BCAT1 increases sensitivity to Cytarabine and confers CXXC motif derived resistance to pro-oxidant treatment in Acute Myeloid Leukaemiacells

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Introduction

Recently increased branched-chain amino transferase (BCAT1) expression has been associated with poor prognosis in AML making it an attractive target for novel therapies.[1] BCAT1 has been shown to increase proliferation in a variety of cancer cell lines. [2] We theorised the proliferative advantage of BCAT1 expressing cells may render them vulnerable to front line chemotherapeutic Cytarabine (Ara-C) which interrupts cell division. Secondly, BCAT1 features a CXXC motif, a feature common to antioxidant enzymes. We previously demonstrated BCAT1 CXXC motif can metabolise reactive oxygen species (ROS) [3]. Given excessive ROS can induce apoptosis we hypothesised BCAT1 CXXC antioxidant properties may protect against pro-oxidant treatment.

Key Findings

Firstly we over-expressed BCAT1-CXXC and BCAT1-CXXS mutant in U937 cell lines (**Fig 1**). BCAT1 overexpression increased the proportion of G2M phase cells. Concurrently BCAT1 increased sensitivity to Ara-C treatment compared to controls(**Fig 2**). Rotenone treatment revealed the LD50 was significantly higher for BCAT1-CXXC compared to BCAT1-CXXS. Moreover, BCAT1-CXXC displayed significantly reduced intracellular ROS compared to BCAT1-CXXS. (**Fig 3**).

Methods

- Cysteine → Serine substitution of the CXXC motif was performed by site directed mutagenesis creating BCAT1-CXXS mutant, eliminating CXXC motif derived antioxidant activity.
-Following stable transduction of BCAT1 expression was quantified by qPCR. Protein expression was confirmed western blot analysis.
- Cycle cycle analysis was performed by Propridium Iodide staining.
- Cell viability assays viable/dead cells were distinguished by Viacount Easyfit cluster analysis. LD50 Dose response analysis was performed by non-linear regression using Prism

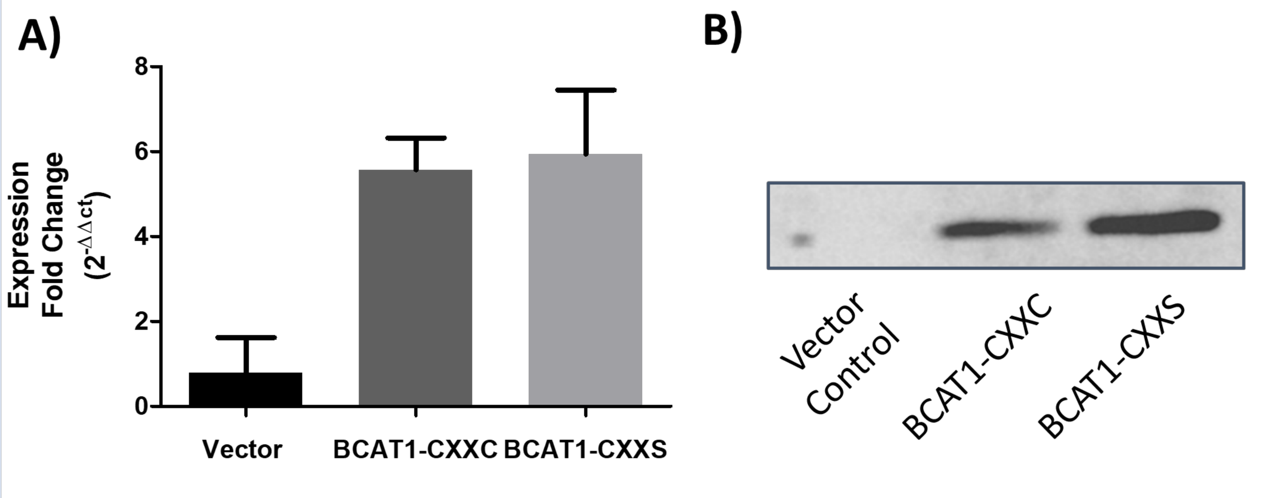

Figure 1: Overexpression of BCAT1 in U937 cells. A) Following stable transduction of U937 cells fold expression change of *BCAT1* transcript was determined by qPCR. BCAT1-CXXC (5·57 ± 1·31 fold increase) and BCAT1-CXXS (5·93 ± 2·6 fold increase)**B)** Expression at the protein level was confirmed by Western Blot analysis. 5·57 ± 1·31 fold increase) and BCAT1-CXXS (5·93 ± 2·6 fold increase)

