Zarei and Saadat
*Egyptian Journal of Medical Human Genetics* (2023) 24:63
https://doi.org/10.1186/s43042-023-00445-7

Egyptian Journal of Medical Human Genetics

RESEARCH

Open Access

# Metformin reduces the cellular DNA repair capacity and enhances the effect of curcumin on the induction of apoptosis in AGS gastric cancer cells

Ehsan Zarei[1] and Iraj Saadat[1*]

## Abstract

**Background** Cancer results from the accumulation of mutations in critical genes, such as DNA repair genes. But these genes are a double-edged sword, because the basis of current cancer treatment is DNA damage from chemotherapy and radiation, and the repair system can slow the healing process by repairing the induced damage. Therefore, any substance that can reduce the DNA repair capacity of cancer cells can make the cells more sensitive to treatment. Metformin and curcumin, as low-complication compounds, can play this role well.

**Methods** In the present work, changes in the expression of *CASP3*, *BAX*, and *BCL2L1* apoptotic genes, and nine genes involved in DNA repair pathways (*XRCC1*, *XRCC2*, *XRCC3*, *XRCC4*, *XRCC5*, *XRCC6*, *XRCC7*, *BRCA1* and *BRAC2*) were measured comparatively by real-time PCR in AGS gastric cancer cell line under single and co-treatments with metformin and curcumin.

**Results** Our findings showed that co-treatment of metformin and curcumin induced decreasing the expression of anti-apoptotic *BCL2L1* and increasing expression of proapoptotic *CASP3* and *BAX*. Metformin decreased the expression levels of seven genes, while curcumin did not alter the expression levels. The co-treatment of metformin and curcumin showed that although the *XRCC2*, *XRCC3*, *XRCC4*, *XRCC5*, *XRCC6*, *XRCC7*, *BRCA1*, and *BRCA2* were down-regulated, there was no difference between metformin and co-treatment for mRNA levels.

**Conclusion** Our results suggest that metformin increases the sensitivity of cancer cells to anticancer drugs by suppressing several DNA repair pathways and that curcumin may induce apoptosis.

**Keywords** Metformin, Curcumin, DNA repair gene, Apoptosis

## Introduction

Alterations in cellular DNA repair capacity play a critical role in the onset, progression, and even response to cancer treatment [1]. Because anti-cancer therapies, including ionizing radiation and chemotherapy, are based on DNA damage, regulating the DNA damage response may lead to tumor susceptibility to treatment or resistance to genotoxic agents. Therefore, targeting DNA repair pathways may be a potential therapeutic approach to cancer treatment [2].

Metformin is the first-line treatment for type 2 diabetes mellitus and the most widely used drug for this disease worldwide. Numerous epidemiologic studies have shown a significant association between the use of metformin in people with type 2 diabetes mellitus and a reduction in the risk of many types of cancer [3, 4]. Metformin, in

*Correspondence:
Iraj Saadat
isaadat@shirazu.ac.ir
[1] Department of Biology, School of Science, Shiraz University, Shiraz 71467-13565, Iran

addition to its protective role in cancer, induces apoptosis and inhibits the proliferation of many cancer cells, tumorigenesis, tumor progression in vivo, metastasis, and angiogenesis [5, 6]. On the other hand, metformin improves the response to chemotherapy and radiotherapy, reduces the anticancer drug resistance in diabetic patients with breast cancer, and increases the sensitivity of cancer cells to drugs [7]. Also, metformin protects the skin against UVB damage by reducing its DNA repair capacity [8].

Curcumin is the main active ingredient of turmeric (*Curcuma longa*) and has antioxidant, antiseptic, antiseptic, antiseptic effects [9]. Epidemiological studies attribute the reduction in cancer incidence to the curcumin-rich diets [10]. The results of treating cells with curcumin show that it inhibits cell proliferation and induces apoptosis [11]. Also, studies proved the induction of DNA damage and the reduction of the expression of DNA repair genes by curcumin in different cancer cell lines [12]. Although the anticancer effects of curcumin have been confirmed in population-based studies regardless of its molecular pathways, there are inconsistencies in the performance of curcumin in various laboratory studies. Studies indicate its protective role against DNA damage as well as reduced DNA repair capacity, especially in the expression of genes involved in double-strand break (DSB) repair pathways [13].

