The impact of Combretastatin A-4 on cancer cell viability and function: α multi-assay approach

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Abstract

Background: Combretastatin A-4 (CA-4) is a microtubule-disrupting agent with anti-tumor activity through modulation of cytoskeletal dynamics. This study evaluated the effect of a CA-4 analog on multiple cancer cell lines, and the circulating tumor cell line: CTC-MCC-41. It is widely accepted that CTCs often respond differently to drugs compared to primary tumors, thus, it is important to identify new drugs for these metastatic precursors.

Methods: H1299 (non-small cell lung cancer), MDA-MB-231 (triple-negative breast cancer), HT-29 (colorectal cancer), and CTC-MCC-41 (patient-derived colon CTC line) were enrolled in this study. The effect of CA-4 (10 μM) after 24 and 48 hours of treatment on cell viability, migration, and colony formation was assessed using MTT, Boyden chambers, and clonogenic assays, respectively. Western blot analysis examined vimentin and α/β-tubulin expression. The TetherChip assay assessed McTNs formation (cell protrusions implicated in CTC adhesion and metastasis), with wheat germ agglutinin (WGA) staining.

Results: The CA-4 analog reduced colony formation, migration, and cell viability across all cell lines in a statistically significant manner. Specifically, clonogenic assays confirmed a statistically significant reduction in colony formation capacity in all cell lines (mean reduction range across cell lines: 33–40% at 24h; 34–58% at 48h). Cell motility was also significantly lowered at 24h and 48h (mean reduction range: 31–62% and 45–89%, respectively). Cell viability was reduced, with moderate-to-strong effects observed across all cell lines (mean viability range: 46–79% at 24h; 49–71% at 48h). Western blot experiments revealed reduced expression of both vimentin and α/β-tubulin at 48h across all cell lines (vimentin reduction range: 7–73%; α/β-tubulin: 40–48%). TetherChip analysis after 1h CA-4 treatment showed a strong decrease in McTN formation (reduction range: 60–90%), indicating a potential anti-metastatic effect.

Conclusions: The CA-4 analog exerts strong anti-tumor activity across cancer cell lines and CTCs by impairing viability, migration, and colony formation. Furthermore, the significant decrease in McTN formation suggests a potential role in limiting CTC adhesion and metastatic spread. These findings strengthen the potential of CA-4 to enter clinical practice as a microtubule-targeting agent and warrant further investigation for its effect in metastatic disease.

Introduction

Microtubules are dynamic cytoskeletal elements essential for mitosis, intracellular transport, and maintenance of cell shape. Their central role in cell division has established them as key therapeutic targets in oncology, with agents such as taxanes and vinca alkaloids widely used in clinical practice. However, resistance to these agents frequently arises, underscoring the need for alternative microtubule-targeting compounds [1].

Combretastatin A-4 (CA-4), a natural product that binds the colchicine site of tubulin, causes microtubule depolymerization and disruption of cellular architecture. Beyond its recognized cytotoxic and anti-angiogenic activity, recent work suggests CA-4 may also interfere with metastatic traits by impairing cytoskeletal integrity, cell motility, and adhesion [2].

