The Marine Natural Product Mimic MHP88 Shows Anticancer Activity and has the Potential to Cause Immunogenic Cell Death

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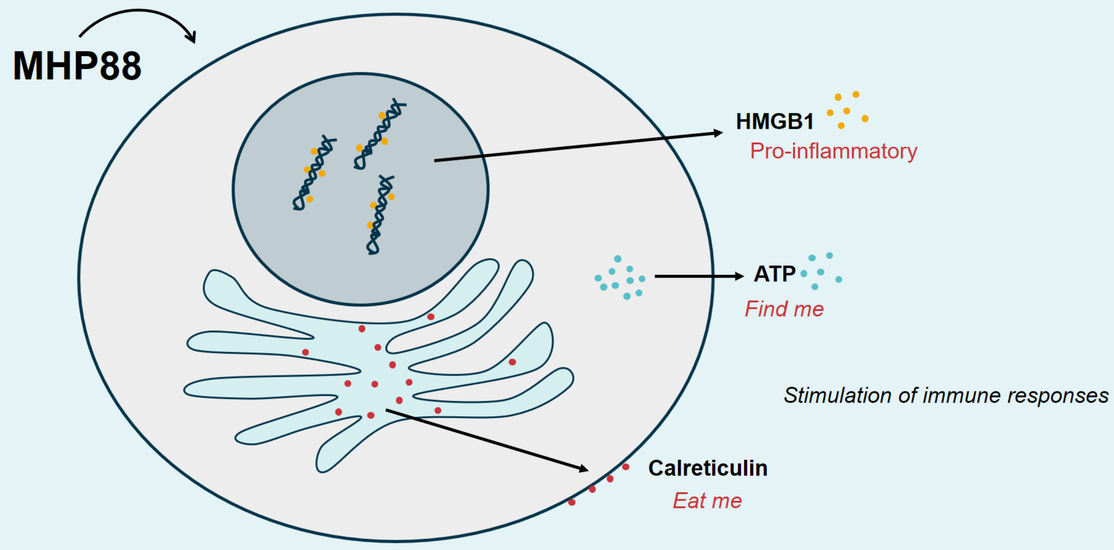
Background

The marine natural product mimic MHP88 is a novel synthetic molecule based on unique structures found in molecules from an arctic marine bryozoan (1, 2). Initial studies showed that MHP88 kills cancer cells efficiently, but is not hemolytic. In this study, we look closer at the mode of death induced by MHP88 in oral cancer (HSC-3) and lymphoma (Ramos) cell lines. Immunogenic cell death (ICD) is a mode of death that can be induced by some anticancer molecules and is characterized by the release and expression of certain damage-associated molecular patterns (DAMPs), which have immune stimulating effects. Specifically, the release of high mobility group box 1 (HMGB1) and ATP, as well as the translocation of calreticulin from the ER lumen to the outside of the cell membrane, constitute the major hallmarks of ICD (3). *In vivo*, ICD induced in cancer cells has a vaccination effect, protecting the host from future challenge with the same cancer cells. Animal studies on the molecule LTX-401, which has similar properties as MHP88, have successfully demonstrated this effect (4).



Hypothesis

MHP88 can induce immunogenic cell death

Research Question

Does MHP88 cause release and exposure of DAMPs related to ICD?

Screening of Anticancer Activity

Table 1 A colorimetric MTS assay was employed to determine the concentration of MHP88 needed to inhibit the growth of a panel of cell lines by 50% upon 4 hours of stimulation (IC_{50}^{4h}).

Cell Line	Type*	Site of Origin	IC_{50}^{4h} ± SD (µM)
B16F1	A	Murine Melanoma	13.72 ± 0.61
SK-N-AS	A	Neuroblastoma	15.94 ± 0.23
A375	A	Melanoma	14.52 ± 0.22
HEPG2	A	Hepatocellular Carcinoma	17.26 ± 2.50
HT-29	A	Colorectal Adenocarcinoma	15.68 ± 0.33
MCF-7	A	Breast Adenocarcinoma	14.06 ± 2.71
HSC-3	A	Oral Squamous Cell Carcinoma	8.53 ± 0.57
MRC-5	A	Non-Malignant Lung Fibroblast	18.54 ± 2.68
HUVEC	A	Non-Malignant Umbilical Endothelium	10.73 ± 3.63
GL261-Luc2	A	Glioblastoma	11.04 ± 2.88
Ramos	S	B Cell Lymphoma	7.53 ± 2.01
Jurkat	S	T Cell Leukemia	6.62 ± 1.60
PBMC	S	Non-Malignant White Blood Cells	4.13 ± 0.31