X-ray cross-complementing (XRCC) and breast cancer (BRCA) genes play a role in various pathways of DNA repair pathways [14, 15]. XRCC1 is involved in the base excision repair (BER) pathway; in addition, XRCC2, XRCC3, BRCA1, and BRCA2 are involved in the homologous recombination (HR) pathway, and XRCC4, XRCC5, XRCC6, and XRCC7 are active in non-homologous end joining (NHEJ). BRCA1 works jointly in NHEJ repair, the nucleotide excision repair (NER), HR pathways [14]; however, BRCA2 has a more specific role in DNA repair [15]. Damaged eukaryotic cells are not repaired, but are removed by apoptosis. Increasing the expression of proapoptoses, especially caspase-3 (CASP3) as a "point of no return", and/or decreasing anti-apoptotic proteins in the Bcl-2 protein family, such as BCL2L1, can shift the balance in favor of cell death after DNA damage treatment [16]. We have previously observed the dose-dependent cytotoxic effect of metformin and curcumin on AGS gastric cancer cell line. Also, based on Chou-Talalay method, it was found that metformin and curcumin with a ratio of 1:625 in 72 h could have a strong synergistic interaction. Furthermore, the results of our previous study showed that metformin and curcumin could inhibit the EMT mechanisms by impeding cell migration and invasion, as well as colony formation; these changes were significantly higher in combination treatments. Furthermore, the combination of metformin and curcumin significantly and selectively increased the cytotoxic effects of chemotherapy drugs on the cancer cell line compared to the normal cell line [17]. According to these results and as the changes in DNA repair capacity and cell apoptosis potential are affected by EMT changes and cancer progression, changes in the expression of genes involved in these pathways were investigated.

Due to the contradictions in the studies and the lack of reports on the effect of curcumin and metformin combination on DNA repair genes in a gastric cancer cell line, our present study aimed at investigating this area.

## Materials and methods

### Reagents

Metformin powder (Santa Cruz, USA) was dissolved in phosphate buffer solution (PBS) (Merck, Germany), and curcumin powder (Merck, Germany) was dissolved in DMSO (Shellmax, China). To test cytotoxicity, Cell Proliferation Kit (MTT) was prepared from Roche Company. Human AGS gastric cancer cell line was prepared from National Cell Bank of Iran and cultivated in RPMI 1640 culture medium (Bio-idea, Iran) enriched with (10% v/v) heat-inactivated fetal bovine serum (FBS, Gibco) and 100 units/mL penicillin and 100 µg/mL streptomycin (Bio-idea, Iran) under standard conditions of a humidified 5% $CO_2$ incubator at 37 °C. RNX-Plus Solution for total RNA isolation was purchased from Cinagen (Iran) and cDNA synthesis kit from Takara (Japan). RealQ Plus Master Mix Green was provided by Ampliqon (Denmark).

### RNA extraction, cDNA synthesis, and gene expression analysis

The concentrations used for metformin (0.625 mM) and curcumin (1 µM) were selected based on the Chou synergistic relationship with a combined index <1 (CI = 0.3), so that the viability of the cells under single treatments was more than 97%, while it decreased to about 60% in combined treatments. Based on previous observations, these values were used to examine the gene expression changes [17]. AGS gastric cancer cells ($5 \times 10^5$) were treated with metformin, curcumin, and their combination for 72 h. Total RNA was extracted and cDNA was synthesized. Altered gene expression patterns were detected by real-time quantitative polymerase chain reaction (qPCR) in a Rotor-Gene 6000 instrument (Corbett, Australia) with the thermal profile as follows one step of 95 °C for 15 min, 40 cycles of 95 °C for 5 s and 60 °C for 30 s. *XRCC1*, *XRCC2*, *XRCC3*, *XRCC4*, *XRCC5*, *XRCC6*, *XRCC7*, *BRCA1*, and *BRAC2* genes as well as *CASP3*, *BAX* and *BCL2L1* genes were studied in our study. Primer sequences designed with the aid of AllelID

