Doxorubicin Induced Oxidative Stress is a Double Edged Sword for Life and Death of Cancers Regulated by p53-Dependent Dual Function of SRC kinase

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Introduction: Doxorubicin (DOX) is a baseline regimen for osteosarcoma. The use of antioxidant supplement during chemotherapy is controversial, because it may reduce cardiac toxicity caused by DOX-induced ROS but may reduce cytotoxic effect on cancer cells at the same time. To clarify this issue, we screened for the cytotoxicity of combination therapy of DOX and antioxidant, NAC (N-acetyl cysteine) in various cancer cells. We found DOX induced ROS differentially affect life and death in cancers. In this study, we proved it is determined by the expression of wild type P53. We show that the dual function of SRC kinase is behind this phenomenon.

Methods: Various cancer cell lines were used to compare the effect of DOX, NAC and SRC inhibitor. Monolayer cells were treated with DOX or in combination of DOX and NAC or DOX and SRC inhibitor. And cell viability was measured by MTT assay.
We used the Bliss and Loewe additivism to quantify the level of synergy in drug combination experiments. Apoptosis was evaluated by Annexin V-FITC analysis and western blot using a-caspase7 and a-PARP Western blot was performed to measure the expression and activation of p53, JNK, SRC and STAT3. And beta-Actin was used as an internal control.
HCT 116 -/- and +/- (5x 105 cells) were injected subcutaneously in the back of six weeks old athymic mice. Two weeks later, mice were treated with doxorubicin, NAC, SRC inhibitor.

Results: In P53 null cancers, NAC significantly synergize DOX (Fig1A,D), reduced tumor size in vivo xenograft model (Fig1B), increased apoptosis (Fig1C). In contrast, NAC antagonize DOX (Fig1A,D), inhibit apoptosis (Fig1C) in P53 wild type by suppressing phosphorylation of P53 (Fig1E). which is reversed by knockdown of P53 using shP53 RNA (Fig1F).
SRC kinase was activated by both DOX and H2O2 in all cell lines tested (Fig2A,D). DOX increased intracellular ROS level (Fig2B). DOX induced SRC activation was attenuated by NAC (Fig2C) indicating DOX/ROS/SRC activation. Constitutively active SRC (CA-SRC) decreased apoptosis and apoptotic signal in p53 null MG63 cell line (Fig2E,F). CA-SRC increased cell survival in a DOX treated p53 null cancer. During DOX treatment, SRC activated JNK and P53 in P53 wild type cancer leading apoptosis. However, SRC activated STAT3 in P53 null type cancer leading cell survival (Fig3A). In xenograft model, SRC inhibitor reduced tumor size of p53 null type but increased that of p53 wild type (Fig3B,D). SRC inhibitor synergized DOX in P53 dysfunctional cancers cell lines, but antagonized DOX in P53 wild type cancer cell lines (Fig3C,E). SRC inhibition showed synergic effect to DOX in a P53 null leiomyosarcoma primary cell (Fig3F).

Discussion: We clarified that DOX-induced oxidative stress is cytotoxic only for normal cells or cancers which express wild type P53. DOX-induced oxidative stress paradoxically enhances chemo-resistance in P53-dysfunctioning cancers through ROS/SRC signaling. In the same context, our in vitro screening and in vivo data suggest antioxidant can be safely used for the patients who have P53-dysfunctioning
cancers, which reduces the risk of DOX-induced heart failure. It limitedly hampers chemotherapeutic effects only in P53 wild cancers. As such, DOX-induced oxidative stress is a double edged sword for life and death of cancer cells. We found that P53-dependent dual function of SRC kinase was behind this phenomenon. DOX-induced activation of SRC kinase showed a dual role for cell survival. It increased phosphorylation of P53 by activating JNK signaling, which enhanced apoptosis in p53 wild type cancers. On the other hand, DOX-induced activation of SRC kinase increased cell survival by STAT3 activation in p53 null osteosarcoma. Thus, SRC inhibitor can be used to synergize Dox for P53-dysfunctioning cancers, but not for P53 wild type cancers. Our data suggest P53 be a new determinant factor for a genome based strategy to guide personalized combination therapy.

**Significance:** SRC kinase showed dual function depending on functional status of P53. Therefore, the status of P53 should be considered during cancer treatment using SRC inhibitors.
Figure 2