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 that stabilizes and activates wt p53 in human cancers (1). However, most lymphoma tumors harbor wild-type (wt) p53 (3).
- Nutlin-3a is a recently discovered small molecule with a Ki value of 10 nM, and its activity is enhanced by a factor of 3.5 when incubated with MDM2 (4).
- Our study analyzed the proteomic profile of anaplastic large cell lymphoma (ALCL) cells before and 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, isolated, labeled, separated, and analyzed by mass spectrometry using LC-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 IPI human protein database, whilst Relative Quantitation along with further data evaluation were executed using Mascot Distiller.

RESULTS

- Treatment with Nutlin-3a resulted in cell growth inhibition in SUP-M2 & DEL cells as shown by MTS assay.
- Treatment with Nutlin-3a also leads to cell death assessed by the trypan blue exclusion assay (upper panel) & apoptosis assessed by Annexin V staining & flow cytometry (lower panel).
- DAPI staining & fluorescence microscopy show apoptotic cells.

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 (HSP90 & HSP70) in DEL cells, & these findings are confirmed by Western Blot analysis in vitro system.

REFERENCES