Activation and Stabilization of p53 by Nutlin-3a Leads to Downregulation of HSP90 and Synergistic Effects with 17-AAG in Anaplastic Large Cell Lymphoma (ALCL)

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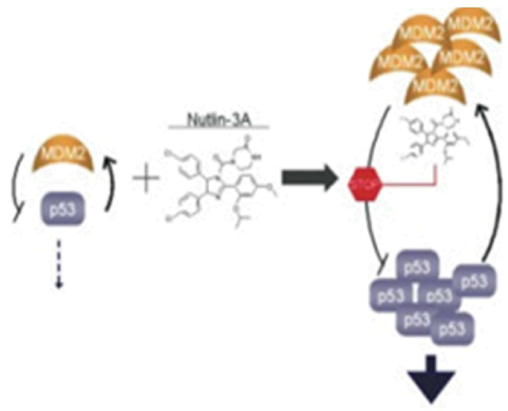
BACKGROUND

- p53 is the most frequently mutated tumor suppressor gene in human cancers (1). However, most lymphoma tumors harbor wild type (wt) p53 (2).
- Nutlin-3a is a recently discovered small molecule disrupting the interaction of p53 with its inhibitor MDM2, that results in stabilization of p53 & non-genotoxic activation of the p53 pathway (3,4).
- Our study analyzed the proteomic profile of anaplastic large cell lymphoma (ALCL) cells before & after stabilization & activation of wt p53 by nutlin-3a and investigated its possible synergy with HSP90 inhibitors.



METHODS

SUPM2, DEL, Karpas 299 & SU-DHL1 cell lines were grown, using Nutlin-3a for non-genotoxic stabilization & activation of p53. Cell viability & proliferation assays were assessed by MTS or colony formation assay, trypan blue exclusion assay, Annexin-V binding, fluorescence microscopy & DAPI staining. Expression levels of proteins were analyzed by Western blot analysis. Proteins were extracted, isotopically labeled (5), separated by SDS-PAGE, in-gel digested (6) & analyzed by mass spectrometry using nLC-MS/MS (Ion Trap). MS/MS data were processed by DataAnalysis & BioTools (Bruker Daltonics). Protein ID was found using Mascot search engine (Matrix Science Ltd) against the IPIhuman protein database, whilst Relative Quantitation along with further data evaluation were executed using Mascot Distiller.

RESULTS

Figure 1

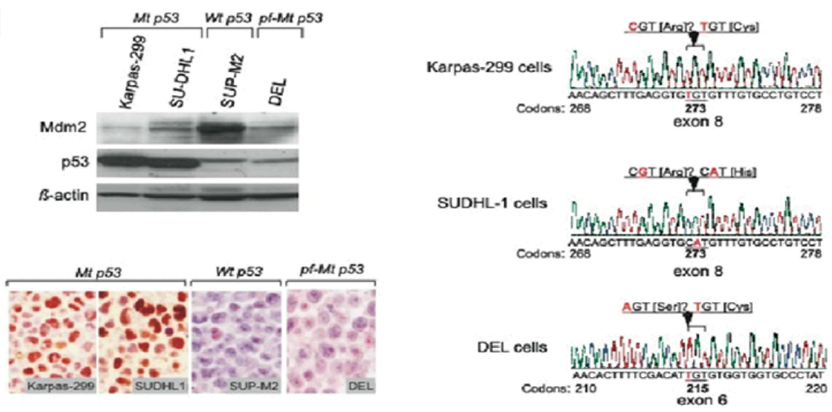


Figure 1: Our *in vitro* study system for ALK+ALCL included 4 cell lines. SUPM2 cells carry a wt p53-gene. DEL harbors a mutated but partially functional p53 gene, while Karpas 299 & SU-DHL1 cells carry a mutated & inactive p53 gene product. Mutations & expression of p53 protein in ALK+ALCL cell lines are shown.

Figure 2

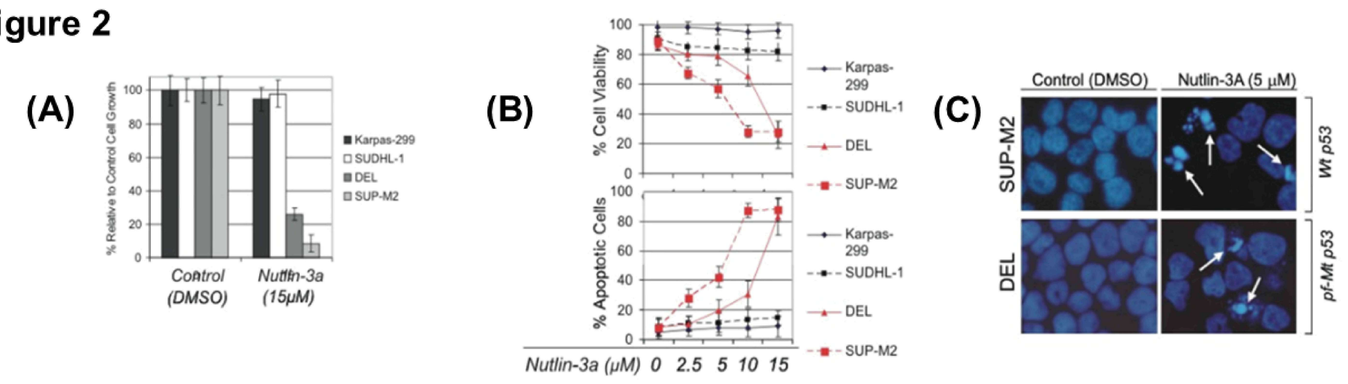


Figure 2: Activation of the p53-pathway in ALK+ALCL cell lines after treatment with Nutlin-3a. (A) Treatment with Nutlin-3a resulted in cell growth inhibition in SUP-M2 & DEL cells as shown by MTS assay. (B) Treatment with Nutlin-3a also leads to cell death assessed by trypan blue exclusion assay (upper panel) & apoptosis assessed by Annexin V staining & flow cytometry (lower panel). (C) DAPI staining & fluorescence microscopy show apoptotic cells.