software version 7.0 are summarized in Table 1. *GAPDH* gene was considered as the reference gene and alterations in gene expression were determined by $2^{-\Delta\Delta Ct}$ method [18].

### Statistical analysis

All experiments were done in triplicate. The data are expressed as the mean ± standard deviation (SD). Statistical analyses were carried out via one-way analysis of variance (ANOVA) and Duncan's post hoc test using SPSS 16.0 (SPSS, Inc., USA) with a significance level of less than 0.05 ($p < 0.05$).

## Results

### Combination of metformin and curcumin alters the expression of apoptotic genes in favor of apoptosis

Our results demonstrated that treatment with metformin and curcumin induced decreasing the expression of anti-apoptotic *BCL2L1* gene and increasing expression of proapoptotic *CASP3* and *BAX* genes. Although these expressional alterations were observed in single treatments, co-treatment with metformin and curcumin significantly changed the expression patterns towards apoptosis (Fig. 1). In summary, metformin treatment did not cause a significant change in the expression of apoptotic genes; however, curcumin treatment increased the expression of *BAX* gene by 8.6-fold and decreased the expression of *BCL2L1* anti-apoptotic gene by 0.57-fold compared to the untreated control cell. However, their combination treatment showed a significant increase in proapoptotic *CASP3* (2.8-fold) and *BAX* (15-fold) genes and a decrease in *BCL2L1* anti-apoptotic expression (0.76-fold). The *BAX/BCL2L1* ratio in curcumin (20-fold) and co-treatment (86-fold) increased significantly (Fig. 1).

### Metformin and curcumin reduce the DNA repair capacity in AGS cancer cells

In the present study, changes in the mRNA levels of nine genes involved in DNA repair pathways were examined

**Table 1** The primer sequences used in this study

| Genes | Sequence (5′ to 3′) | Product length (bp) |
|---|---|---|
| *XRCC1* (MIM: 194360; NM_006297.2) | F: AAGGGAAGAGGAAGTTGGAT<br>R: GTTGGAGCTGGCAATTTAGG | 109 |
| *XRCC2* (MIM: 600375; NM_005431.2) | F: TTCGGGGCGATGTGTAGTG<br>R: TTCAAGAATATCACCATGCA | 144 |
| *XRCC3* (MIM: 600675; NM_001100119.2) | F: CGTGCAATTAAGAAAGCCAAACTG<br>R: CTCAGCAAGTGCCAGACCT | 113 |
| *XRCC4* (MIM: 194363; NM_022550.3) | F: GGACATCAAACAAGAAGGGGAAACT<br>R: AGCTGAAGCCAACCCAGAGA | 127 |
| *XRCC5* (MIM: 194364; NM_021141.4) | F: GCAGTGTCACCTCTGTTGGA<br>R: TATGAGCTGGTTACTCGCTTCCT | 101 |
| *XRCC6* (MIM: 152690; NM_001288978.1) | F: GCGTTGATTGGGACCGAGTA<br>R: CATGTTGGCTACTGCTCACTTTG | 101 |
| *XRCC7* (MIM: 600899; NM_006904.7) | F: GTCATTACTTGTGATGAGCTACTCC<br>R: TGGTTCTTGGGCACGAATGT | 111 |
| *BRCA1* (MIM: 113705; NM_007294.4) | F: TTGCCAGAAAACACCACATCAC<br>R: GGTCACCCAGAAATAGCTAACTACC | 158 |
| *BRCA2* (MIM: 600185; NM_000059.3) | F: CAAGTGGTCCACCCCAACTA<br>R: ACAATTAGGAGAAGACATCAGAAGC | 100 |
| *CASP3* (MIM: 605265; NM_004346.4) | F: AAGCGAATCAATGGACTCTGG<br>R: CTGTACCAGACCGAGATGTC | 134 |
| *BAX* (MIM: 600040; NM_001291428.2) | F: GCCCTTTTGCTTCAGGGTTTCA<br>R: CAGCTTCTTGGTGGACGCAT | 108 |
| *BCL2L1* (MIM: 600039; NM_138578.3) | F: TGCATTGTTCCCATAGAGTTCCA<br>R: CCTGAATGACCACCTAGAGCCTT | 79 |
| *GAPDH* (MIM: 138400; NM_002046.6) | F: ACATCGCTCAGACACCAT<br>R: GGCAACAATATCCACTTTACCA | 112 |

