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Synergistic impact of autocrine motility factor and curcumin on colorectal cancer cell proliferation

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Abstract

Colorectal cancer (CRC) presents a formidable challenge, characterized by a steadily increasing incidence. Current approaches to manage CRC, including chemotherapy and targeted therapies, are burdened with significant limitations such as resistance development, adverse events, and high costs. Hence, there is an urgent demand for a more promising alternative. Autocrine motility factor (AMF), known for its role in promoting cancer cell motility, exhibits a unique ability to selectively impede the growth of cancer cells. In our study, we have elucidated the specific inhibitory effect of AMF derived from DU145 prostate cancer cells (D-AMF) on the proliferation of CRC cells. D-AMF effectively downregulated the expression of glucose-6-phosphate dehydrogenase (G6PD) at both the mRNA and protein levels, resulting in a concurrent increase in the generation of reactive oxygen species (ROS). Notably, the combination of D-AMF and curcumin proved highly effective in eliminating curcumin-resistant CRC cells. Therefore, the use of D-AMF in conjunction with curcumin holds promise as an alternative treatment approach for CRC.

Keywords Autocrine motility factor, Colorectal cancer cells, Curcumin, Glucose-6-phosphate dehydrogenase, Reactive oxygen species

Introduction

Glucose-6-phosphate isomerase (GPI) plays a dual role in cellular processes. In its dimeric form, GPI acts as an enzyme, facilitating the conversion of glucose-6-phosphate to fructose-6-phosphate, a critical step in glycolysis. However, when existing as a monomer, GPI assumes the functions of AMF and neuroleukin [1]. Cancer cells employ the secretion of AMF to boost their motility and proliferation through autocrine mechanisms, while also promoting angiogenesis and metastasis via both autocrine and paracrine pathways. AMF triggers the activation of PI3K/AKT and/or MAPK/ERK signaling pathways and is internalized into the endoplasmic reticulum through an AMF receptor (AMFR)-mediated, dynamin-dependent, cholesterol-sensitive raft pathway [2]. Clinically, elevated levels of AMF and AMFR hold prognostic significance in various cancers, including gastric cancer, non-small cell lung cancer, melanoma, and colorectal cancer [3]. In addition to its oncogenic effects, AMF has been found to selectively induce apoptosis in cancer cells in an AMF type-dependent manner, possibly involving interactions between different AMF types [4–6]. Therefore, AMF has the potential to operate as either a signal for cell death or as a factor promoting cell survival during the competition among cancer cells, which may not effectively differentiate between self and non-self AMF molecules. Cell competition, positioned as a downstream process within developmental signaling pathways, plays a pivotal role in assessing cellular fitness and selectively

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eliminating less fit cells [7]. Considering the close association between cell competition and cancer progression [8], it is plausible to consider that AMF might function as a secreted signal capable of instigating cell competition, thereby influencing the destiny of cancer cells. Curcumin, derived from the rhizomes of *Curcuma longa* L., possesses a wide range of valuable properties, encompassing anti-cancer, anti-inflammatory, antimicrobial, and antioxidant effects. Its ability to influence numerous biological pathways pertinent to cell proliferation, survival, and metastasis has demonstrated its efficacy against a variety of cancer cell lines [9]. Recent advancements in curcumin derivatives have significantly enhanced their historically limited bioavailability and absorption [10]. Furthermore, there is a growing interest in the synergistic effects of curcumin when combined with established cancer drugs in clinical settings [11]. Expanding upon previous research that has illuminated the varying effects of AMFs sourced from different cancer cell lines on distinct cancer cell types [4–6], our study reinforced the unique ability of D-AMF to significantly suppress the proliferation of HT29 and SW620 CRC cells. This effect was accompanied by a downregulation of G6PD expression, an increase in ROS production, and the near-complete eradication of curcumin-surviving CRC cells. These findings underscore the potential of D-AMF as a valuable component in combination therapy, offering a promising, effective, and cost-efficient strategy to enhance therapeutic efficacy and counteract resistance development in CRC treatment.

