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Elucidating mechanisms of resistance against the anticancer thiosemicarbazone COTI-2 by structural modifications and metal complex formation

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Aim of the study

COTI-2 is a thiosemicarbazone (TSC) that is currently tested as an anticancer therapeutic in a clinical phase I trial. The best-studied anticancer TSC is Triapine, against which cancer cells form resistance (a major obstacle in anticancer therapy) that strongly depends on the primary amino group in the structure of Triapine. The anticancer activity of TSCs is influenced by their interaction with endogenous metal ions, such as copper and iron. Therefore, in this study the effect of terminal N_4-modifications and metal complexation of COTI-2 on anticancer activity and resistance formation was investigated.

1. Establishing a COTI-2-resistant cell model

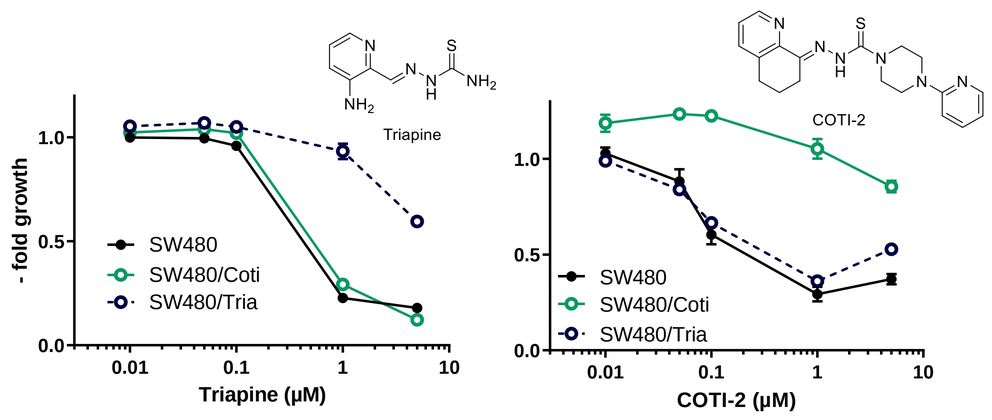


Figure 1: Resistance of SW480/Tria and SW480/Coti cells against Triapine and COTI-2. Anticancer activity of the drugs was tested by MTT viability assay after 72 h. Means ± standard deviations (SD) were derived from triplicates of one representative experiment out of three.