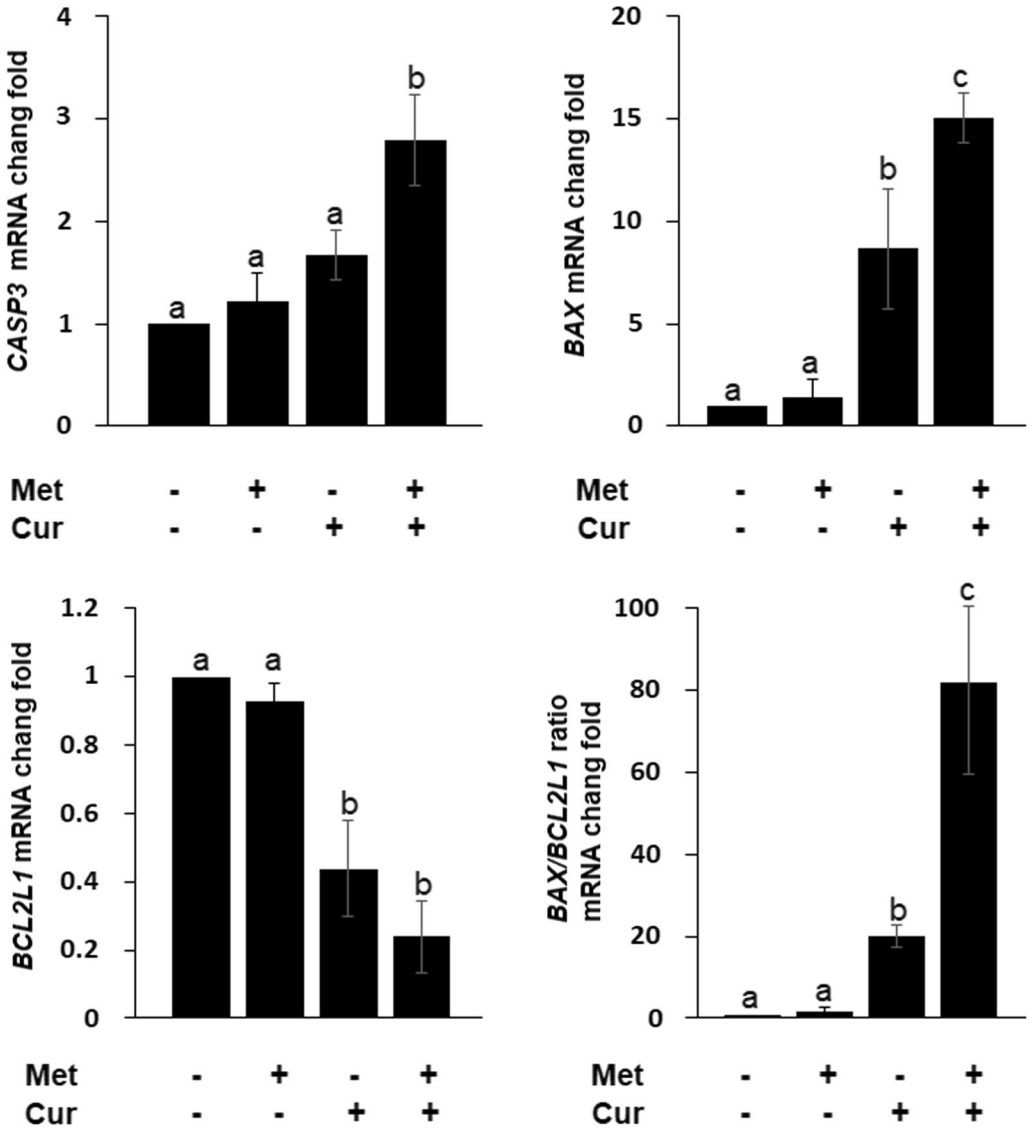


**Fig. 1** Diagram of changes in *CASP3*, *BAX* and *BCL2-L1* gene expression as well as the *BAX/BCL2-L1* ratio expression after treatment with 0.625 mM metformin, 1 μM curcumin and their combination over a 72 h time interval. Gene expression was normalized with *GAPDH* as internal control. The results (mean ± SEM) were obtained from 3 independent experiments. All experiments were performed independently in triplicate. Statistical analysis was performed using one-way ANOVA and Duncan's post-hoc test. In each panel, similar letters indicate no statistically significant difference

in AGS cells treated with metformin and curcumin (Fig. 2). Single treatment with metformin during 72 h showed that the expression levels of *XRCC2*, *XRCC3*, *XRCC5*, *XRCC6*, *XRCC7*, *BRCA1*, and *BRCA2* significantly decreased. In single treatment with curcumin, the alteration of mRNA levels was not statistically significant. The combined treatment of metformin and curcumin also demonstrated a significant decrease in the expression of *XRCC2*, *XRCC3*, *XRCC5*, *XRCC6*, *XRCC7*, *BRCA1*, and *BRCA2* genes. There was no significant difference between the studied mRNA levels of metformin and co-treatment of metformin and curcumin, indicating that curcumin has no effect on the expression levels of the examined DNA repair genes.

## Discussion

In the present report, we provided evidence of the effect of metformin and curcumin on the expression levels of three genes involved in apoptosis and nine genes involved in DNA repair pathways. In this study, the selected DNA repair genes show a wider range of activity in DNA damage response.

Combination therapy and interests in low-complication and natural compounds are new topics in cancer