Results and discussion

In this study, the AMFs used were cloned from various cancer cell lines, including AsPC-1 pancreatic cancer cells (AS-AMF, Genbank: MW664917), DU145 prostate cancer cells (D-AMF, Genbank: MW664916), HeLa cervical cancer cells (H-AMF, Genbank: KY379509), HepG2 liver cancer cells (HG-AMF, Genbank: MW664918), HT29 colon cancer cells (HT-AMF, Genbank: MW843569), MCF-7 breast cancer cells (M-AMF, Genbank: MW664919), SKOV3 ovarian cancer cells (SK-AMF, Genbank: MW664910), and A549 lung cancer cells (A-AMF, Genbank: BC004982) (Fig. 1a). We examined the influence of these distinct AMFs on the proliferation of two CRC cell lines: the highly adherent HT29 and the metastatic SW620 cells. At a concentration of 2 μg/mL, D-AMF exhibited a significantly greater inhibitory effect on both CRC cell lines compared to other AMFs. Furthermore, D-AMF displayed a dose-dependent growth inhibition pattern (Fig. 1b). In a clonogenic assay, the use of 4 μg/mL D-AMF resulted in a significant 68% reduction in HT29 cell growth and an even more pronounced 73% reduction in SW620 cell growth compared to untreated control cells (Fig. 1c, d). Building on our previous research findings [6], we investigated the impact of D-AMF on the expression levels of G6PD mRNA in CRC cells. G6PD, a crucial enzyme within the pentose phosphate pathway (PPP), is responsible for generating ribose and NADPH. It plays a critical role in regulating various aspects of cancer cell behavior, including survival, migration, angiogenesis, proliferation, and metastasis. Elevated activity of this enzyme has been consistently observed in a wide range of tumor types [12], which may explain the increased resistance of cancer cells to anticancer drugs inducing oxidative stress [13]. Consequently, inhibiting G6PD has been documented to enhance the susceptibility of cancer cells to anticancer drugs that induce oxidative stress [14]. In this context, it became evident that D-AMF significantly suppressed the mRNA expression of the target gene in CRC cells (Fig. 2a). These findings were further supported by Western blot analysis (Fig. 2b). These findings suggest the existence of a common mechanism driving the potent actions of AMF against specific cancer cell targets. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a pivotal enzyme responsible for converting NAD + to NADH within the glycolytic pathway, is considered a housekeeping enzyme and a standard internal reference in Western blotting experiments. However, its overexpression has been associated with the development of various cancers, including CRC. This has led to an increased interest in the exploration of GAPDH inhibitors [15]. It was observed that D-AMF did not affect the expression of GAPDH (Fig. 2b), suggesting that D-AMF specifically impacts the PPP without interfering with glycolysis. In light of the observed downregulation of G6PD, we conducted an assessment of ROS production after an 8-h treatment with D-AMF. The results demonstrated a significant time-dependent increase in ROS levels in CRC cells, while untreated control cells exhibited no such changes. This elevation in ROS levels was visually confirmed through confocal fluorescence microscopy images (Fig. 2c). Inhibiting G6PD is a promising avenue to address chemotherapy resistance. Several compounds, including dehydroepiandrosterone, 6-aminonicotinamide, polygonin, and polydatin, have been identified as potential G6PD inhibitors. However, their efficacy remains uncertain [12, 14]. The discovery of D-AMF-induced G6PD downregulation and increased ROS levels holds great potential for mitigating drug resistance. Furthermore, the AMF-induced ROS pathway provides an intriguing avenue for exploring the fundamental mechanisms of cell competition, given the pivotal role of ROS in influencing cellular fate [16]. In our combination studies, it was observed that D-AMF exhibited the capability to enhance the efficacy of various natural compounds against CRC cells (Fig. 3). Notably,