*A = adherent cells, S = suspension cells

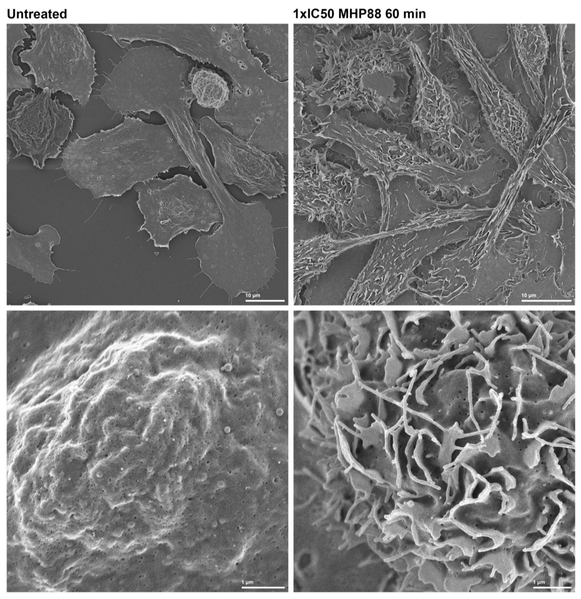


Figure 1 Scanning electron microscopy images of HSC-3 cells treated with MHP88 at the concentration corresponding to 1xIC_{50} show major ultrastructural changes, particularly on the cell membrane, after only one hour.

MHP88 Induces All Major Hallmarks of Immunogenic Cell Death

HMGB1

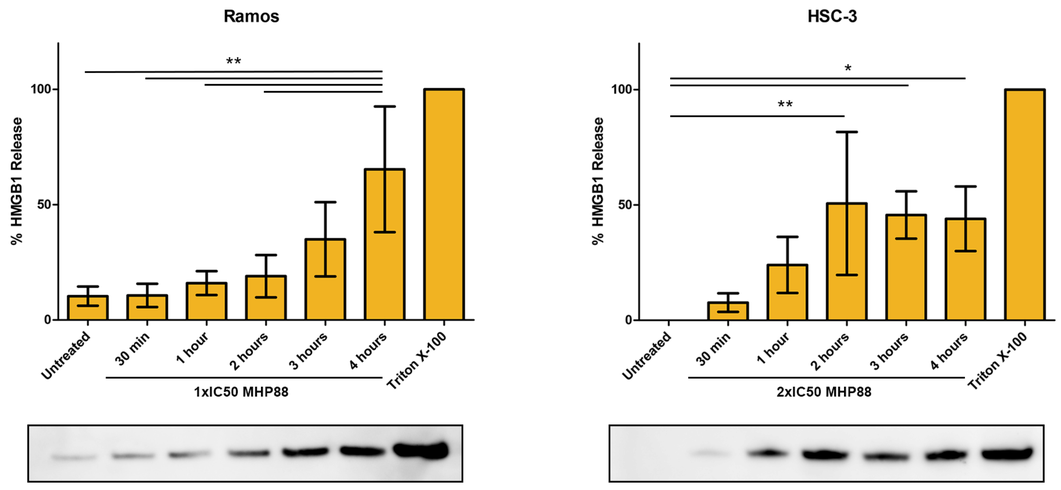


Figure 3 Release of HMGB1 from Ramos and HSC-3 cells treated with MHP88 was assessed by Western blotting. Cells treated with RPMI alone or with 1% Triton-X100 served as negative and positive control, respectively. Bars represent the mean of three independent experiments and error bars represent the standard deviation.

ATP

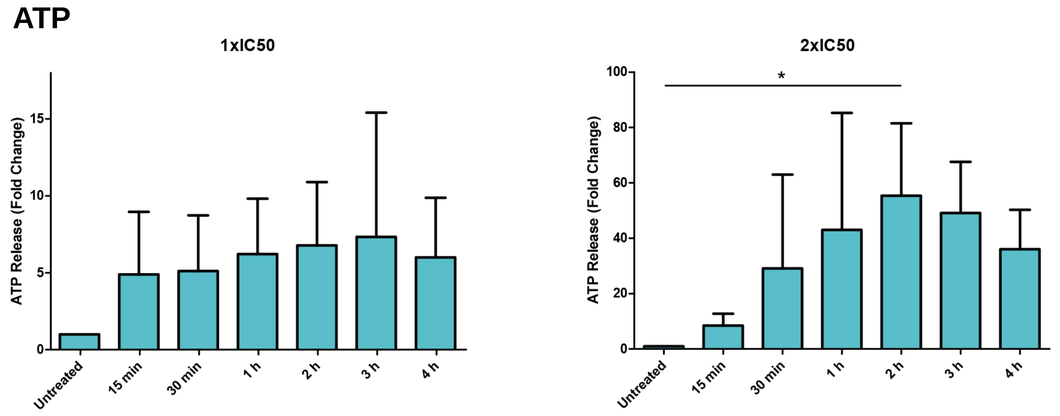


Figure 4 Release of ATP from HSC-3 cells treated with MHP88 was assessed by means of a luciferase based luminescence assay. Cells were treated with MHP88 in concentrations corresponding to 1xIC_{50} or 2xIC_{50} MHP88 for up to 4 hours and the medium was assessed for presence of ATP. Bars represent the mean of three independent experiments with triplicates of each sample. Error bars represent the standard deviation.

