Role of curcumin as an anti-proliferative agent and apoptosis regulator in colorectal cancer cells

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Abstract

Curcumin is a polyphenolic compound abundant in turmeric (*Curcuma longa L.*) rhizomes, which is a popular spice in Asian cuisines. Curcumin exhibits health beneficial properties against inflammation, cancer, and neural damage. We studied the effects of curcumin in colorectal cancer cell (CRC) proliferation and its relationship with selected pro-apoptotic genes. The objectives of this study were to test the effects of curcumin on 1) CRC cell proliferation, 2) the expressions of selected pro-apoptotic genes (i.e., activating transcription factor 3-ATF3 and poly-adenosine-diphosphate-ribose polymerase-PARP), and 3) the transcriptional regulation of ATF3. Four CRC cell lines (HCT116, SW480, HCT15, and HT29) were tested against six doses of curcumin (0, 5 µM, 10 µM, 20 µM, 30 µM, and 40 µM) for their proliferation capacity over 48 or 72 hours post-treatment. Interestingly, 20 µM or more of curcumin suppressed the proliferation of all CRC cells significantly. Western blotting showed that curcumin increased the expression of 1) ATF3 in a dose- and time-dependent manner and 2) PARP cleavage in a dose-dependent manner in HCT116 cells. Moreover, 30 µM of curcumin treatment enhanced the ATF3 promoters significantly indicating the role of curcumin in transcriptional regulation of ATF3 in HCT116 cells. However, none of the cell signaling pathways were related to curcumin-induced ATF3 expression. This study re-emphasized the role of curcumin as an antiproliferative agent and apoptotic gene regulator in colorectal cancers.

Methods

Cell Proliferation:

- HCT116, SW480, HCT15, and HT29 cells were plated (5000 cells/well) in 96 wells.
- Cells were treated with 0, 5 µM, 10 µM, 20 µM, 30 µM, and 40 µM of curcumin with five replicates per treatment.
- Cells were incubated for 48-72 hours post-treatment.
- Cell proliferation was studied using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT assay).

ATF3 and Cleaved PARP Expressions:

- HCT116 cells were plated ($2*10^6$ cells/well) in 6 well-plates.
- <u>For dose dependency studies:</u>
  - Cells were treated with 0, 5 µM, 10 µM, 20 µM, 30 µM, and 40 µM of curcumin.
  - Cells were lysed 24 hours post-treatment
  - Western blotting was conducted to determine expression changes of ATF3 and cleaved PARP proteins.

Methods Continued…

- <u>For the time dependency study:</u>
  - Cells were treated with 30 µM of curcumin for 0, 0.5, 1, 2, 4, 6, 8, and 24 hours.
  - Cells were lysed post each incubation period.
  - Western blotting was conducted to determine the expression changes of ATF3.
- ***ATF3 Promoter Activity:***
  - HCT116 cells were plated (50000 cells/well) in 12 well plates.
  - Cells were transfected with ATF3 promoters and treated with 30 µM of curcumin 24 hours post-transfection.
  - Promoter expression was determined using Luciferase Assay 24 hours post-treatment.