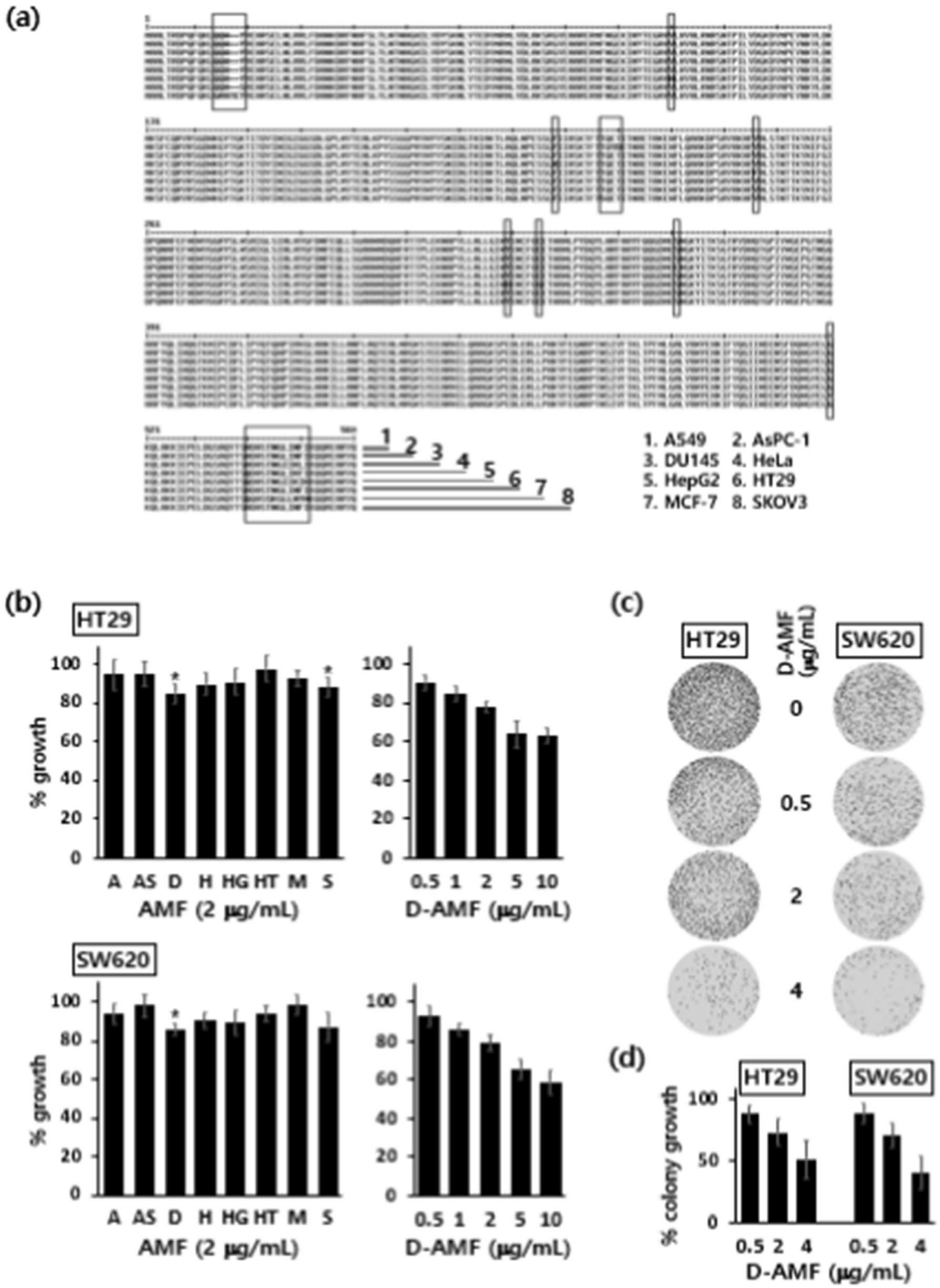

Fig. 1 Assessment of the influence of AMF on CRC cell growth. **a** Comparative analysis of AMF sequences revealing altered amino acid positions. **b** Proliferation effects of AMFs and D-AMF on CRC cells. **c** Colony formation in CRC cells treated with D-AMF. **d** Comparative analysis of colony growth in CRC cells treated with D-AMF

