Association of Heat Shock Proteins as Chaperone for STING: A potential link in a key immune activation mechanism revealed by a novel anticancer agent PV-10

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BACKGROUND

- The activation of stimulator of interferon (IFN) genes (STING) is an intracellular receptor system located in the endoplasmic reticulum that has been shown to augment antitumor immunity through the induction of pro-inflammatory cytokines and chemokines.
- Several STING agonists have been developed and studied in preclinical investigations to treat refractory malignancies.
- Our previous preclinical studies have identified PV-10 (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein) as a novel therapeutic agent with potent activity following intra-tumoral injection in a spectrum of aggressive tumors.
- Here we describe a previously unidentified mechanism of STING activation by PV-10 which may facilitate sustained immune activation and therapeutic antitumor activity.

METHODS

- The well-established acute monocytic leukemia (AML) cell line THP-1 model was used to study STING activation *in vitro*.
- THP-1 cells were treated with increasing doses of PV-10 and the induction of STING was evaluated by western blot analysis using cGAMP as a positive control.
- The culture supernatants from PV-10 treated THP-1 cells were analyzed for the induction of a panel of 42 immune and inflammatory cytokines and chemokines using a Bio-Plex multiplex bead-based assay system.
- Specific antibodies were used to immuno-precipitate STING associated proteins in the presence of PV-10. STING associated protein upon PV-10 treatment were analyzed by mass spectrometry (LC-MS/MS) and results were analyzed using the Mascot database.

RESULTS

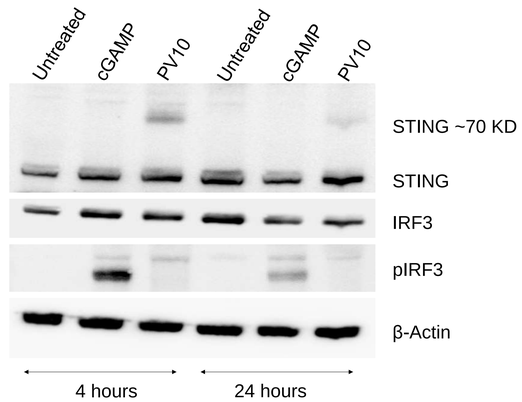


Figure 1. THP-1 cells were treated with cGAMP (activator of STING) [positive control] or 100 μM PV-10 for indicated duration. Western blot analysis show presence of STING ~70KD band in samples treated with PV-10. No phosphorylation of IRF3 was observed in these samples compared to cGAMP control.

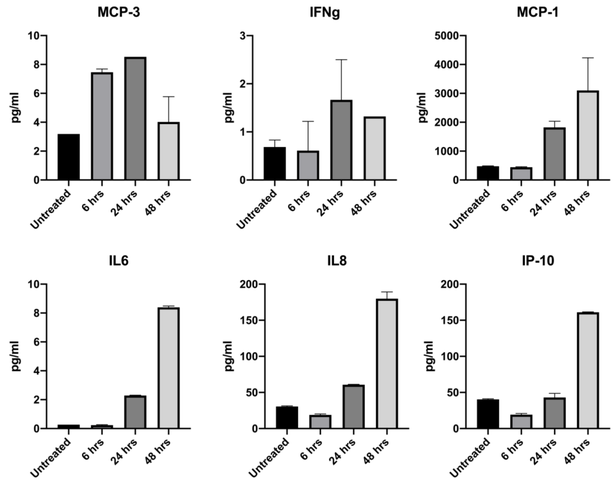
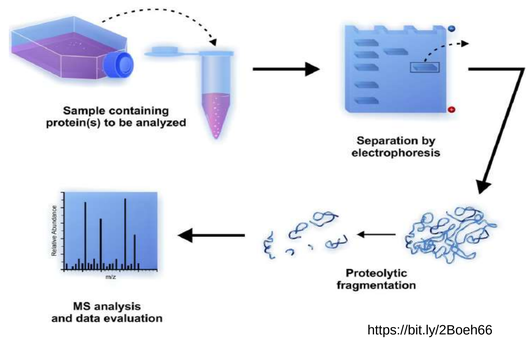


Figure 2. THP-1 cells were treated with 100 μM PV-10 and culture supernatant were collected at 6, 24 and 48 hours and analyzed for presence of various cytokines and chemokines on a 42-plex panel using a Bio-plex multiplex bead assay system. Results show an increase in IFN γ at 24 hrs. Upregulation of MCP-3, MCP-1, Il-6, IL-8 and IP-10 also noted at various time points.



Rank	Protein name
1	Heat Shock Protein 70
2	Polyadenylate binding protein 1
3	Stimulator of Interferon genes 1
4	Tubulin
5	Heat Shock Protein 60
8	Heat Shock Protein 90

Figure 3. STING (70 KD) bands were cut from protein gel of PV-10 treated THP-1 cells and subjected to proteolytic cleavage and LC-MS/MS analysis to identify proteins in the complex. The identified proteins include heat shock protein 70, 60 and 90.

CONCLUSIONS

- PV-10 treatment results in formation of a 70 KD STING complex which may result in immune activation and anti-cancer activity.
- Multiplex cytokine analysis of the media supernatant from THP-1 cells treated with PV-10 show activation of various cytokines involved in immune system activation and inflammation.
- Mass spectrometric analysis of the 70 KD STING complex confirmed the presence of STING along with HSP 70 and 60.
- This is the first experimental data, we are aware of , reporting the potential involvement of HSPs in STING mediated immune activation. 5-Future studies are needed to test the role of PV-10 as a single agent immunotherapeutic or in combination.

ACKNOWLEDGEMENTS

This research was funded in part by Provectus Biopharmaceuticals Inc., the Kids Cancer Care Foundation of Alberta (KCCFA), and the Alberta Children's Hospital Foundation.

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