient deprivation) on MCF-7 proliferation:

- initial cell concentration $5x10^4$ cells/mL; V=100 µL of cell suspension per well (96-well plate) → cell viability determined after 96 h by MTT method

Cells cultured in:

- DMEM media with high Glc concentration (4500 mg/L) - DMEM_HGlc
- DMEM media with low Glc concentration (1000 mg/L) – DMEM_LGlc
- DMEM_HGlc and DMEM_LGlc media diluted with HBSS or PBS in ratios 1:1 and 2:1



Breast cancer (MCF-7) cells

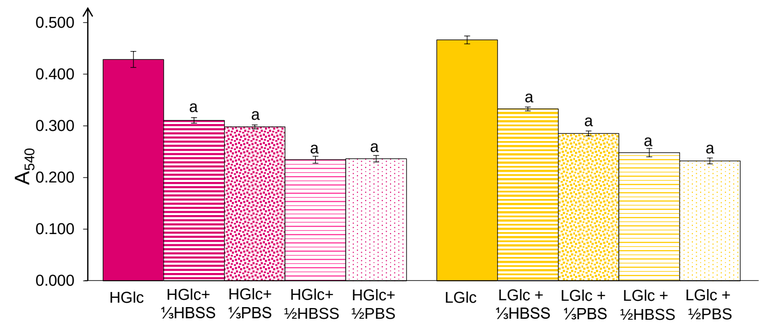


Fig. 1 MCF-7 cells cultured in different media (DMEM with high (4500 mg/L; DMEM_HGlc) or low (1000 mg/L; DMEM_LGlc) glucose concentration, and these media diluted with HBSS or PBS in ratios 1:1 (½DMEM_HGlc+½HBSS; ½DMEM_HGlc+½PBS; ½DMEM_LGlc+½HBSS; ½DMEM_LGlc+½PBS) and 2:1 (⅔DMEM_HGlc+⅓HBSS; ⅔DMEM_HGlc+⅓PBS; ⅔DMEM_LGlc+⅓HBSS; ⅔DMEM_LGlc+⅓PBS). MCF-7 viability was assessed after 96 h with MTT assay. Data are presented as mean ± SEM. Statistical significance (Student's *t*-test) *vs.* DMEM_HGlc or DMEM_LGlc: $^a p<0.001$

Influence of nutrient deprivation and curcumin on MCF-7 viability and proliferation:

- initial cell concentration $5x10^4$ cells/mL; V=100 µL of cell suspension in 2/3 DMEM_HGlc+1/3 PBS or 2/3 DMEM_LGlc+1/3 PBS per well (96-well plate)

Treatment with curcumin (CRC):

- 24 h after seeding treatment of cells with 2.5–100 µM CRC
- Control – DMEM_HGlc+DMSO or DMEM_LGlc+DMSO

Determination of cell viability:

- 72 h after treatment by MTT method

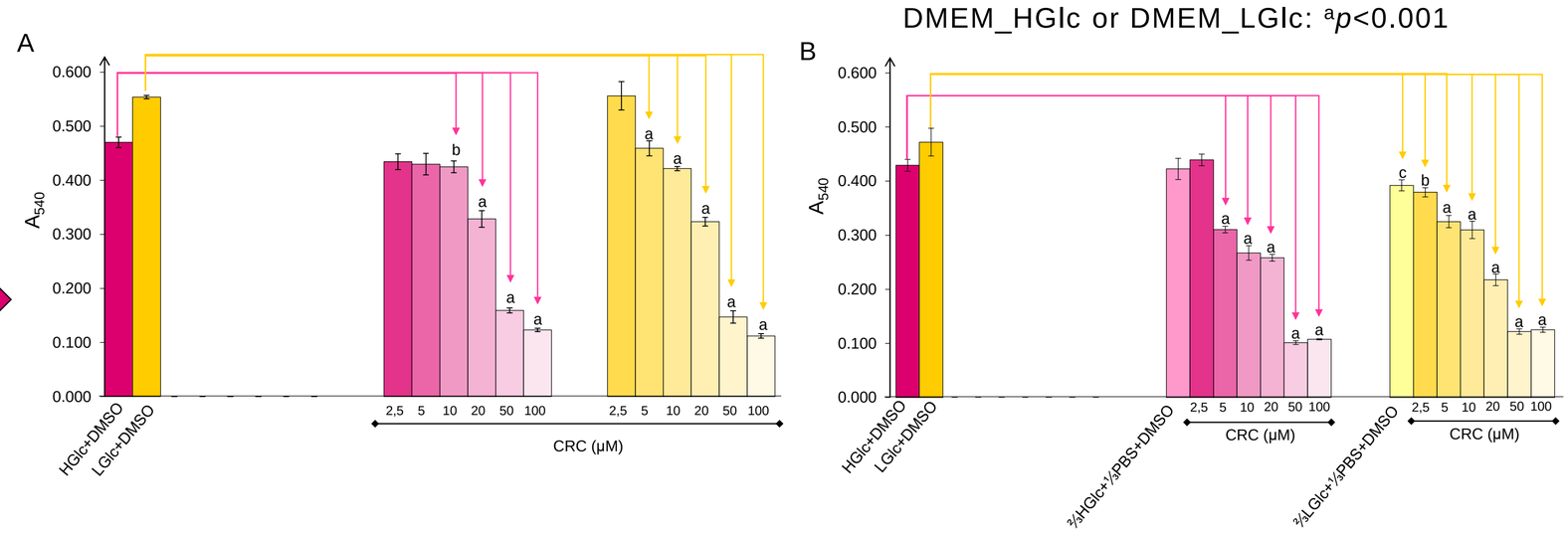


Fig. 2 Effects of 2.5 – 100 µM curcumin (CRC) on MCF-7 viability and proliferation after 72 h of exposure obtained with MTT assay (A) MCF-7 cells cultured in DMEM_HGlc and DMEM_LGlc; (B) MCF-7 cells cultured in ⅔ DMEM_HGlc+⅓PBS and ⅔ DMEM_LGlc+⅓PBS. Data are presented as mean ± SEM. Statistical significance (Student's *t*-test) *vs.* respective control: $^a p<0.001$, $^b p<0.05$

Influence of nutrient deprivation and curcumin on MCF-7 cell death:

- initial cell concentration $1x10^5$ cells/mL; V=4 mL of cell suspension in 2/3 DMEM_HGlc+1/3 PBS or 2/3 DMEM_LGlc+1/3 PBS per well (6-well plate)

Treatment with curcumin (CRC):

- 24 h after seeding treatment of cells with 5 – 100 µM CRC
- Control – DMEM_HGlc+DMSO or DMEM_LGlc+DMSO

Determination of cell death:

- 72 h after treatment by cytofluorimetric analysis (Muse Cell Analyzer, Merck Millipore)