Figure 3

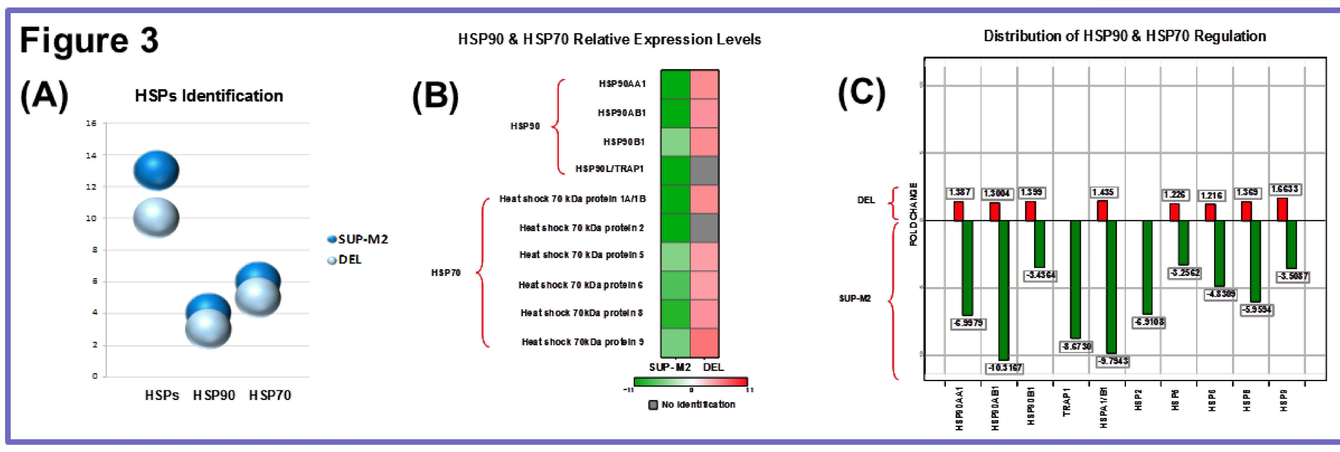


Figure 3: Differential proteomic analysis was applied in 2 ALK+ALCL cell lines, SUP-M2 and DEL, before & after Nutlin-3a treatment. (A) Schema of the HSPs, HSP90 & HSP70 identification in SUP-M2 & DEL cell lines. (B) Color representation of relative expression levels of HSP90 & HSP70 family members. Rows =HSPs, columns = cell lines. The color scale extends from bright red (max up-regulation) to bright green (max down-regulation). (C) The effect of nutlin-3A treatment induced *down-regulation* (green) in SUP-M2 & *no or marginal up-regulation* (red) in DEL cell line.

Figure 4

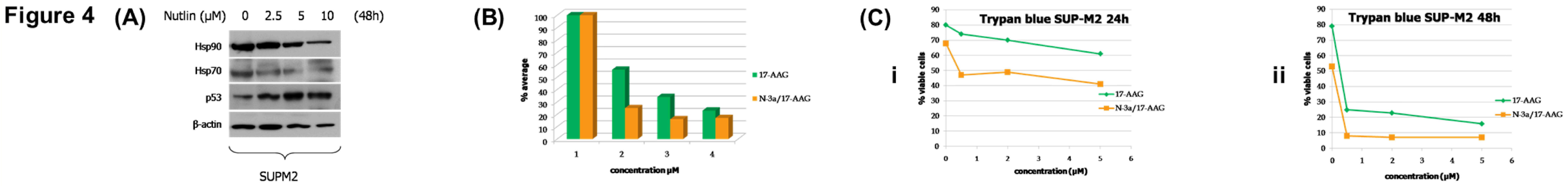


Figure 4: Down-regulation of HSP90 & synergistic effects with 17-AAG (A) Treatment with nutlin-3a resulted in down-regulation of HSP90 in SUP-M2 cells as shown by WB. (B) Growth of viable cells assessed by MTS assay after treatment with 17-AAG alone or combined with Nutlin-3a. (C i,ii) Cell viability assessed by trypan blue exclusion assay after treatment with 17-AAG alone or combined with Nultin-3a (24 & 48hrs).

CONCLUSIONS

- p53 activation by the MDM2 inhibitor Nutlin-3a, induces substantial apoptotic cell death of ALK+ ALCL cells carrying a wt, or a mutant but partially functional p53 gene.
- Mass Spectrometry-based proteomic analysis reveals that stabilization & activation of p53 by Nutlin-3a leads to down-regulation (SUP-M2) & no or marginal up-regulation of HSP70 & HSP90 (DEL) in ALK+ALCL cell lines, & these findings are confirmed by Western Blot analysis in our *in vitro* system.
- Combined treatment with Nutlin-3a & 17-AAG shows synergistic effects in p53-induced apoptotic cell death in ALK+ALCL cells harboring wt-p53 gene.
- Activation of p53 using MDM2 inhibitors such as Nutlin-3a combined with HSP inhibitors may be a novel targeted therapeutic approach for patients with ALK+ALCL.

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