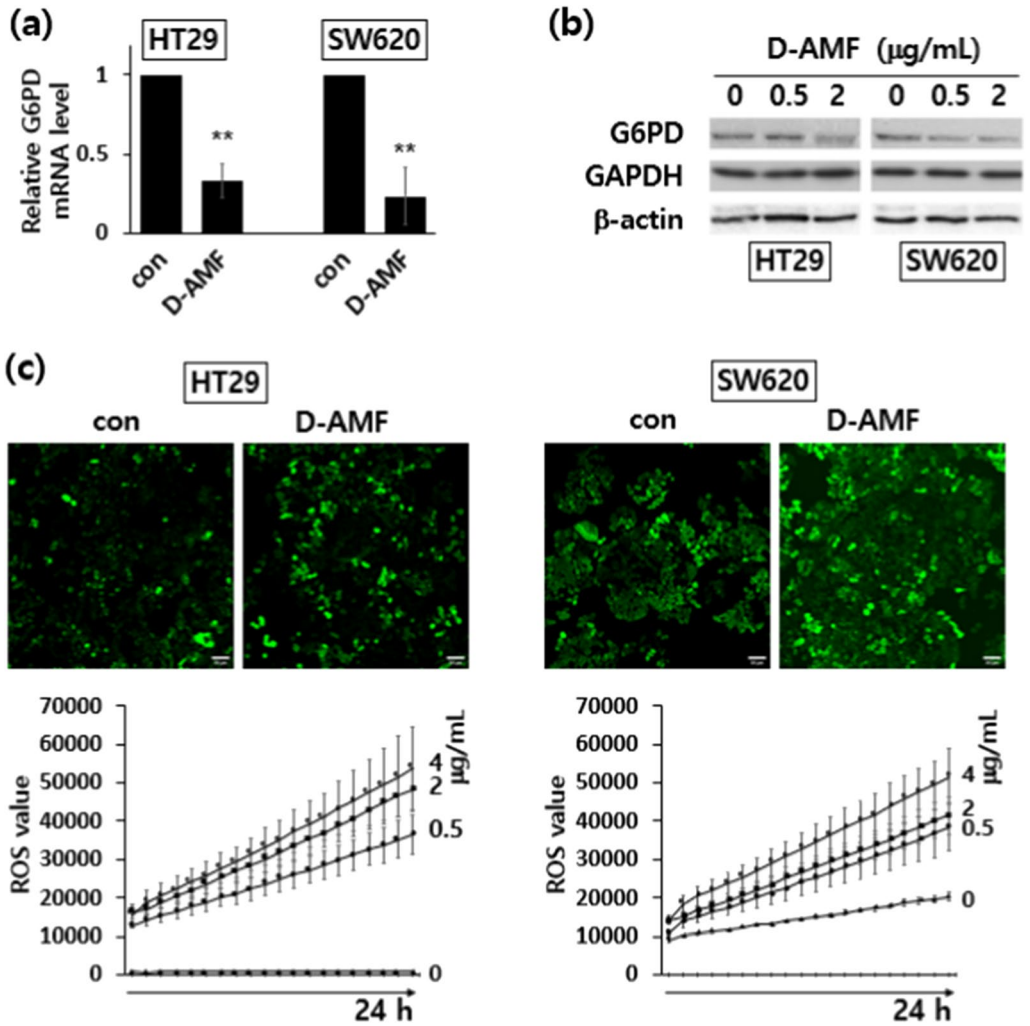

Fig. 2 Impact of AMF on CRC cell expression. **a** Influence of D-AMF on G6PD mRNA Expression. **b** Impact of D-AMF on G6PD and GAPDH protein expression. **c** Generation of ROS in CRC cells induced by D-AMF. Values are the mean ± SEM. Statistical differences were tested using one-way ANOVA analysis. *p < 0.05, **p < 0.01