Turmeric powder

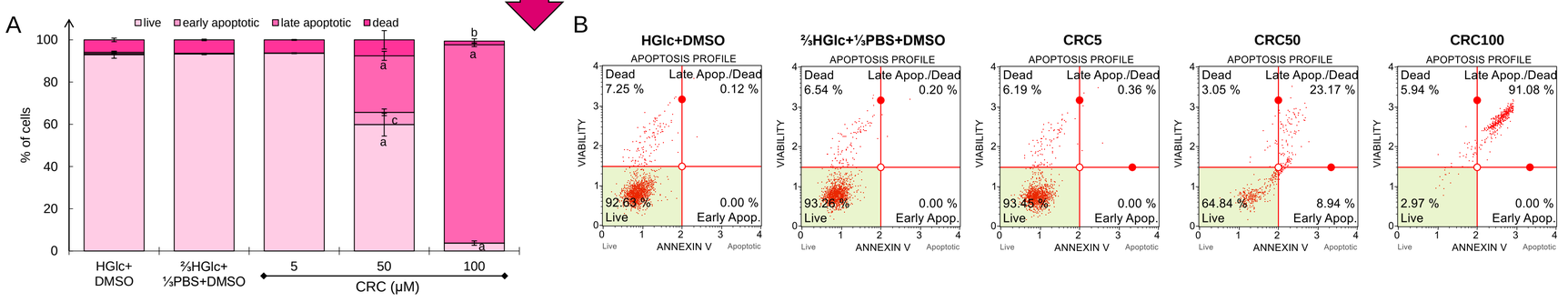


Fig. 3 Flow cytometric analysis of apoptosis/necrosis in MCF-7 cells cultured in ⅔DMEM_HGlc+⅓PBS after exposure to 5 – 100 µM curcumin (CRC) for 72 h. (A) Percentage of live, early and late apoptotic, and dead cells in population; mean ± SEM. Statistically significant (Student's *t*-test) $^a p<0.001$; $^b p<0.005$, $^c p<0.01$ *vs.* control. (B) Representative dot-plots of control and CRC treated cells.

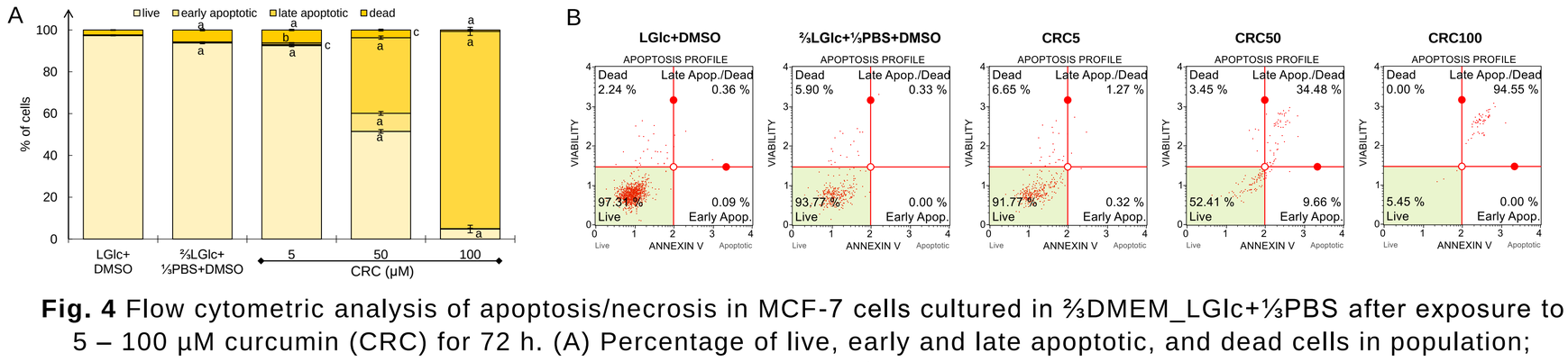


Fig. 4 Flow cytometric analysis of apoptosis/necrosis in MCF-7 cells cultured in ⅔DMEM_LGlc+⅓PBS after exposure to 5 – 100 µM curcumin (CRC) for 72 h. (A) Percentage of live, early and late apoptotic, and dead cells in population; mean ± SEM. Statistically significant (Student's *t*-test) $^a p<0.001$; $^b p<0.005$, $^c p<0.025$ *vs.* control. (B) Representative dot-plots of control and CRC treated cells.

CONCLUSION

- DMEM (high or low glucose) diluted with ⅓ PBS significantly inhibited MCF-7 proliferation (determined by MTT method), while the viability was still sufficient to set up experiments of nutrient deprivation combined with curcumin.
- Curcumin (2.5 – 100 µM) additionally enhanced the effect of nutrient deprivation in a dose-dependent manner. When cells were grown in PBS-diluted media (either high or low glucose), curcumin at doses ≥ 50 µM caused a strong growth inhibition (75 % compared to control).
- In addition, the proportion of apoptotic cells in the culture apparently increased with increasing curcumin concentration.
- Dilution of all nutrients with PBS had a pronounced effect on reducing cancer cell growth, in contrast to the amount of glucose in the medium, which did not appear to be a limiting parameter for cell viability.

***Curcuma longa* L.**

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