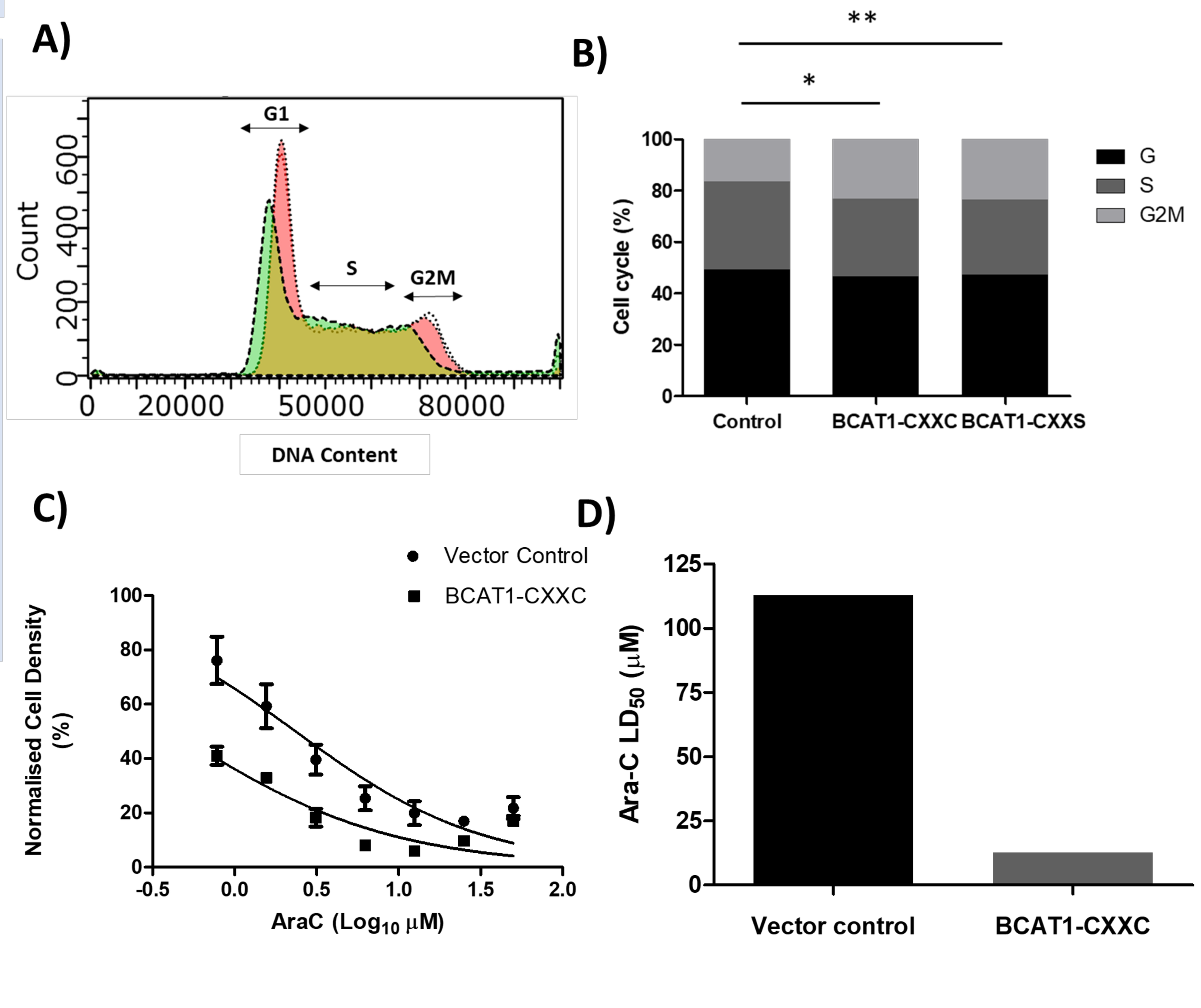

Figure 2: BCAT1 expression increases G2M phase cells and sensitivity to Ara-C treatment A) Representative Histograms displaying representative cell cycle data. U937-BCAT1-CXXC (No fill) and U937-BCAT1-CXXS (Red fill) cells display higher proportion of G2M phase cells compared to Vector Control (Green fill) **B)** Stacked bar chart displaying percentage of cells in G, S and G2M for U937 cell lines.(n=4, *p=0.05, **p=0.01) **C)** Dose response curve displaying cell density in response to Ara-C treatment normalised to no treatment control. **D)** Bar chart displaying IC50 concentration for BCAT1-CXXC(0·442 ± 1·34 μmol/l) compared to Vector control (2·36 ± 1·18 μmol/l) (n = 3 P < 0·0001).

