SUPPLEMENTARY MATERIAL

Histone deacetylase inhibitor enhances the efficacy of MEK inhibitor through NOXA-mediated MCL1 degradation in triple-negative and inflammatory breast cancer

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Materials and Methods

Co-immunoprecipitation

Cultured SUM190PT and SUM149PT cells were collected after described treatments, and were washed with pre-chilled PBS, followed by cell lysing with a cold non-denaturing lysis buffer (50 mM Tris-HCl, pH 7.4, 1% NP-40, 150 mM NaCl, 10% glycerol, 1.5 mM $MgCl_2$, 1 mM EDTA, and phosphatase and protease inhibitors). To immunoprecipitate MCL1 proteins, lysate (500 µg) was mixed with 5 µg MCL1 antibody (R&D Systems) for 2 hours at 4°C with rotation. Protein G Sepharose 4 Fast Flow beads (GE Healthcare, Marlborough, MA) were added to the antigen–antibody complex and incubated for 1 hour at 4°C with rotation. Beads were recovered by low-speed centrifugation and washed with lysis buffer. LDS sample buffer and sample reducing agent (Novex Life Technologies, NuPAGE®) were added to each sample and incubated at 70°C for 10 minutes, centrifuged, and subjected to SDS-PAGE. After appropriate separation, gels were transferred and analyzed for co-immunoprecipitation using standard immunoblotting techniques as described previously

TUNEL staining

DNA fragmentation in mouse tissue samples was measured by *in situ* terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) using the DeadEnd TUNEL kit (Promega, Madison, WI). The mouse tumor xenografts' TUNEL-positive cells were evaluated in 3 randomized fields at 20x magnification, and the average was expressed as the number of apoptotic cells for each sample.

Chromatin Immunoprecipitation

Acetylation levels of the *NOXA* gene promoter following entinostat treatment (1 μM, 48 hours) were measured by chromatin immunoprecipitation (ChIP) assay using the Simple ChIP Enzymatic Chromatin IP Kit (magnetic beads) following manufacturer's protocol (Cell Signaling Technology, Beverly, MA). Briefly, untreated and entinostat treated SUM149PT and SUM190PT cells ($4x10^6$ cells per sample) were nuclease digested and sonicated (3 cycles of 30 seconds) to shear DNA to approximately 150-900 bp in size. Sonicated chromatin samples were incubated with antibodies against acetylated-lysine, Histone H3 as positive control, or normal rabbit IgG as negative control (Cell Signaling Technology, Beverly, MA), overnight at 4°C with rotation. Two percent was used of total genomic DNA from nuclear extracts as input. Purified immunoprecipitation and input DNA were used as templates for RT-qPCR with the following primers to amplify the *NOXA* promoter region (Forward: 5'-AGTAATTTCGGGGCCGAGC-3' and Reverse: 5'-GGCGTTATGGGAGCGGAC-3'). Quantification of DNA by RT-qPCR was done following manufacturer's protocol.