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Cripto-1 promotes resistance to drug-induced apoptosis by activating the TAK-1/NF-κB/survivin signaling pathway

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ABSTRACT

Cripto-1 is an oncogenic protein that belongs to the epidermal growth factor (EGF)-cripto-1/FRL1/cryptic (CFC) family. It has been shown to stimulate tumorigenesis and metastasis by promoting cancer cell proliferation, epithelial-to-mesenchymal transition (EMT), and tumor angiogenesis. However, the role of Cripto-1 in cell survival and apoptosis remains largely undefined. In the present study, we found that Cripto-1 is significantly upregulated in a number of human cancer cell lines. The membrane-associated but not the soluble form of Cripto-1 promotes resistance to drug-induced caspase-3 cleavage, an indicator of apoptosis. Consequently, Cripto-1 silencing sensitizes human cancer cells to chemotherapy drugs including cytarabine, cisplatin and taxol. Our mechanistic studies revealed that Cripto-1 promotes apoptosis resistance by inducing NF-κB-mediated Survivin expression through activation of TAK-1. We also found that Cripto-1 silencing does not affect growth of un-treated cancer cells, and Cripto-1 forms self-assembled punctiforms and changes its subcellular distribution upon cytarabine treatment. Thus, the anti-apoptotic activity of Cripto-1 could be an inducible function that can be activated by external stimuli such as drug stimulation. Our findings suggested that targeting the Cripto-1/TAK-1/NF-κB/Survivin pathway may be an effective approach to combat apoptosis resistance in cancer.

1. Introduction

Many anti-cancer drugs kill malignant cells by triggering apoptosis, a programmed cell death promoted by caspases. However, activation of anti-apoptotic and/or survival pathways in cancer cells often leads to therapeutic failure [1]. Understanding the molecular mechanisms responsible for apoptosis resistance is critical in the design and development of novel therapeutics against cancer. Cripto-1, the original member of the epidermal growth factor (EGF)-cripto-1/FRL1/cryptic (CFC) family of signaling proteins, has been linked to tumorigenicity [2]. Structurally, the EGF-CFC proteins contain an N-terminal signal sequence for extracellular secretion, a EGF-like domain, a cysteine-rich CFC domain, and a hydrophobic carboxy-terminus with sequences for glycosylphosphatidylinositol (GPI) attachment for membrane association [3,4]. Cripto-1 can function either as a membrane-anchored protein or as a soluble ligand after GPI removal [13]. Cripto-1 is markedly upregulated in a variety of human cancers such as colon, breast, lung, ovarian, and pancreatic cancer [4–7]. Functionally, it has been shown to promote tumorigenesis and metastasis by increasing cancer cell proliferation, enhancing epithelial-to-mesenchymal transition (EMT), and stimulating tumor angiogenesis [3,5–7]. However, whether Cripto-1 regulates cancer cell survival and/or apoptosis is largely unclear.

Nuclear factor (NF)-κB, a master regulator of gene transcription, was activated downstream of a number of key oncogenic pathways to regulate the expression of genes associated with immune response, inflammation, survival and proliferation, inhibit apoptosis, and enhance metastasis [8–11,16]. In particular, the anti-apoptotic function of NF-κB has been documented [10,12]. In previous report, the anti-apoptotic activity of NF-κB was suggested on EGFR dependent manner [18]. Inhibition of NF-κB activation decreases expression anti-apoptotic genes and induces genes associated with apoptosis. It has been suggested that PKC-a, which is upstream of and activates IκB kinase (IKK) and induce NF-κB DNA binding activity, could enhances anti-apoptosis, which reversed by PKC selective inhibitors, indicating appropriate NF-κB signaling pathway may be required for drug-induced apoptosis [18]. TGF-β-activated kinase (TAK)-1, like PKC-a, is another important regulator for the activation of NF-κB [22–24], which was considered as a key activator of IκB kinase (IKK). TAK-1 has been shown to mediate cancer cell metastasis and apoptosis resistance through NF-κB [14]. Various gene products, including cell growth(cyclin D1 and cyclooxygenase-2)

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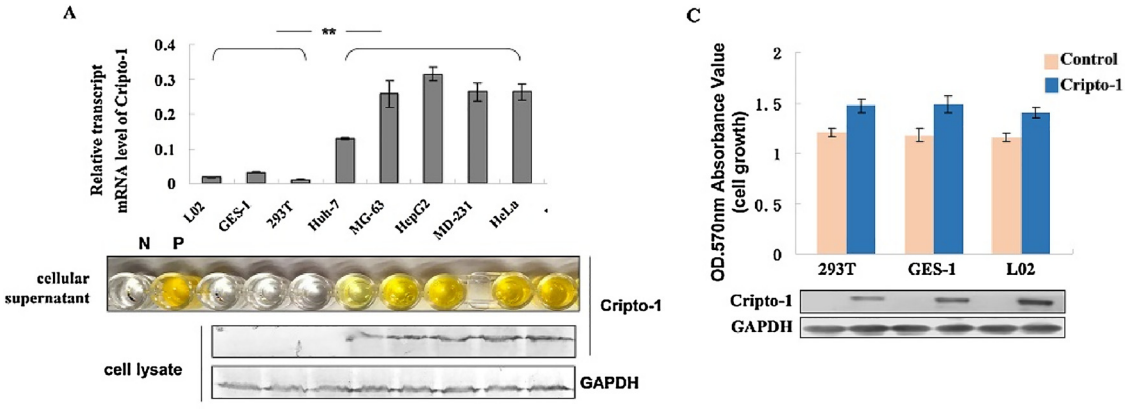