Results

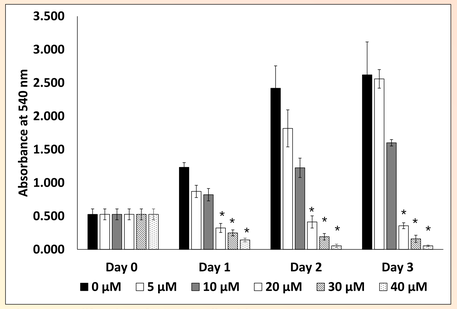


Figure 1. Proliferation of HCT116 cells with treatments

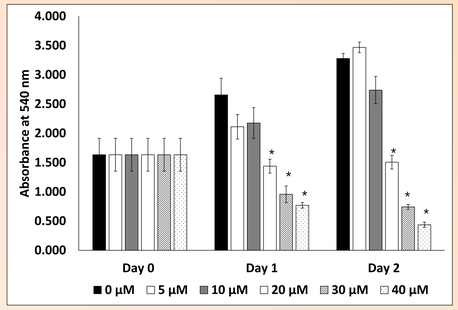


Figure 2. Proliferation of SW480 cells with treatments

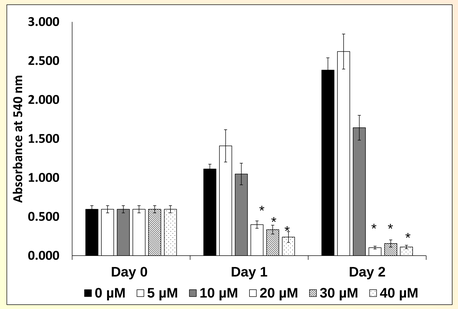


Figure 3. Proliferation of HCT15 cells with treatments

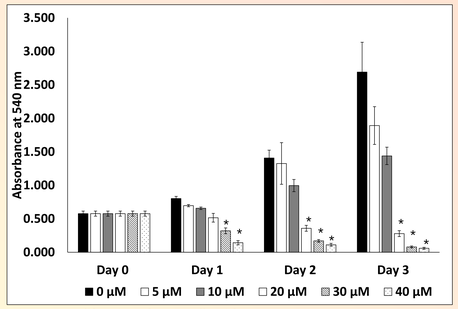
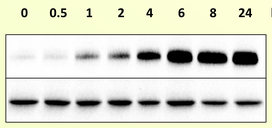
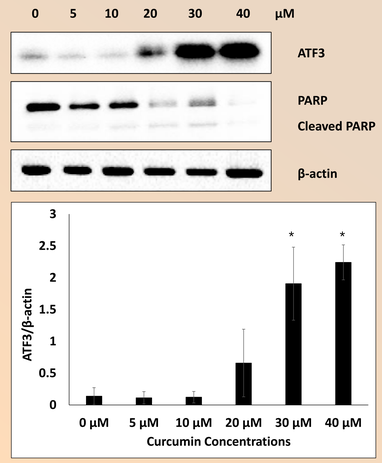


Figure 4. Proliferation of HT29 cells with treatments

Cell proliferation of all CRC cells was significantly inhibited at 20 µM or more of curcumin starting from 24 hours post-treatment



Curcumin induced ATF3 expression in HCT116 cells in a time-dependent manner.



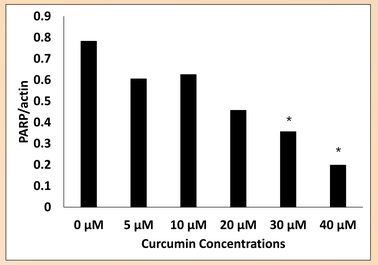


Figure 5. Western Blotting images for dose dependent, curcumin-induced ATF3 and cleaved PARP

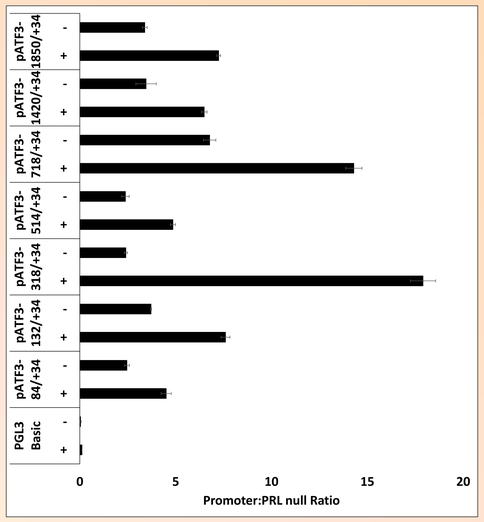


Figure 6. Curcumin-induced transcriptional activity in ATF3 gene

Conclusion: Curcumin is an antiproliferative agent and apoptotic gene regulator in CRC cells.