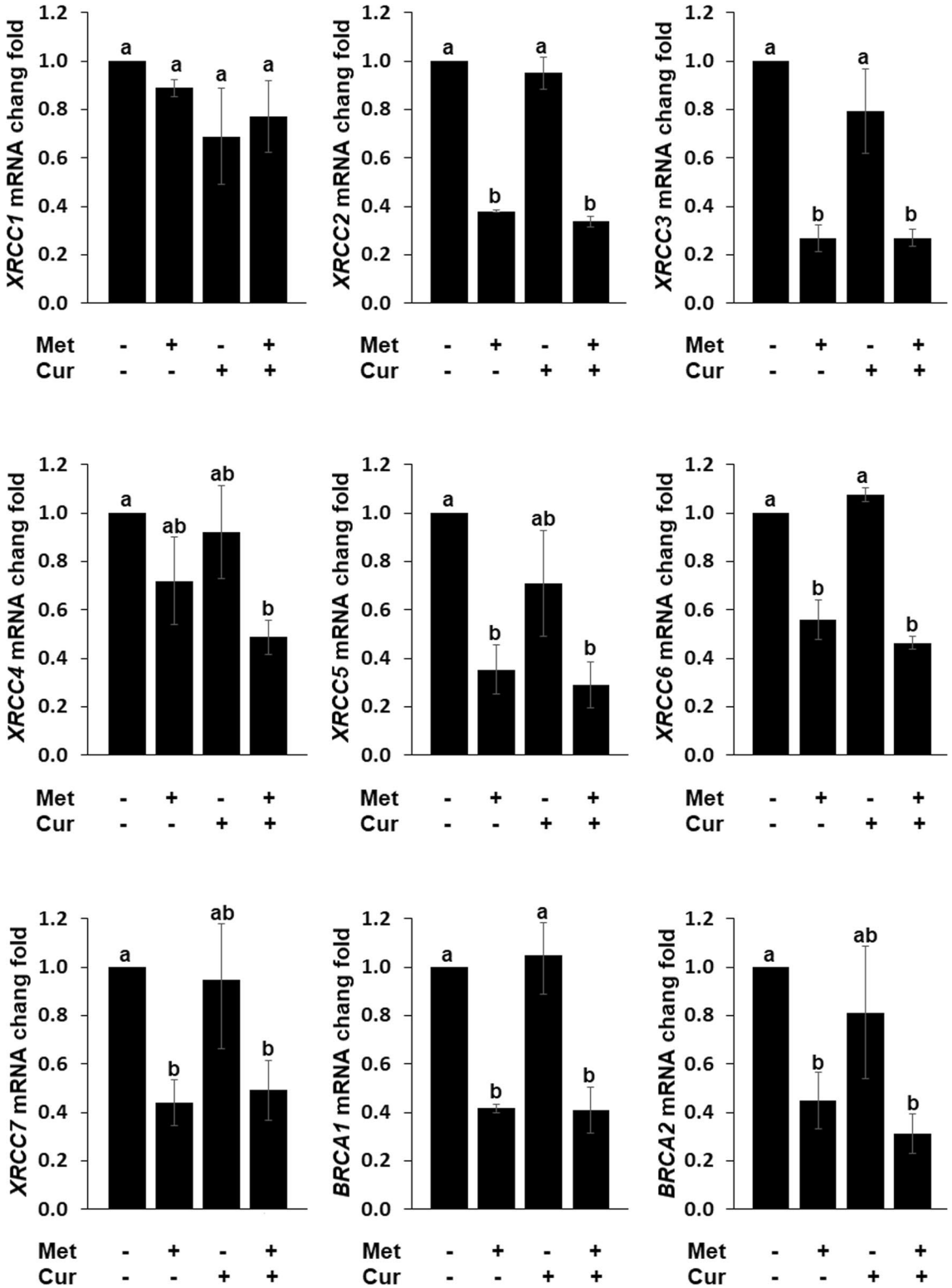


**Fig. 2** Combination of 0.625 mM metformin and 1 μM curcumin inhibits DNA repair genes expression in AGS cell line. Alterations in the expression of genes involved in DNA repair were measured using real-time qPCR after 72-h treatments with metformin, curcumin, and their combination. Graphs represent mean ± SEM for three independent experiments conducted in triplicates. Statistical analysis was performed using one-way analysis of variance (ANOVA) and Duncan's post hoc test. In each panel, similar letters indicate no statistically significant difference. All experiments were performed independently in triplicate

research and treatment, and the study of the simultaneous effect of curcumin and metformin in recent years has received special attention. Our previous results showed that metformin and curcumin could synergistically reduce the survival of gastric cancer cells and significantly inhibit migration, invasion, and colony formation. Therefore, changes in the expression of genes involved in DNA repair and apoptosis were expected [17].

Examination of apoptotic genes expression patterns showed that single treatment with curcumin induced apoptosis due to up-regulation of *BAX* and down-regulation of *BCL2L* genes. In co-treatment of metformin and curcumin, *CASP3* and *BAX* genes were up-regulated and *BCL2L1* gene was down-regulated. The *BAX/BCL2L1* ratio, an index of apoptosis induction [16], increased more than 4 times compared to single-curcumin treatment and up to 82 times compared to the untreated control cells (Fig. 1). Therefore, co-treatment may increase apoptosis in the AGS cells. This is in accordance with the results of the cytotoxicity test in MTT assay and explains those results. In a similar study, it has been reported that the combination of metformin and curcumin induced apoptosis in LNCaP [19].