Calreticulin

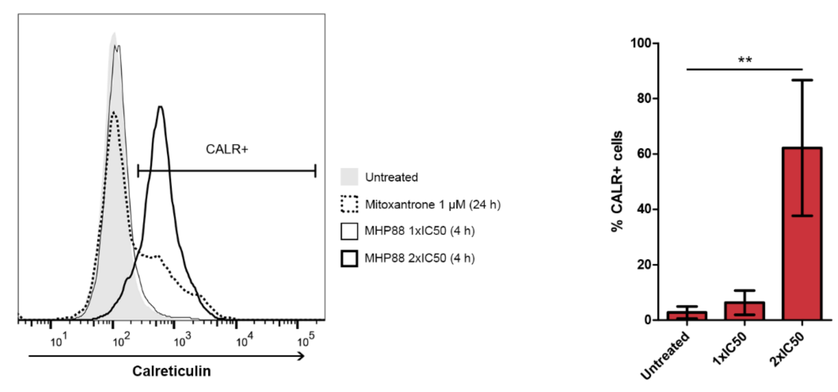


Figure 5 Translocation of calreticulin to the outside of the cell membrane of HSC-3 cells treated with MHP88 was assessed by flow cytometry. Cells were treated with with MHP88 in concentrations corresponding to 1xIC_{50} or 2xIC_{50} MHP88 for 4 hours and stained with a fluorescently labeled anti-calreticulin antibody and PI before analysis. Untreated and mitoxantrone treated cells served as negative and positive controls, respectively. The histogram shows calreticulin expression in live (PI negative) cells and the bar graph shows the mean percentage of calreticulin expressing cells in three separate experiments with error bars representing standard deviation.

MHP88 Induces Necrosis in Cancer Cells

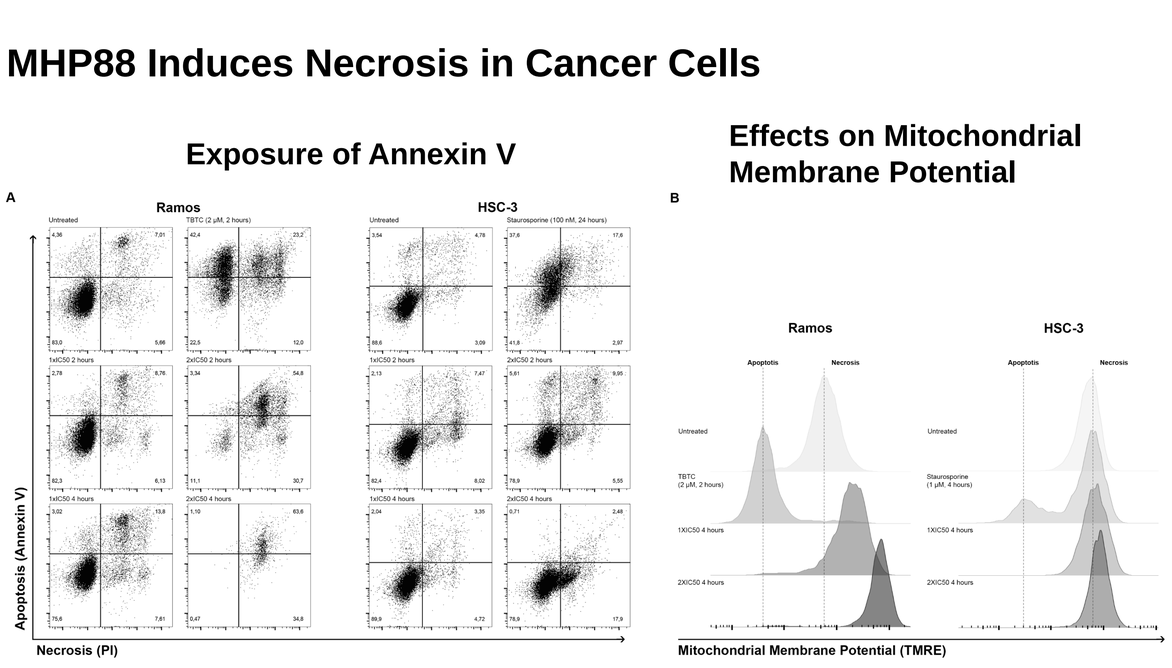


Figure 2 Flow cytometric analyses were used to determine the mode of death induced by MHP88. Ramos and HSC-3 cells were treated with MHP88 in concentrations corresponding to 1xIC_{50} or 2xIC_{50} MHP88 for up to 4 hours. Fluorescent staining was applied to indicate apoptosis (Annexin V), necrosis (PI) and collapse of mitochondrial membrane potential (TMRE). TBTC and Staurosporine were used as apoptosis controls for Ramos and HSC-3 cells, respectively. Both assays indicate that MHP88 induces necrosis.

Conclusion

Our studies indicate that the mode of death induced in cancer cells by MHP88 is of immunogenic nature and therefore might be able to turn cold tumors hot. This indicates that MHP88, as well as other marine natural product mimics, have the potential to be used as therapeutic agents in the future.

References

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