Supplementary Materials

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

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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; *, $p \le 0.05$; **, $p \le 0.01$; ***, $p \le 0.001$. 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- β 1 (10 ng/ μ l) and/or SB505124 (2.5 μ M). (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- β 1 (10 ng/ μ l) and/or SB505124 (2.5 μ M). 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; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$. Scale bars, 100 μ m. 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 μ M). 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.

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				DU	145 KR				в					DU	145 KR				
CHX	+	+	+	+	+	+	+	+		CHX	+	+	+	+	+	+	+	+	
DTX	+	+	+	+	+	+	+	+		DTX	-	-	-	-	-	-	-	-	
TGF-p	- 3	-	-	-	+	+	+	+		TGF-β	-	-	-	-	+	+	+	+	
_	0	8	12	16	0	8	12	16	(hours)		0	8	12	16	0	8	12	16	(hours)
	-				-		-		Bcl-2		-	-	-	-	-	-	-	-	Bcl-2
[-	-	-	-	-	-	-	-	β-acti	n l	1	-	-	-	-	-		-	β-actin

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Mechanisms Regulating BcI-2 Stablity.								
Function	Regulation Type	Findings	Referrence					
Stablize	S-nitrosylation	In H460 cells, nitric oxide induced S-nitrosylation on cystein residues. In H460 cells, Cys158 and Cys229 of Bcl-2 were nitrosylated.	Chanvorachote et al., 2006 Azade et al., 2006					
	Phosphorylation	In HeLa cells, the MAP kinase sites Thr74 and Ser87 of BcI-2 are phosphorylated by MAP kinase.	Breitschopf et al., 2000					
	Mono-ubiquitination	In 293 cells, Parkin induced monoubiquitination on C-terminus of Bcl-2.	Chen et al., 2010					
Destablize	S-denitrosylation	In HeLa, A375, and 7860, MDA-7/IL24 induced s-denitrosylation on BcI-2.	Tian et al., 2012					
	Dephosphorylation	In HUVEC cells, TNF-alpha induced dephosphorylation on Thr 56, Thr 74, and Ser 87 (major role) of BcI-2. In H460 cells , Cisplatin/hydrogen peroxide/hydroxyl radical induced dephosphorylation of BcI-2.	Dimmeler et al., 1999 Wang et al., 2008 Luanpotpong et al., 2011					
	Poly-ubiquitination	In H460 cells, Cr(VI)-I induced ROS led to polyubiquintinated BcI-2. In SW480, PPAR-alpha induced polyubiquintination on Lysine 22 with BcI-2. In HeLa and MEF, lysine 17 of BcI-2 was ubiquintinated by E3 ligase XIAP. In HEK293, Keap1:CuI3-Rbx1 induced polyubiquintnation on lysine 17. In 293T, FBXO10 polyubiquintinated BcI-2.	Azad et al., 2008 Gao et al., 2015 Edison et al., 2017 Niture et al., 2011 Chiorazzi et al., 2013					
	Caspases Cleavage	In Huh7, caspase 3 (major role) and caspase 9 cleaved Bcl-2, FKBP38 prevented the cleavage by binding to flexible loop domain of Bcl-2.	Choi et al., 2010					



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/ μ l), and cycloheximide (CHX, 10 μ M) 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 μ M for 3 hours before protein collection. Cytotoxicity assays were performed in triplicate, and error bars represent the standard errors of the means. ns, $\rho > 0.05$; *, $\rho \leq 0.05$; **, $\rho \leq 0.01$; ***, $\rho \leq$ 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/ μ l), 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; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \geq 0.0$ 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*^{low}, mRNA expression z-score less than median; *KLF5*^{high}, mRNA expression z-score greater than median, TGF-β^{high}, TGFB1 and either TGFBR1 or TGFBR2 greater than median; and TGF-β^{low}, TGFB1 and either TGFBR1 or TGFBR2 equal to or smaller than median.