In the present study, investigation of changes in the expression of DNA repair genes shows that although metformin alone and in low non-cytotoxic doses can reduce the repair capacity, curcumin and its combination with metformin did not significantly alter the expression of DNA repair genes. Consistent with our results, other studies show that curcumin has no effect at concentrations below 14 μM [20] and the effects of curcumin are dose- and time-dependent [21], as reflected in our MTT results.

Our results showed that curcumin alters the expression of apoptotic genes, while having no effect on the expression of DNA repair genes. In contrast, metformin only reduced the expression of DNA repair genes. Inhibiting the DNA repair pathway and inducing apoptosis are ways to make cancer treatment more effective. Based on the link between DNA repair pathways and cancer progression, a new treatment strategy is being considered to increase the effect of DNA-damaging agents through DNA repair inhibitors. Our findings suggested that metformin increased the sensitivity of cancer cells to anticancer drugs by suppressing several DNA repair pathways, and that curcumin could induce apoptosis and accelerate cancer cell death. As demonstrated in our previous study, metformin and curcumin enhanced the effectiveness of chemotherapy drugs [17]. This could be the basis for further studies to investigate the anti-cancer effects of metformin and curcumin.

## Conclusions

Our current results show that curcumin may increase the expression of proapoptotic genes and by downregulation of the anti-apoptotic gene, it can sensitize AGS cells to apoptosis. Metformin potentiated this effect of curcumin. Metformin also reduced DNA repair capacity by reducing the expression of genes involved in DSB repair. Reducing the repair capacity and facilitating the onset of apoptosis can provide insights into cancer treatment.

### Abbreviations

| | |
|---|---|
| ANOVA | ANalysis of VAriance |
| BAX | Bcl-2-Associated X |
| BCL2L1 | Bcl-2-like 1 |
| BER | Base Excision Repair |
| BRCA | BReast CAncer gene |
| CASP3 | Caspase 3 |
| DSB | Double-Strand Break |
| HR | Homologous Recombination |
| $IC_{50}$ | Half maximal Inhibitory Concentration |
| NER | Nucleotide Excision Repair |
| NHEJ | Non-Homologous End Joining |
| SEM | Standard Error of Mean |
| SD | Standard Deviation |
| XRCC | X-Ray Cross-Complementing |

### Acknowledgements

We would like to thank all who participated in this study.

### Author contributions

E.Z. performed the experiment and data analysis, and writing—original draft preparation. I.S. designed the research and performed writing and editing the manuscript and supervision.

### Funding

This work was supported by Shiraz University (97GCU3M1740).

### Availability of data and materials

The data are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

This research was approved by the Ethics Committee of Shiraz University with the Ethics Code: ECBD-SU-9430318.

### Consent for publication

Not applicable.

### Competing interests

The authors have no competing interests.

Received: 9 June 2023 Accepted: 13 October 2023
Published online: 21 October 2023

### References

1. Clementi E, Inglin L, Beebe E, Gsell C, Garajova Z, Markkanen E (2020) Persistent DNA damage triggers activation of the integrated stress response to promote cell survival under nutrient restriction. BMC Biol 18(1):1–15
2. Li L-y, Guan Y-d, Chen X-s, Yang J-m, Cheng Y (2021) DNA repair pathways in cancer therapy and resistance. Front Pharmacol 11:2520

