Supplementary Materials

TGF-β causes docetaxel resistance in prostate cancer via the induction of Bcl-2 by acetylated KLF5 and protein stabilization

Li et al.

Figure S1. KLF5 is required by TGF-β to induce DTX resistance in prostate cancer cells. (A, B) Cytotoxicity assay in DU 145 and PC-3 cell variants with concomitant treatments of DTX and TGF-β1 (10 ng/μl) and/or SB505124 (2.5 μM). KLF5 -/-, endogenous KLF5 was knocked out. (C, D) Colony formation assay of KLF5 -/-DU 145 and PC-3 cells with or without wild type KLF5 restoration in Matrigel treated with DTX (1 nM) and/or TGF-β1 (10 ng/μl). Matrigel colony formation assay was performed in duplicate, and error bars represent the standard errors of the means. ns, p > 0.05; *, p ≤ 0.05; **, p ≤ 0.01; ***, p ≤ 0.001. Scale bars, 100 μm. Magnification, X10. DTX: docetaxel; SB: SB-505124.
**Figure S2. KLF5 acetylation at K369 mediates DTX resistance in prostate cancer cells.** (A) Cytotoxicity assay in DU 145 (KLF5-/-) cells expressing wild type KLF5 and acetylation deficient mutant KLF5^K369R (KR) with concomitant treatments of DTX and TGF-β1 (10 ng/μl) and/or SB505124 (2.5 μM). (B, C) Cytotoxicity assay and colony formation assay in Matrigel of DU 145 (KLF5-/-) cells expressing KR and acetylation mimicking mutant KLF5^K369Q (KQ) treated with DTX (1 nM). (D - G) Cytotoxicity assay of DTX (D, E) and colony formation assay with 1 nM DTX (F, G) in DU 145 and PC-3 (KLF5-/-) cells expressing KR and KQ with concomitant treatments of TGF-β1 (10 ng/μl) and/or SB505124 (2.5 μM). Cytotoxicity assay and Matrigel colony formation assay were performed in triplicate, and error bars represent the standard errors of the means. ns, p > 0.05; *, p ≤ 0.05; **, p ≤ 0.01; ***, p ≤ 0.001. Scale bars, 100 μm. Magnification, X10. DTX: docetaxel; SB: SB-505124.
Figure S3. DTX is less effective in inducing early apoptosis in cells expressing acetylated KLF5, and Mcl-1 did not play an apparent role in TGF-β induced DTX resistance in KQ cells. (A, B) Apoptosis and necrosis assays were used to measure early apoptosis response in DU 145 parental cells (A) and KR and KQ cells (B) with DTX treatment (10 nM). (C, D) Cytotoxicity assay of DTX-treated DU 145 (KLF5−/−) cells expressing KR and KQ with or without concomitant treatment of S63845 (1 μM). Cytotoxicity assay was performed in triplicate, and error bars represent the standard errors of the means. ns, $p > 0.05$; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$. DTX: docetaxel.
Figure S4. TGF-β induces DTX resistance by stabilizing Bcl-2 protein in DU 145 cells expressing acetylation-deficient KLF5. (A, B) Detection of Bcl-2 protein level by Western blotting in DU 145 KR cells treated with different combinations of DTX (10 nM), TGF-β (10 ng/μl), and cycloheximide (CHX, 10 μM) for indicated time. (C) A literature review of molecular mechanisms that regulate Bcl-2 stability. (D) Western blotting analysis of Bcl-2 protein in DU 145 KR cells after 16 hours of DTX treatment. MG-132 treatment was applied at 10 μM for 3 hours before protein collection. Cytotoxicity assays were performed in triplicate, and error bars represent the standard errors of the means. ns, \( p > 0.05 \); *, \( p \leq 0.05 \); **, \( p \leq 0.01 \); ***, \( p \leq 0.001 \). DTX: docetaxel.
**Figure S5.** TGF-β induces DTX resistance through apoptosis inhibition.

Detection of PARP and cleaved PARP protein levels by Western blotting (A) and Annexin V +/PI – cell percentage by flow cytometry analysis (B) in DU 145 KQ cells treated with different combinations of DTX (10 nM), TGF-β (10 ng/μl), and ABT-199 (1000 μM) for 20 (A) or 16 hours (B). Flow cytometry analysis was performed in triplicate, and error bars represent the standard errors of the means. ns, \( p > 0.05 \); *, \( p \leq 0.05 \); **, \( p \leq 0.01 \); ***, \( p \leq 0.001 \). DTX: docetaxel.

**Figure S6.** Bcl-2 mediates DTX resistance in prostate cancer cells. (A, B)

Detection of KLF5, Bcl-2, and acetylated-KLF5 in parental cells and Docetaxel Resistant 50 and 100 (DDR50 and DDR100) cells of the DU 145 cell line. (B) Cytotoxicity assay of DTX in DDR50 cells with or without ABT-199 treatment (500 nM). (C) Cytotoxicity assay of DTX in DDR50 cells with or without KLF5 silencing by siRNA. Cytotoxicity assays were performed in triplicate, and error bars represent the standard errors of the means. ns, \( p > 0.05 \); *, \( p \leq 0.05 \); **, \( p \leq 0.01 \); ***, \( p \leq 0.001 \). DDR50: DTX-resistant cell lines tolerated a final DTX concentration of 50 nM;
DDR100: DTX-resistant cell lines tolerated a final DTX concentration of 100 nM.

Figure S7. Higher TGF-β signaling activity and higher KLF5 mRNA level correlate with poorer survival of prostate cancer patients. (A) Kaplan-Meier estimates of disease free survival in 492 patients with advanced prostate cancer (TCGA, Provisional). (B, C) Kaplan-Meier estimates of overall survival in 57 (B) and 43 (C) patients with castration-resistant prostate cancer. $KLF5^{\text{low}}$, mRNA expression z-score less than median; $KLF5^{\text{high}}$, mRNA expression z-score greater than median, TGF-β$^{\text{high}}$, TGFβ1 and either TGFBR1 or TGFBR2 greater than median; and TGF-β$^{\text{low}}$, TGFβ1 and either TGFBR1 or TGFBR2 equal to or smaller than median.