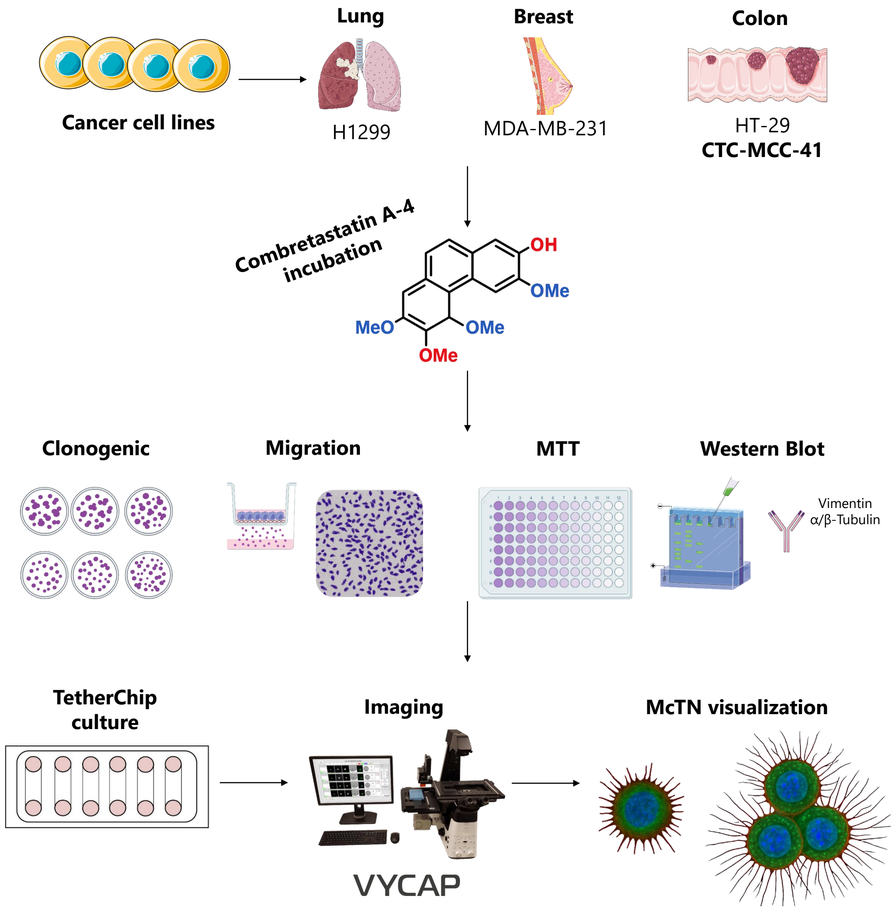
A particularly relevant feature of circulating tumor cells (CTCs), the primary mediators of metastasis, are microtentacles (McTNs). These tubulin-based protrusions promote adhesion, survival in circulation, and reattachment to secondary sites. Since McTNs rely heavily on intact microtubules, microtubule-disrupting agents like CA-4 provide a promising strategy to suppress this metastatic phenotype [3].

In this context, we investigated the effects of a CA-4 analog across multiple cancer cell models, including H1299 (NSCLC), MDA-MB-231 (TNBC), HT-29 (colorectal), and the patient-derived colon CTC line CTC-MCC-41. By assessing proliferation, viability, migration, cytoskeletal protein expression, and McTN formation, we aimed to determine whether CA-4 can impair both tumor growth and metastatic potential.

Aims

- ❖ Assess the effect of CA-4 on colony formation, migration, and viability across cancer cell lines, including the patient-derived CTC-MCC-41 colon cancer cell line
- ❖ Investigate changes in cytoskeletal protein expression (vimentin and α/β-tubulin)
- ❖ Determine whether CA-4 disrupts microtentacle (McTN) formation, a metastatic trait dependent on microtubules

Methods



Results

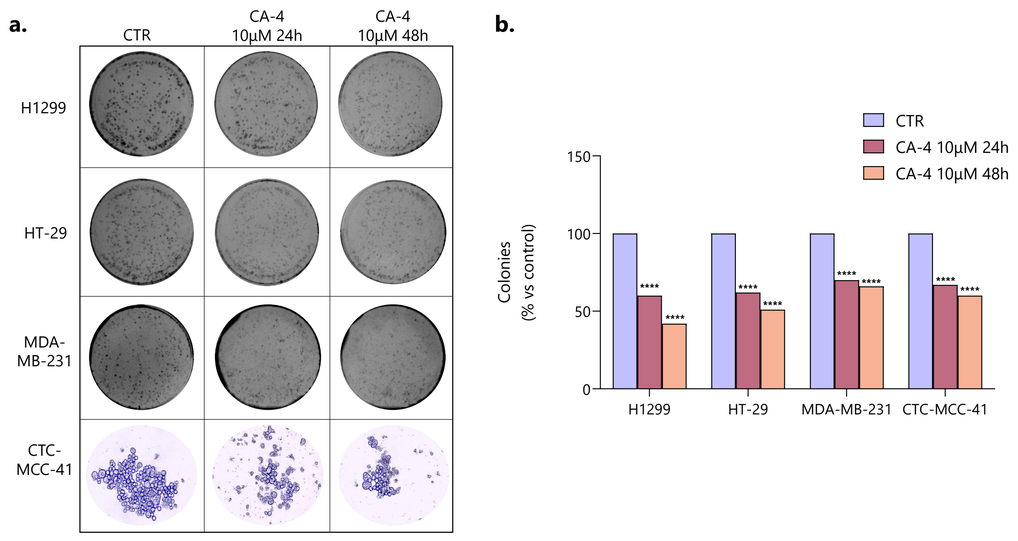


Figure 1. Combretastatin A-4 (CA-4) treatment impairs colony formation in cancer cell lines. **a.** Representative images of colony formation assays in H1299, HT-29, MDA-MB-231, and CTC-MCC-41 cells treated with CA-4 (10 μM) for 24 and 48 hours. **b.** Quantification of colony formation shown as a percentage relative to the control (CTR).