2. Less cell cycle arrest and paraptosis in resistant cells

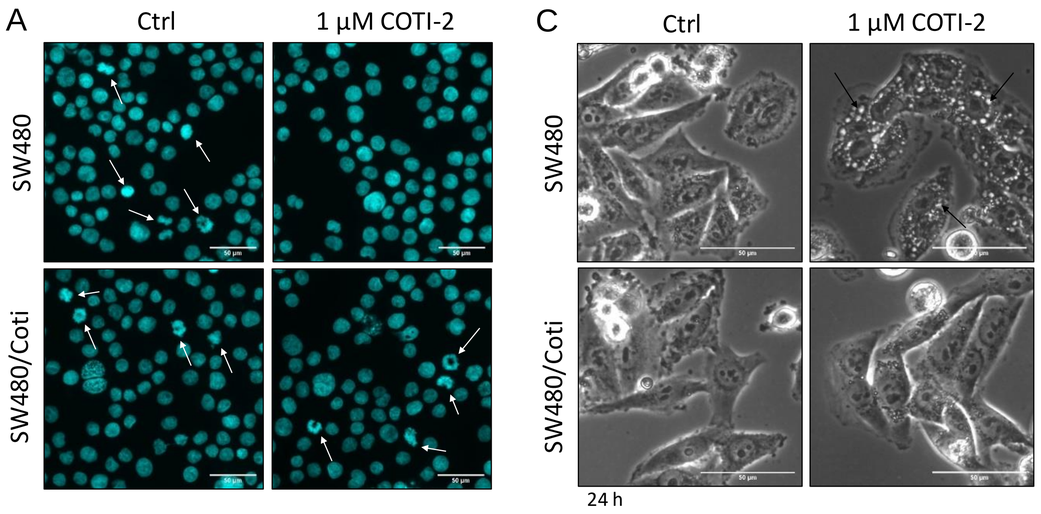
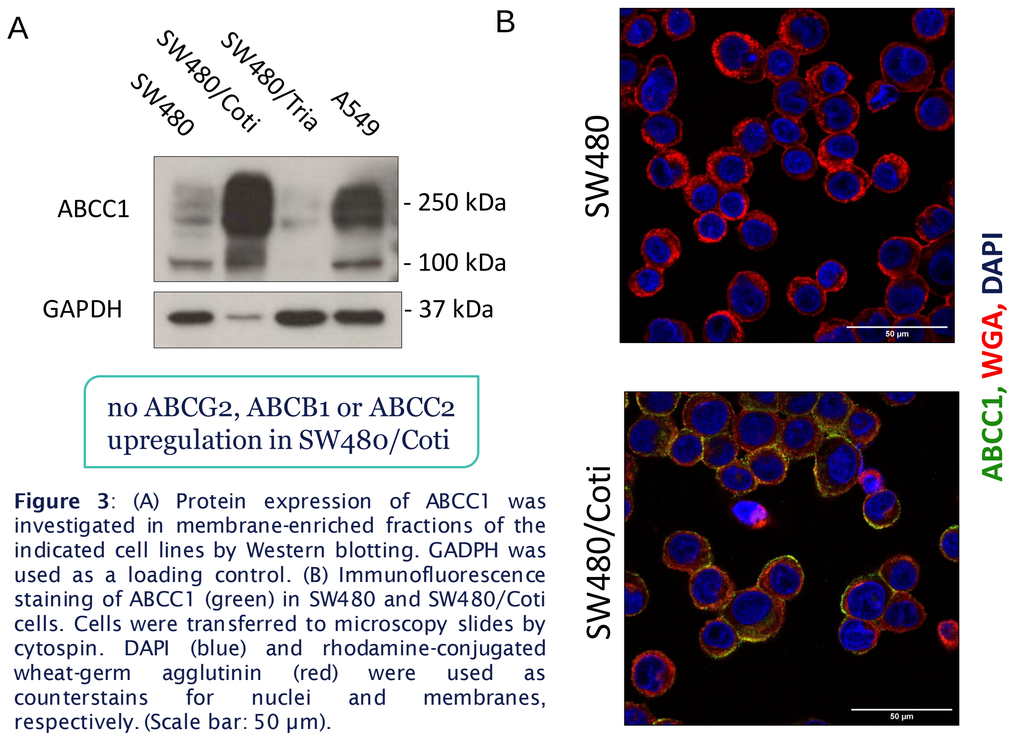


Figure 2: Cell cycle arrest and paraptotic cell death induction by COTI-2 in SW480 and SW480/Coti cells after 24h treatment. (A) Mitotic cells (arrows) were visualized by DAPI staining (scale bar: 50 µm). (B) The percentage of cells in G2/M phase was analyzed by propidium iodide (PI) staining and flow cytometry. Mean ± SD was derived from three independent experiments. Significance between cell lines (asterix between bars) was calculated by Two-way ANOVA and Sidak`s multiple comparison test. Significant difference to control group (indicated by asterix above bar) was calculated by One-way ANOVA and Dunnett`s multiple comparison test ($p < 0.05$ *). (C) Representative microscopy images of paraptotic vesicle formation (arrow) in cells (scale bar: 50 µm).

3. SW480/Coti cells express ABCC1



no ABCG2, ABCB1 or ABCC2 upregulation in SW480/Coti

Figure 3: (A) Protein expression of ABCC1 was investigated in membrane-enriched fractions of the indicated cell lines by Western blotting. GADPH was used as a loading control. (B) Immunofluorescence staining of ABCC1 (green) in SW480 and SW480/Coti cells. Cells were transferred to microscopy slides by cytospin. DAPI (blue) and rhodamine-conjugated wheat-germ agglutinin (red) were used as counterstains for nuclei and membranes, respectively. (Scale bar: 50 µm).