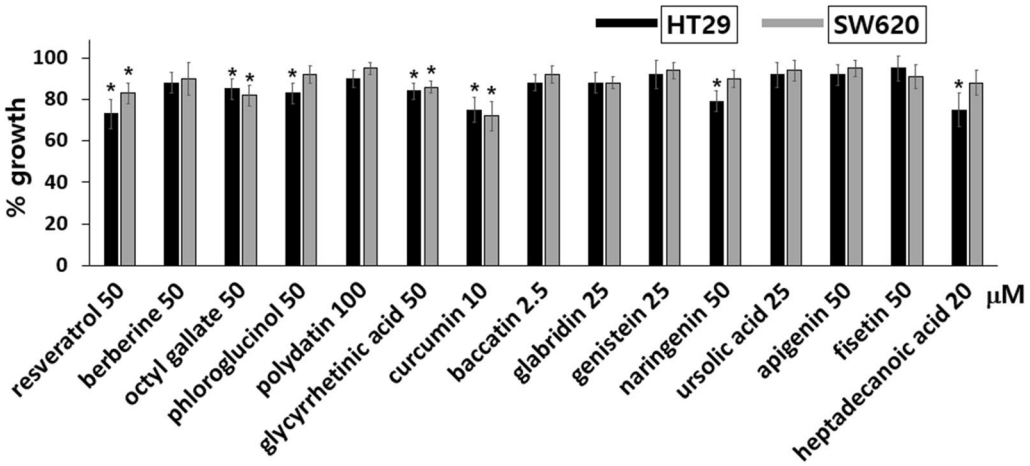

Fig. 3 Effect of natural compounds in conjunction with 0.5 μg/mL D-AMF on CRC cell proliferation

resveratrol, octyl gallate, glycyrrhetinic acid, and curcumin showed promising results. Interestingly, polydatin, a recognized G6PD inhibitor [14], appeared to have minimal effect when used alone to inhibit CRC cell growth. Moreover, the combined application of D-AMF and polydatin did not surpass the effect of D-AMF alone. Our choice of curcumin was based on its well-established history of diverse biological benefits, practical applications, and improved bioavailability [9, 10]. In clonogenic assays, the combination of D-AMF and curcumin outperformed curcumin alone against CRC cells (Fig. 4a). Individually, 5 μM curcumin reduced growth by 33% and 45% in SW620 and HT29 cells, respectively, compared to untreated controls. Co-administering 2 μg/mL D-AMF with 5 μM curcumin resulted in growth reductions of 78% and 73% in SW620 and HT29 cells, respectively, compared to curcumin-only treatment. Co-administering 2 μg/mL D-AMF with 10 μM curcumin nearly completely suppressed colony development, highlighting the exceptional synergy between D-AMF and curcumin against CRC cells (Fig. 4b). This synergy was further underscored in a stress tolerance assay, which revealed an accelerated rate of cell death when D-AMF and curcumin were combined (Fig. 4c). Particularly, under highly stressful conditions, it became evident that the metastatic SW620 cells exhibited significantly reduced tolerance compared to the highly adherent HT29 cells. The co-administration of D-AMF and curcumin resulted in the near elimination of SW620 cells. In the time-dependent ROS production assay, curcumin was observed to increase ROS production, indicating its dual pro-oxidative and anti-oxidative properties (Fig. 4d). Notably, significant synergy was observed with D-AMF at concentrations of 2 and 4 μg/mL, particularly in SW620 cells, although this synergy was less pronounced in HT29 cells. This suggests that the enhanced growth inhibition may not rely solely on ROS production. It is plausible to suggest that the elevated ROS levels induced by D-AMF play a role in sensitizing cells to the process of curcumin-induced apoptosis and autophagy [17], ultimately leading to the near-total eradication of CRC cells.