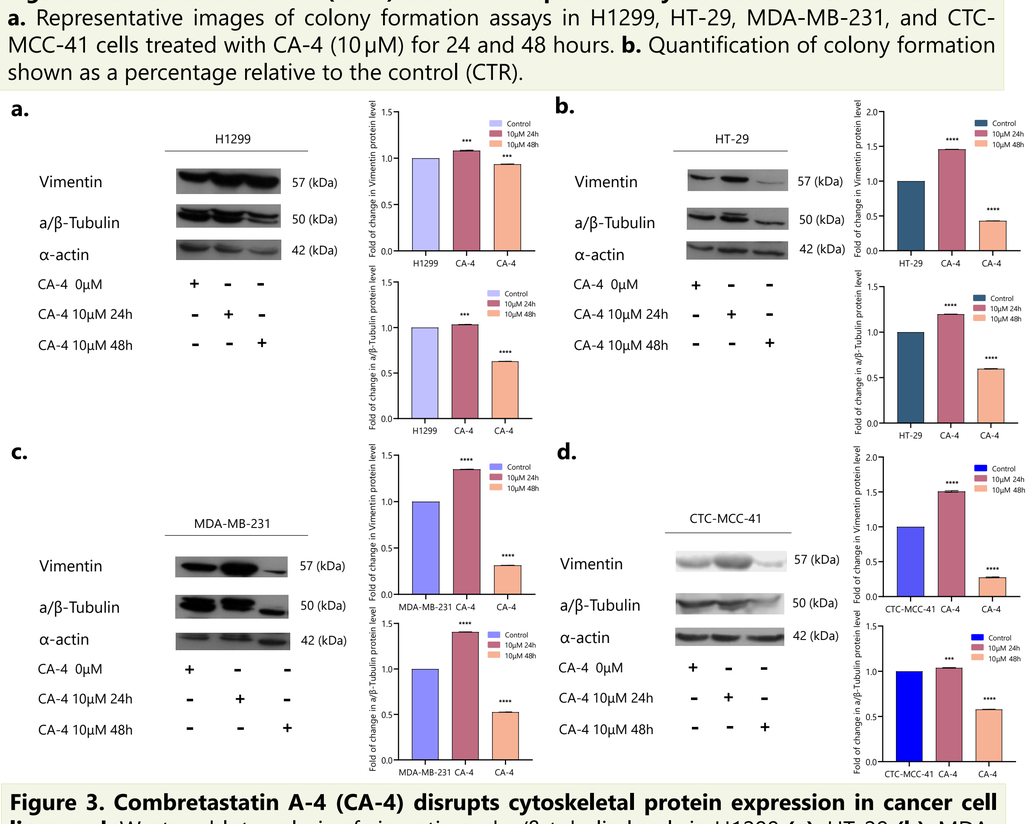


Figure 3. Combretastatin A-4 (CA-4) disrupts cytoskeletal protein expression in cancer cell lines. a-d. Western blot analysis of vimentin and α/β-tubulin levels in H1299 **(a)**, HT-29 **(b)**, MDA-MB-231 **(c)**, and CTC-MCC-41 **(d)** cells following CA-4 treatment (10 μM) for 24 and 48 hours. α-actin was used as a loading control.