4. Confirmation of COTI-2 export by ABCC1

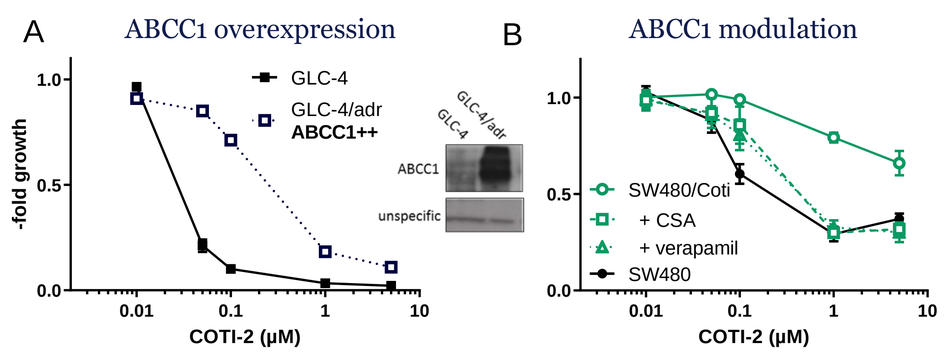


Figure 4: (A) COTI-2 was less active in ABCC1-overexpressing GLC-4/adr cells compared to parental GLC-4 cells. (B) ABCC1 transporter modulation with cyclosporine A (CSA) or verapamil increased sensitivity of SW480/Coti cells to COTI-2. Viability was tested by MTT assay after 72 h. Values given are means ± SD of one experiment out of three performed in triplicates.

5. Effect of derivatization and Cu complex formation on resistance

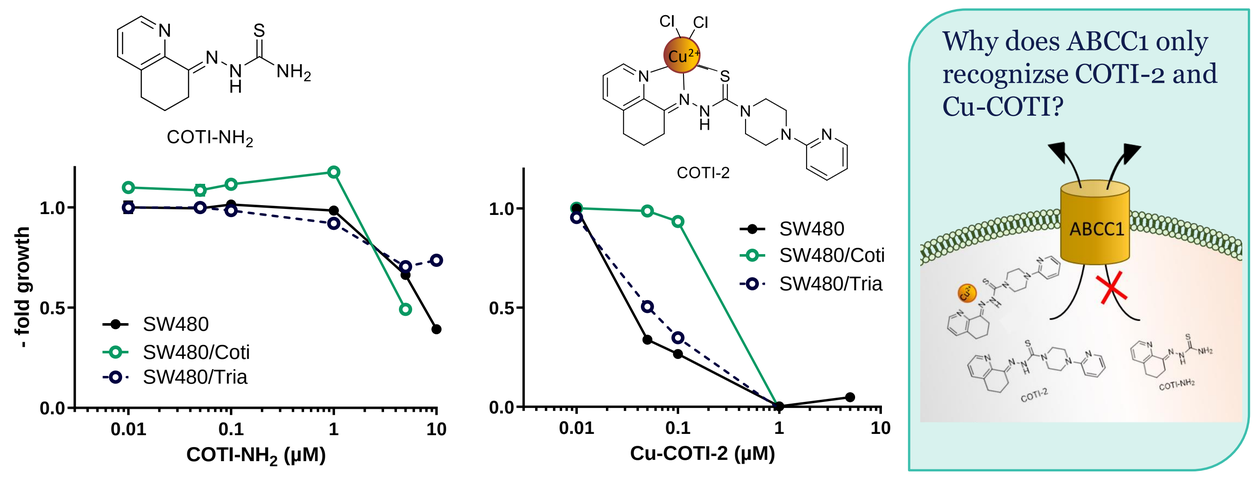


Figure 5: SW480/Tria and SW480/Coti cells are not resistant against the COTI-2 derivative COTI-NH_2, but SW480/Coti cells are resistant against the Cu-complex of COTI-2. Anticancer activity of the drugs was tested by MTT assay after 72 h. Means ± SD were derived from triplicates of one representative experiment out of three.