3. Kim YI, Kim S, Cho SJ, Park JH, Choi IJ, Lee YJ et al (2014) Long-term metformin use reduces gastric cancer risk in type 2 diabetics without insulin treatment: a nationwide cohort study. Aliment Pharmacol Ther 39(8):854–863
4. Zhou X-L, Xue W-H, Ding X-F, Li L-F, Dou M-M, Zhang W-J et al (2017) Association between metformin and the risk of gastric cancer in patients with type 2 diabetes mellitus: a meta-analysis of cohort studies. Oncotarget 8(33):55622
5. Buzzai M, Jones RG, Amaravadi RK, Lum JJ, DeBerardinis RJ, Zhao F et al (2007) Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. Can Res 67(14):6745–6752
6. Vázquez-Martín A, Oliveras-Ferraros C, del Barco S, Martín-Castillo B, Menéndez JA (2009) mTOR inhibitors and the anti-diabetic biguanide metformin: new insights into the molecular management of breast cancer resistance to the HER2 tyrosine kinase inhibitor lapatinib (Tykerb®). Clin Transl Oncol 11(7):455–459
7. Jiralerspong S, Palla SL, Giordano SH, Meric-Bernstam F, Liedtke C, Barnett CM et al (2009) Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. J Clin Oncol 27(20):3297
8. Wu C, Qiang L, Han W, Ming M, Viollet B, He Y-Y (2013) Role of AMPK in UVB-induced DNA damage repair and growth control. Oncogene 32(21):2682
9. Aggarwal BB, Sundaram C, Malani N, Ichikawa H (2007) Curcumin: the Indian solid gold. Adv Exp Med Biol 595:1–75
10. Mohandas K, Desai D (1999) Epidemiology of digestive tract cancers in India. V. Large and small bowel. Indian J Gastroenterol 18(3):118–121
11. Dorai T, Cao YC, Dorai B, Buttyan R, Katz AE (2001) Therapeutic potential of curcumin in human prostate cancer III Curcumin inhibits proliferation, induces apoptosis, and inhibits angiogenesis of LNCaP prostate cancer cells in vivo. Prostate 47(4):293–303
12. Ting C-Y, Wang H-E, Yu C-C, Liu H-C, Liu Y-C, Chiang I-T (2015) Curcumin triggers DNA damage and inhibits expression of DNA repair proteins in human lung cancer cells. Anticancer Res 35(7):3867–3873
13. Cao J, Jia L, Zhou H-M, Liu Y, Zhong L-F (2006) Mitochondrial and nuclear DNA damage induced by curcumin in human hepatoma G2 cells. Toxicol Sci 91(2):476–483
14. Thacker J, Zdzienicka MZ (2003) The mammalian XRCC genes: their roles in DNA repair and genetic stability. DNA Repair 2(6):655–672
15. Tutt A, Ashworth A (2002) The relationship between the roles of BRCA genes in DNA repair and cancer predisposition. Trends Mol Med 8(12):571–576
16. Raisova M, Hossini AM, Eberle J, Riebeling C, Orfanos CE, Geilen CC et al (2001) The Bax/Bcl-2 ratio determines the susceptibility of human melanoma cells to CD95/Fas-mediated apoptosis. J Investig Dermatol 117(2):333–340
17. Zarei E, Sefidi-Heris Y, Saadat I (2021) Synergistic effects of metformin and curcumin on cytotoxicity of chemotherapy drugs using a gastric cancer cell line model. EXCLI J 20:1488–1498
18. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25(4):402–408
19. Eslami SS, Jafari D, Montazeri H, Sadeghizadeh M, Tarighi P (2021) Combination of curcumin and metformin inhibits cell growth and induces apoptosis without affecting the cell cycle in LNCaP prostate cancer cell line. Nutr Cancer 73(6):1026–1039
20. Srinivasan M, Prasad NR, Menon VP (2006) Protective effect of curcumin on γ-radiation induced DNA damage and lipid peroxidation in cultured human lymphocytes. Mutation Res Genetic Toxicol Environ Mutagen 611(1–2):96–103
21. Van Erk MJ, Teuling E, Staal YC, Huybers S, Van Bladeren PJ, Aarts JM et al (2004) Time-and dose-dependent effects of curcumin on gene expression in human colon cancer cells. J Carcinogen 3:8


## Publisher's Note