In our pursuit of novel approaches to CRC treatment, we investigated the individual and combined effects of human-derived AMF and plant-derived curcumin on CRC cell proliferation. Notably, AMF was found to downregulate G6PD, and curcumin exhibited synergistic effects with D-AMF against CRC cells. Moreover, considering the potential of AMF, even in its aggressive form, to be internalized by CRC cells through binding to AMFR while possibly escaping detection as a self-molecule, the combination of AMF and curcumin biotherapy offers a promising alternative for the treatment of drug-resistant CRC.

Materials and methods

Cell growth assay, clonogenic assay, and cell stress tolerance assay

HT29 and SW620 cells were procured from the Korea Cell Line Bank (Korea) and cultured in DMEM supplemented with 10% FBS and antibiotics (penicillin/streptomycin) at 37 ℃ in a 5% CO_2 humidified incubator. To assess cell growth, the MTT assay was employed. For clonogenic assays, cells were seeded in 12-well culture plates at a density of 2000 cells/mL and treated with AMF and/or curcumin for 7 days. The resulting colonies were stained with a 1.25% crystal violet solution and then extracted with 10% acetic acid for measurement at 600 nm. In the cell stress tolerance assay, cells reaching 100% confluency were subjected to new medium with or without AMF and/or curcumin for an additional 3 days. After incubation, cells were rinsed with tap water and subsequently stained with a crystal violet solution.

Quantitative PCR

RNA was extracted using the RNeasy Mini Kit (Qiagen, Germany) and then reverse-transcribed with the SuperScript III cDNA Synthesis Kit (Invitrogen, USA). Quantitative PCR was conducted using the Bio-Rad SYBR Green Supermix (Bio-Rad, USA) with specific primers for two target genes: G6PD (forward: 5′-AAACGGTCG TACACTTCGGG-3′, reverse: 5′-GGTAGTGGTCGA TGCGGTAG-3′) and β-actin (forward: 5′-CATGTA CGTTGCTATCCAGGC-3′, reverse: 5′-CTCCTTAAT GTCACGCACGAT-3′).

AMF Cloning

AMF cDNA clones and AMF proteins were prepared following the established procedure described in prior works [4–6].

Western blot analysis

Protein samples (20 μg per lane) were separated by SDS-PAGE and then transferred to a PVDF membrane for subsequent immunoblotting and chemiluminescence detection. G6PD, GAPDH, and β-actin antibodies were sourced from Santa Cruz Biotech (Santa Cruz, USA).

ROS assay

Cells (3×10^4 cells per well on an 8-well chamber slide) were incubated overnight and then exposed to AMF and/or curcumin for 24 h. Following treatment, they were washed with DMEM and incubated for 30 min at 37 ℃ with 500 μL of 10 μM H2DCFDA in DMEM for fluorescence microscopy (Olympus FV3000, Japan).