6. COTI-2 forms stable ternary complexes with Cu and glutathione

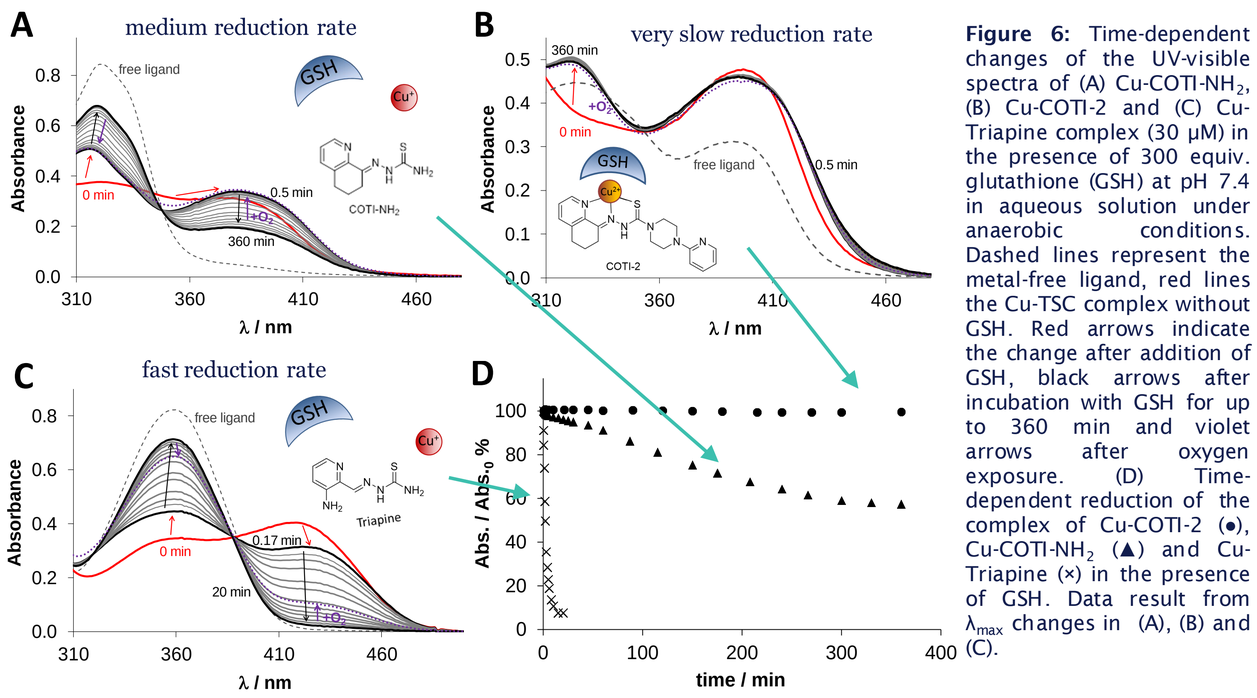
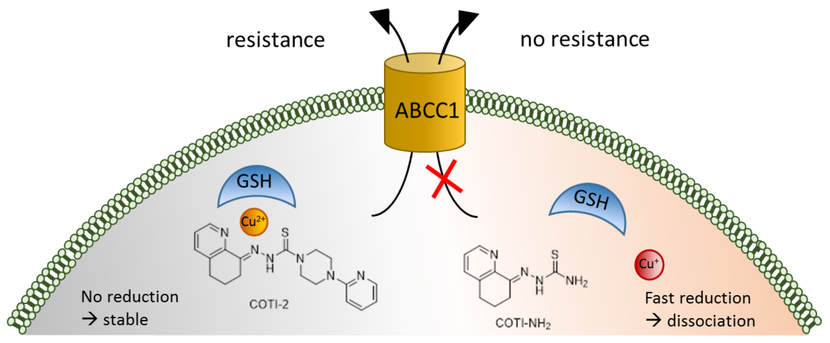


Figure 6: Time-dependent changes of the UV-visible spectra of (A) Cu-COTI-NH_2, (B) Cu-COTI-2 and (C) Cu-Triapine complex (30 µM) in the presence of 300 equiv. glutathione (GSH) at pH 7.4 in aqueous solution under anaerobic conditions. Dashed lines represent the metal-free ligand, red lines the Cu-TSC complex without GSH. Red arrows indicate the change after addition of GSH, black arrows after incubation with GSH for up to 360 min and violet arrows after oxygen exposure. (D) Time-dependent reduction of the complex of Cu-COTI-2 (●), Cu-COTI-NH_2 (▲) and Cu-Triapine (×) in the presence of GSH. Data result from λ_{max} changes in (A), (B) and (C).

Conclusion



1. ABCC1 facilitates resistance against COTI-2 (but not COTI-NH_2 or Triapine), due to formation of a stable ternary complex of COTI-2 with copper(II) and GSH.

2. The formation of copper(II) complexes is not only important for the anticancer activity of TSCs such as COTI-2, but also for acquired resistance due to their export by ABC transporters.

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