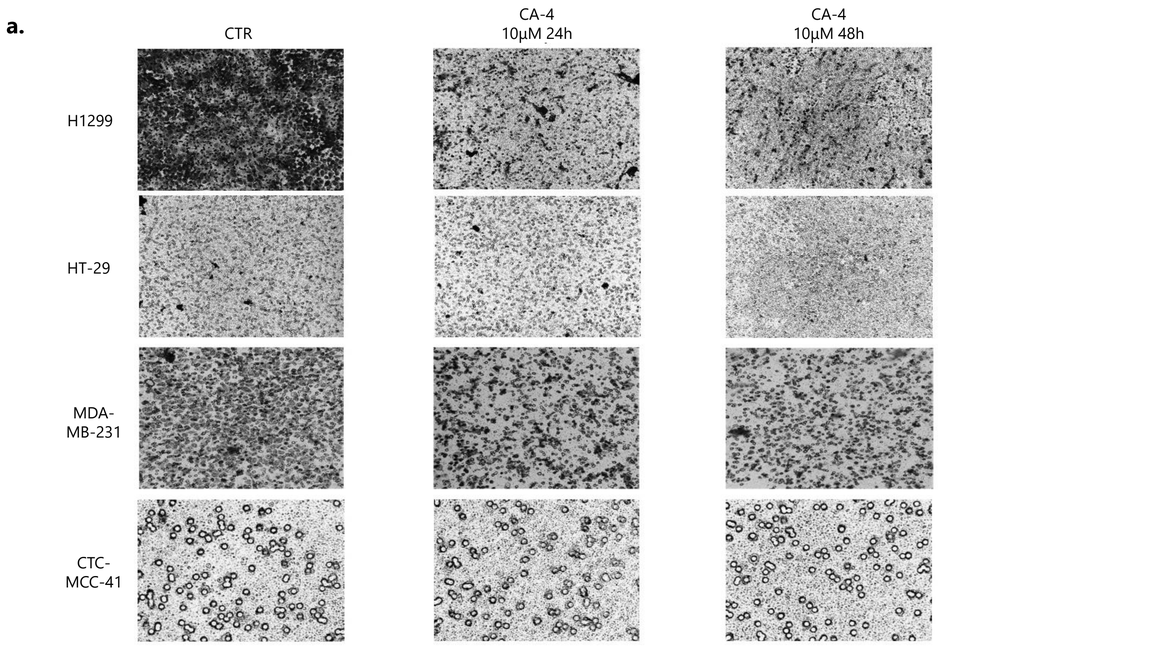


Figure 2. Combretastatin A-4 (CA-4) treatment significantly impairs migration in cancer cell lines. **a.** Representative grayscale images from migration assays showing H1299, HT-29, MDA-MB-231, and CTC-MCC-41 control cells and after Combretastatin A-4 (CA-4) treatment (10 μM) for 24 and 48 hours. **b.** Quantification of cell migration in H1299, HT-29, MDA-MB-231, and CTC-MCC-41 cells treated with CA-4 (10 μM) for 24 and 48 hours.

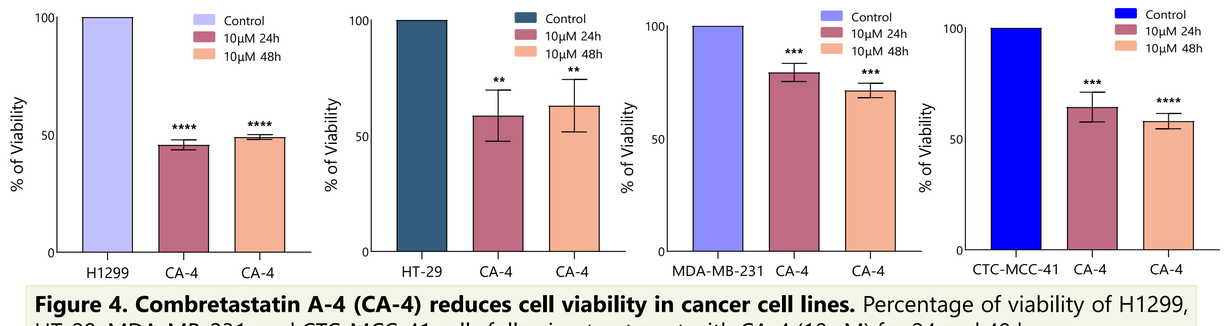


Figure 4. Combretastatin A-4 (CA-4) reduces cell viability in cancer cell lines. Percentage of viability of H1299, HT-29, MDA-MB-231, and CTC-MCC-41 cells following treatment with CA-4 (10 μM) for 24 and 48 hours.