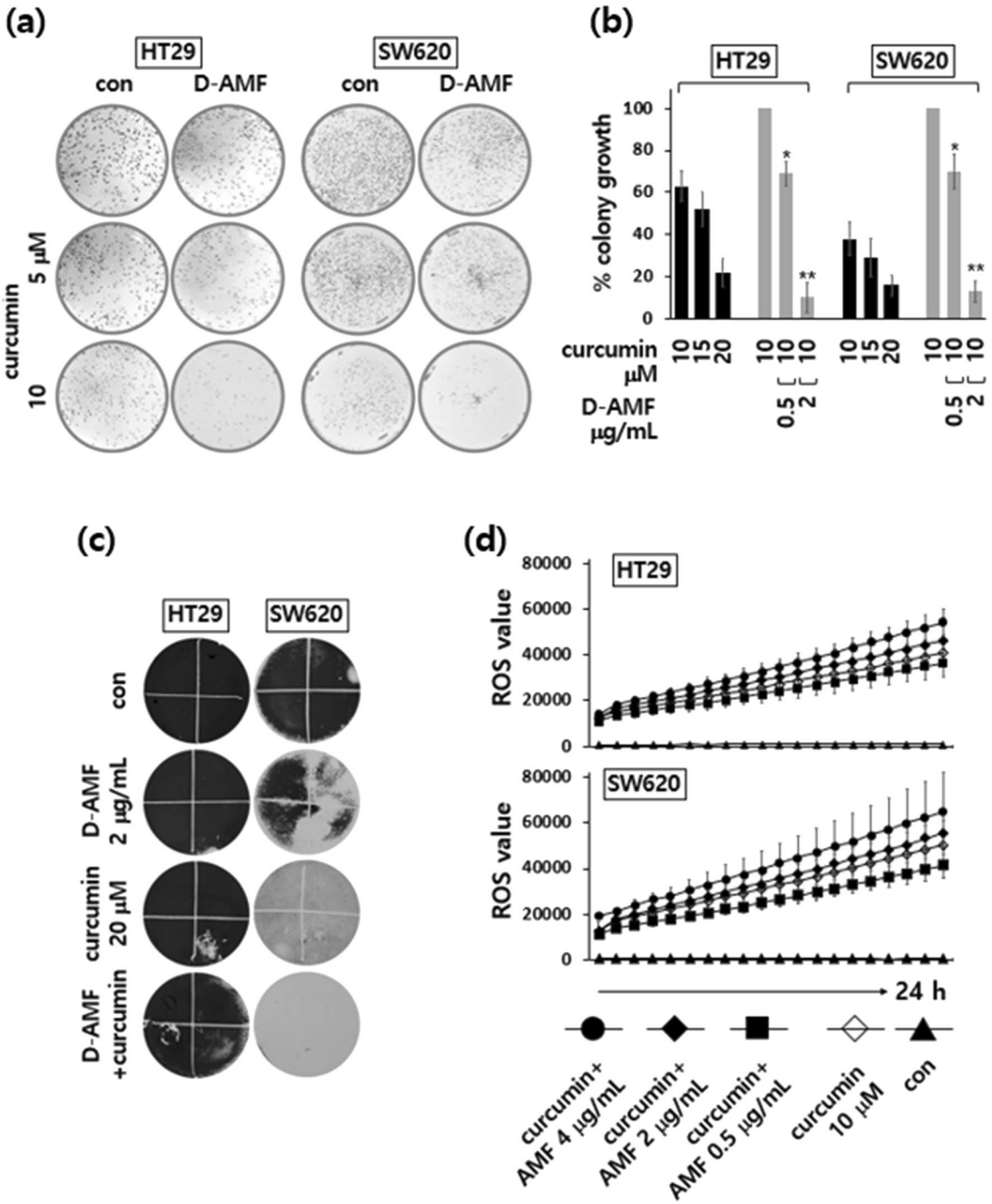

Fig. 4 Evaluation of the synergistic impact of D-AMF and curcumin on CRC cell growth. **a** Colony formation in CRC cells treated with D-AMF and curcumin. **b** Comparative analysis of colony growth in CRC Cells treated with D-AMF and curcumin. **c** Combined effects of D-AMF and curcumin on CRC cells at 100% confluency. **d** Generation of ROS in CRC cells induced by D-AMF and curcumin. Values are the mean ± SEM. Statistical differences were tested using one-way ANOVA analysis. *$p < 0.05$, **$p < 0.01$

Additionally, cells (1×10^4 cells per well on a 96-well plate) were cultured overnight and treated with AMF and/or curcumin for 8 h. Subsequently, H2DCFDA was introduced into the cells, and time-dependent fluorescence was measured over a 24-h period using a BioTek Cytation 7 Cell Imaging Multi-Mode Reader (Agilent Co., USA).

Abbreviations

AMF	Autocrine motility factor
G6PD	Glucose-6-phosphate dehydrogenase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GPI	Glucose-6-phosphate isomerase
PPP	Pentose phosphate pathway

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Author contributions
SJK and HSP designed the study; SJK, YK, and TVD performed the experiments; SJK and HSP performed data analyses; SJK and HSP wrote the manuscript. All authors read and approved the final manuscript.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests
The authors declare that they have no competing interests.

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