

Selective induction of necrotic cell death in cancer cells by β -lapachone through activation of DNA damage response pathway

#4031



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Abstract

Most efforts thus far have been devoted to develop apoptosis inducers for cancer treatment. However, apoptotic pathway deficiencies are a hallmark of cancer cells. We propose that one way to bypass defective apoptotic pathways in cancer cells is to induce necrotic cell death. Here we show that selective induction of necrotic cell death can be achieved by activation of the DNA damage response pathways. While β -lapachone induces apoptosis through E2F1 checkpoint pathways, necrotic cell death can be selectively induced by β -lapachone in a variety of cancer cells. We found that β -lapachone, unlike DNA damaging chemotherapeutic agents, transiently activates PARP1, a main regulator of the DNA damage response pathway, both *in vitro* and *in vivo*. This occurs within minutes of exposure to β -lapachone, resulting in selective necrotic cell death. Inhibition of PAR blocked β -lapachone-induced necrosis. Furthermore, necrotic cell death induced by β -lapachone was significantly reduced in PARP1 knockout cell lines. Our data suggest that selective necrotic cell death can be induced through activation of DNA damage response pathways, supporting the idea of selective necrotic cell death as a therapeutic strategy to eliminate cancer.

Results

Figure 1

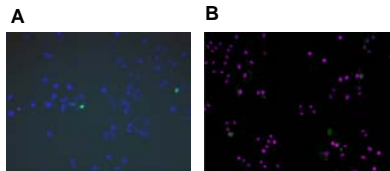


Figure 1. β -Lapachone induces cancer cell death by necrosis and apoptosis

Paca-2 cells in a 96-well plate (6,000/well) were treated with DMSO (A) as a control, or 3.7 μ M β -lapachone (B) for 4 hours before addition of 1:200 Annexin V-FITC (Green) and 1:500 Propidium iodide (Purple, final concentration of 1 μ g/mL). The labeling procedure was allowed to process at 37°C for 20min followed by image acquisition and analysis using IC100 Image Cytometer (Beckman Coulter, Inc.) with 10x amplification.

Figure 2

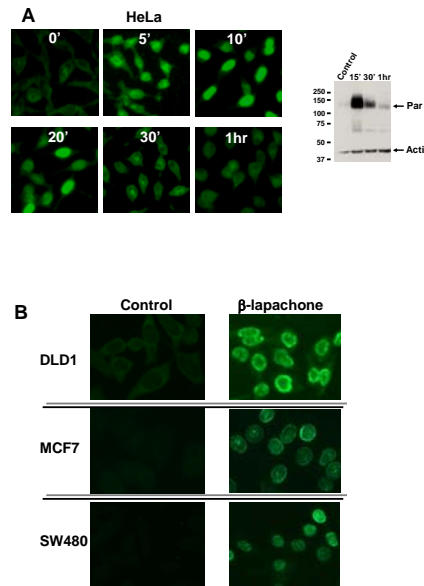


Figure 2. β -Lapachone induces rapid activation of PARP

(A) HeLa cells were grown on coverslips for 24h, then treated with 4 μ M β -lapachone at different time points and fixed with methanol acetone (70/30, v/v) for 10min. Samples were incubated in blocking buffer (5% FBS in PBS) for 10min at room temperature in a humid chamber. Cells were incubated overnight at 4°C with monoclonal anti-poly(ADP-ribose) antibody (10H 1:100). After washing, the cells were incubated for 1h at room temperature with FITC-conjugated anti-mouse antibody (1:1000). Immunofluorescence was evaluated using an immunofluorescence microscope equipped with a CCD camera. In the immunoblotting assay, cell lysates were made at various β -lapachone treatment time points, performed using an anti-PAR antibody. (B) β -Lapachone-induced PARP activity was determined in multiple cancer cells. DLD1, MCF7, and SW480 cells were treated with DMSO and β -lapachone for 10 minutes, followed by PAR immunofluorescence staining.