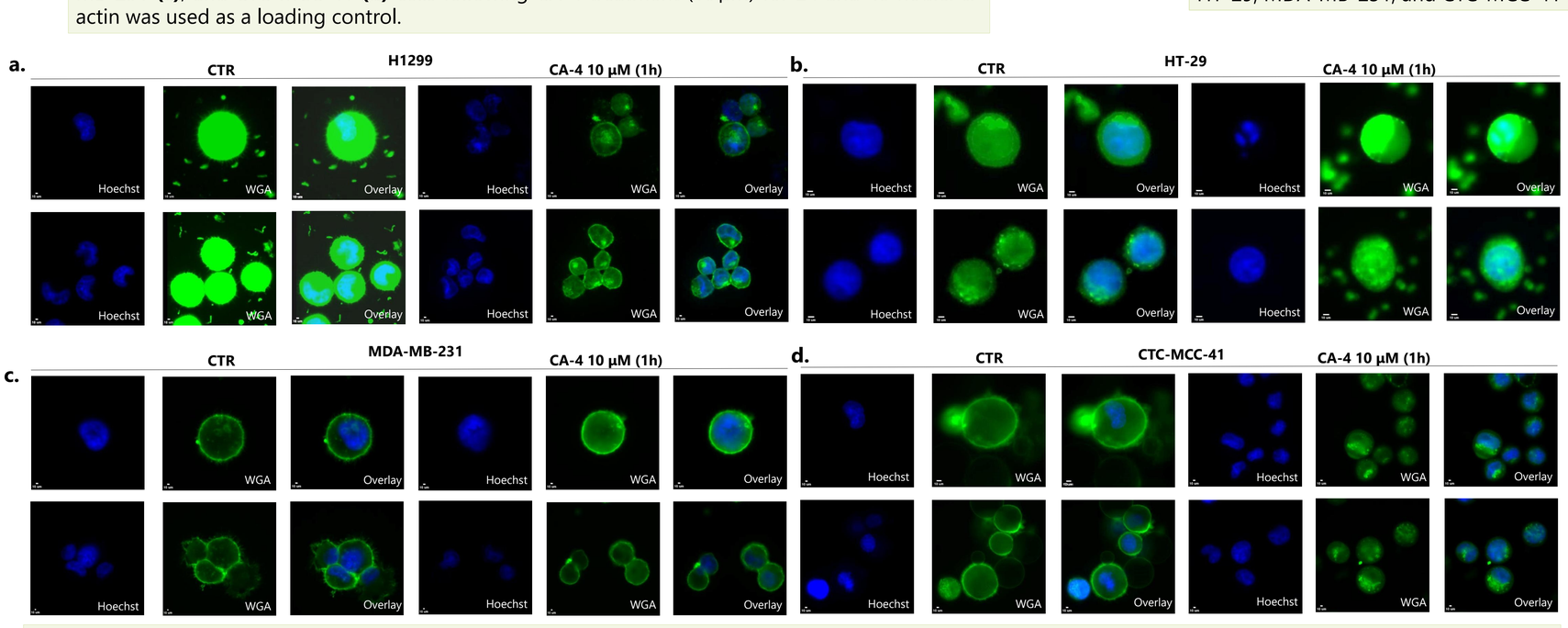


Figure 5. Combretastatin A-4 (CA-4) treatment reduces microtentacle (McTN) formation in H1299, HT-29, MDA-MB-231, and CTC-MCC-41 cells under non-adherent conditions. a-d. Representative immunofluorescence images of (A) H1299 and (B) HT-29 cells seeded on TetherChip nanosurfaces and treated with CA-4 (10 μM) for 1 hour.

Cell line	Control	CA-4 10 μM (1h)
H1299	21	3
HT-29	9	1
MDA-MB-231	18	2
CTC-MCC-41	35	11

Table 1. Total number of cells displaying microtentacles (McTNs) under control conditions and after Combretastatin A-4 (CA-4) treatment (10 μM, 1 h) in each cancer cell line.

Conclusions

- ✓CA-4 significantly **reduced** colony formation, migration, and viability across multiple cancer cell lines, including the **patient-derived CTC-MCC-41 cell line**
- ✓Treatment **disrupted cytoskeletal integrity**, with decreased expression of **vimentin and α/β-tubulin**
- ✓CA-4 strongly **inhibited McTN formation**, suggesting a potential role in **limiting CTC adhesion and metastatic spread**

Acknowledgments

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