B

Cell	293T**		GES-1**		L02**	
	control	Cr-1	control	Cr-1	control	Cr-1
IC50 (ug/ml)	1.464±0.3	2.913±0.33	4.59±0.44	12.74±0.38	4.98±0.43	11.64±0.37

Fig. 1. Cripto-1 overexpression inhibits drug-induced cell death. **A**. Cripto-1 expression in human normal and cancer cells. upper, relative transcription of Cripto-1 mRNA dectected by realtime-PCR. The relative transcript level equals to 2^{-n}(n = D-value). Statistical analysis was performed between normal group and cancer group, using the Student's *t*-test; lower, intracellular and supernatant levels by Western Blot analysis and ELISA, respectively. **B**. The IC_{50} values change of cytarabine for wildtype and Cripto-1-overexpressing cell lines (293 T,GES-1 L02). The cells, both control and the Cripto-1 overexpressing were treated with various concentrations of cytarabine (from 0.5 to 80 u g/ml, doubling dilution) for 48 h. Cell viability was determined by the MTT assay and IC50 value was calculated using the corresponding formula. The Cripto-1 ectopic expression cells were transfected with pcI-neo-Cripto-1 plasmid for 24 h prior to drug treatment. C. The cell viability of the control group and the Cripto-1 overexpression cells. Protein levels were determined by Western Blot analysis. Each experiment was repeated for three times, $^{**}p < 0.01$.

A

Cell	HepG2*		HeLa*	
	control	siRNA-Cr-1	control	siRNA-Cr-1
IC50 (ug/ml)	3.635±0.644	2.356±0.384	5.717±0.163	3.745±0.46

B

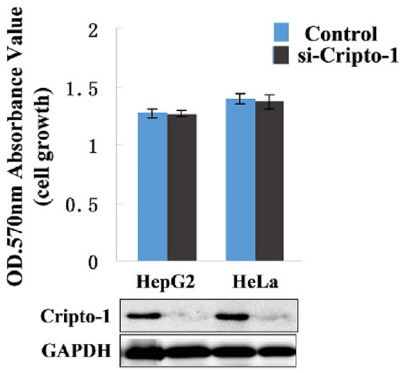

Fig. 2. Cripto-1 silencing enhances drug-induced cell death of human cancer cells. **A**. The IC_{50} values of cytarabine for wildtype and Cripto-1-silenced HepG2 and HeLa cells. Wildtype and Cripto-1-silenced HepG2 and HeLa cells were treated with various concentrations of cytarabine (from 0.5 to 80 u g/ml, doubling dilution) for 48 h. PBS was used as control. Cell viability was determined by the MTT assay. B. The cell viability was determined by MTT after transfected with siRNA-Cripto-1 construct and the protein level was determined by Western Blot analysis. Each experiment was repeated for three times. $^{*}p < 0.05$.

[19,20], survival (Bcl-2,FLIP, cIAPs and Survivin) [21], invasion (ICAM-1 and MMP-9), and angiogenesis (VEGF) have been suggested to be regulated by the TAK-1/NF-κB pathway [24–26]. Survivin, a member of the inhibitor of apoptosis (IAP) family, is the target gene of NF-κB pathway. Survivin inhibits the activation of caspase-3 and caspase-7 and thereby promotes cancer cell resistance against radiation and drug-induced apoptosis [21] Survivin has been shown to be regulated by NF-κB on TAK-1-dependent manner [17]. These findings supported the TAK-1/NF-κB/Survivin signaling as a potential contributing mechanism for apoptosis resistance of cancer cells.

We have previously found that Cripto-1 modulates phagocytic activity and cytokine secretion of macrophages by activating NF-κB [27]. Our mechanistic studies revealed increased IKK phosphorylation and p65 nuclear translocation upon Cripto-1 treatment. Based on these findings, we speculated that Cripto-1 may activate NF-κB in cancer cells to regulate cancer cell survival and apoptosis resistance. In the present study, we investigated the role of Cripto-1 in apoptosis resistance of a variety of human cancer cells. We found that the membrane-associated but not the soluble form of Cripto-1 promotes cancer cell resistance to drug-induced apoptosis through activation of the TAK-1/NF-κB/Survivin signaling cascade.