Figure 3

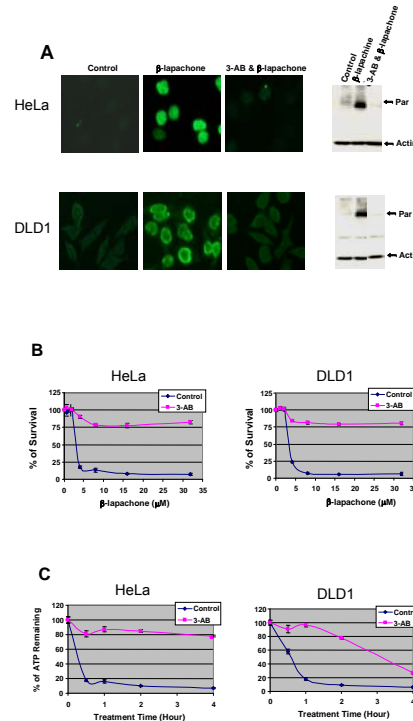


Figure 3. β -Lapachone-induced PARP activation is blocked by PARP inhibitor

(A) HeLa and DLD1 cells were grown on coverslips for 24h, pretreated with 5mM PARP inhibitor 3-AB or DMSO for 1h, then exposed to 4 μ M β -lapachone for 10min, followed by PAR immunofluorescence staining. In the immunoblotting assay, cell lysates were performed using an anti-PAR antibody. (B) β -Lapachone-induced cell death is blocked by PARP inhibitor. HeLa and DLD1 cells were plated in 96-well plates at 10,000 cells per well, cultured for 24h in complete growth medium, pre-treated with PARP inhibitor 3-AB (5mM) or equal volume of DMSO for 1h, and then exposed to β -lapachone at various concentrations for a further 4h, followed by MTT assay to measure cell death. (C) HeLa and DLD1 cells were incubated in the absence or presence of PARP inhibitor 3-AB and then treated with 4 μ M β -lapachone for 0 to 4 hours. The ATP level was measure by ATP assay.

Figure 4

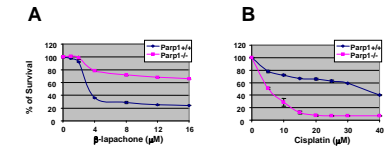


Figure 4. Effect of PARP1 on different chemotherapeutic reagents induced cell death

(A) β -Lapachone induces PARP1-mediated cell death. PARP1+/+ and PARP1-/- cells were treated various concentrations β -lapachone for 4 hours, followed by MTT assay. (B) PARP1 results in resistance to cisplatin-induced cell death. PARP1+/+ and PARP1-/- cells were treated with various concentrations cisplatin for 48 hours, followed by MTT assay to measure cell death.

Figure 5

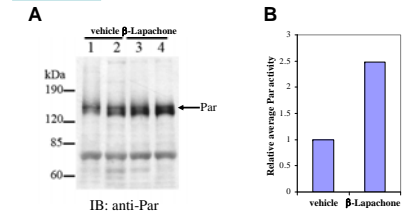


Figure 5. β -Lapachone induces PAR activity *in vivo*

Four Nu/Nu mice (Charles River Labs) with xenografted HT29 tumors were administered with either vehicle or 60mg/kg of β -lapachone by ip. Tumor tissues were harvested 30min after drug treatment and processed for PARP activity analysis by western blotting using an anti-PAR antibody.

Summary

1. β -Lapachone triggered selective necrotic cell death in cancer cells.
2. β -Lapachone caused rapid PARP activation in cancer cells.
3. PARP1 activation was necessary for selective necrotic cell death *in vitro*.
4. *In vivo*, PARP activation occurred after treatment of human xenograft tumors with β -Lapachone.
5. Induction of necrotic cell death was independent of p53 or other apoptotic pathway defects.

These data contribute to our understanding of the signaling mechanisms responsible for the acute and broad-spectrum cancer cell killing activity of β -lapachone—particularly in cells with apoptotic defects.