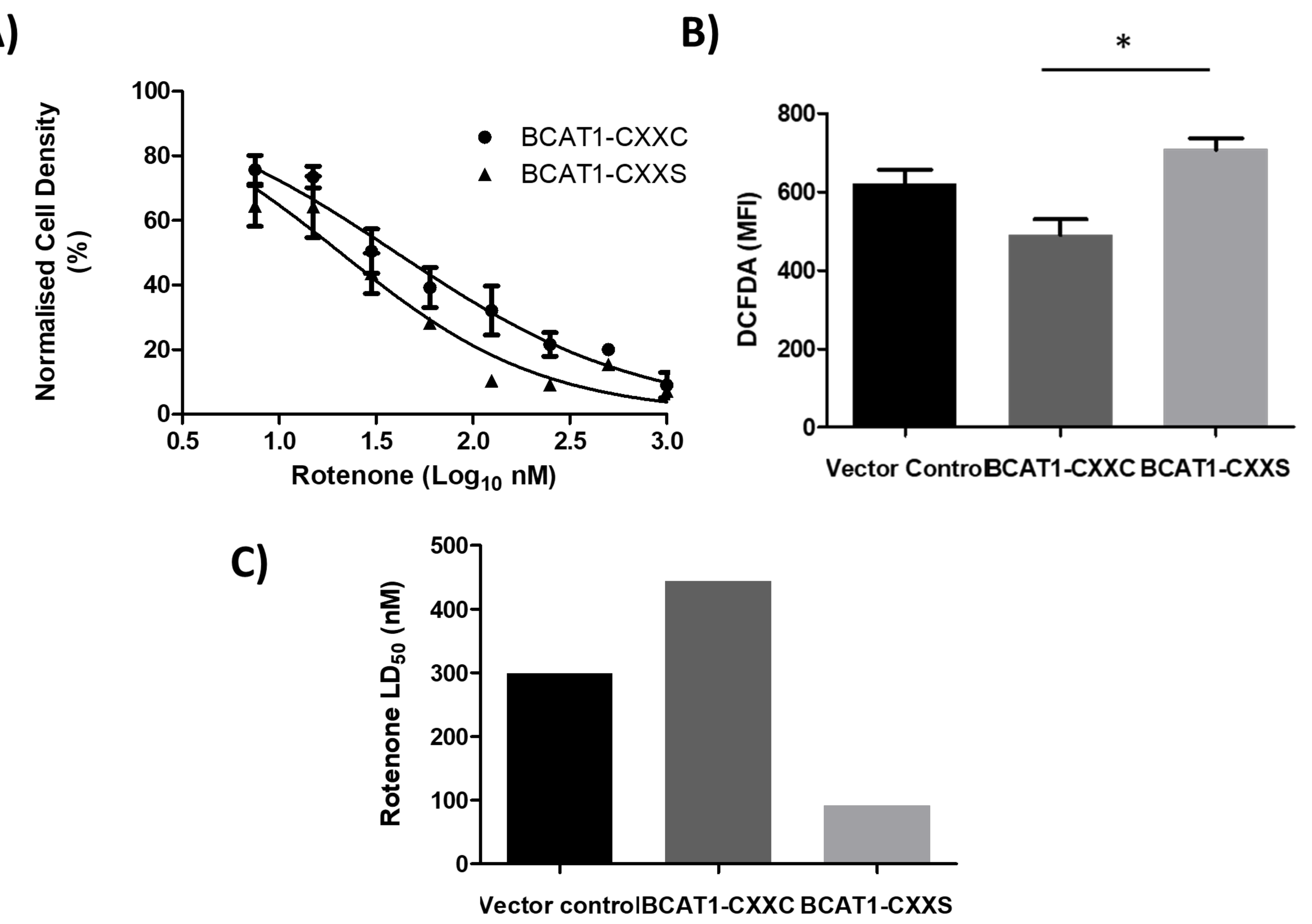

Figure 3: BCAT1 confers CXXC motif derived resistance to pro-oxidant treatment. A) Dose response curve displaying cell density in response to Rotenone treatment for U937-BCAT1(CXXC) and U937-BCAT1(CXXS) cells. **B)** Bar chart displaying DCF signal following treatment with 60 nmol/l Rotenone. BCAT1-CXXC (491 ± 39 nmol/l) significantly decreased ROS signal compared to BCAT1-CXXS(707 ± 29 nmol/l) and Vector control (659 ± 22 nmol/l) (n = 3, P < 0·05). **C)** Bar chart displaying IC50 values for BCAT1-CXXC (40·9 ± 1·14 nmol/l),U937-BCAT1-CXXS(20·8 ± 1·14 nmol/l) and Vector Control (20·7 ± 1·18 nmol/l) (n=3, P<0·001)

Discussion

BCAT1 has attracted significant interest in recent years and is associated with a poor prognostic outlook, therefore there is a pressing need to develop targeted therapies that can be delivered alongside standard chemotherapeutics. Here we have demonstrated for the first time that BCAT1 expression protects against pro-oxidant treatment whilst increasing vulnerability standard therapeutic treatment Ara-C. This data opens up a new therapeutic avenue for clinicians seeking novel strategies in the stratified treatment of BCAT1 high and BCAT1 low expressing patients.

References
1) Coles *et al* (2018) **2)** Hattori *et al (*2017) **3)** Hillier *et al* (2018) https://doi.org/10.1182/blood-2018-99-118823

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