B-08

Quantitative Proteomics Profiling of Silvestrol–treated Nasopharynx Cancer Cell Lines

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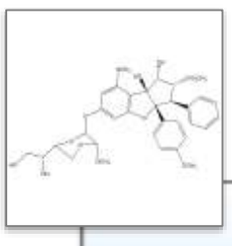
INTRODUCTION

NPC

- Nasopharyngeal carcinoma: 5th most common cancer among Malaysians; prevalent among natives of East Malaysia
- Treatment–resistance, treatment of recurrent NPC & distant metastasis remain challenging; whilst NPC patients have poor survival
- Need new treatment strategies



Silvestrol

- Synergized with cisplatin against NPC cells
- A protein synthesis inhibitor; targets translation initiation step
- Inhibits production of transcription factors and oncogenes; which had not been possible with other inhibitors.
- First–in–class able to target the proto–oncogene, *c–myc*.

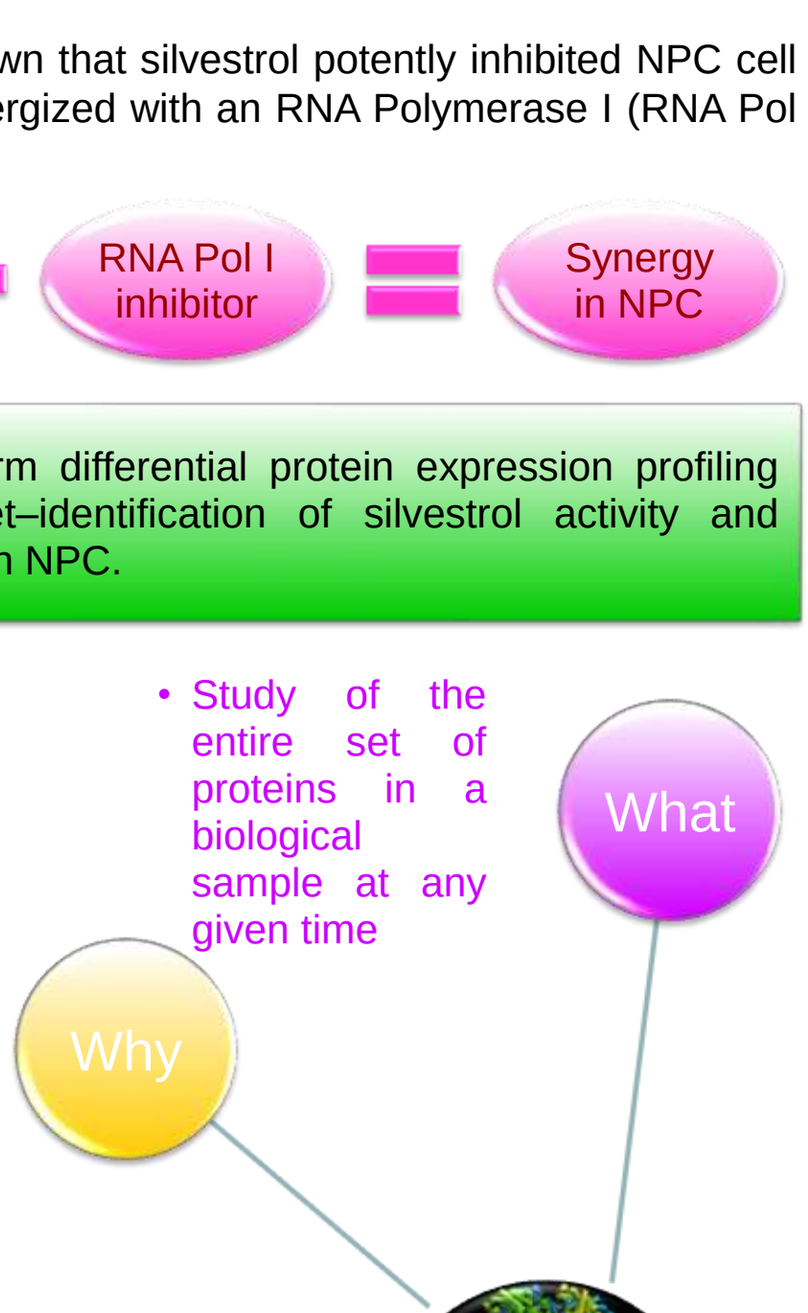
We had previously shown that silvestrol potently inhibited NPC cell lines and its effect synergized with an RNA Polymerase I (RNA Pol I) inhibitor.

Silvestrol + RNA Pol I inhibitor = Synergy in NPC

Objectives

To perform differential protein expression profiling for target–identification of silvestrol activity and efficacy in NPC.

- Comparative protein expression analysis on the effects of combinations of silvestrol & an RNA Pol I inhibitor in NPC
- Proteins are the actual functional molecules in cells & are direct drug targets
- Silvestrol + an RNA Pol I inhibitor (= **combination–treatment**)
- Analysis of: untreated (a) & combination–treated (b) cells
- Cell lines: NPC, C666–1 (i) & HK1 (ii); Non–malignant nasopharyngeal epithelial, NP69SV40T (iii)

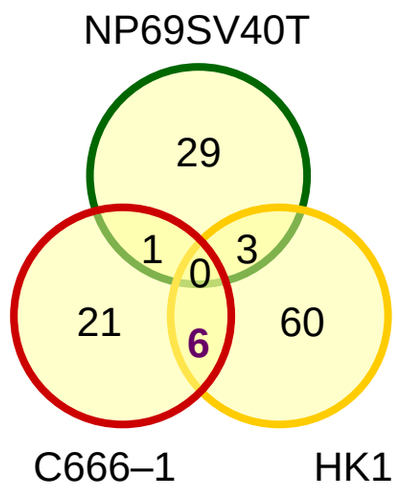


METHOD

Protein quanti–tation → Protein digestion → LC–MS/MS → Protein identifi–cation → Bio–informa–tics analysis

RESULTS

Combination–treatment downregulated 6 proteins in C666–1 & HK1 NPC cell lines, compared to NP69SV40T non–malignant nasopharyngeal epithelial cells (fold change < 0.625; p < 0.05).

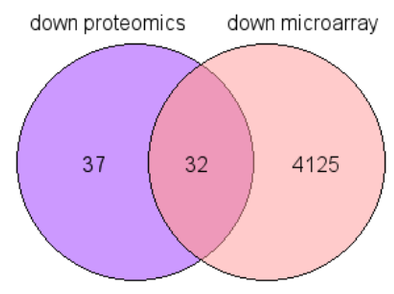


List of 6 proteins downregulated by combination–treatment in both NPC cell lines.

Gene symbol & Gene name
HNRNPH3: heterogeneous nuclear ribonucleoprotein H3
SPTAN1: spectrin alpha, non–erythrocytic 1
EIF3B: eukaryotic translation initiation factor 3 subunit B
EIF5A: eukaryotic translation initiation factor 5A
EIF5A2: eukaryotic translation initiation factor 5A2
EIF5AL1: eukaryotic translation initiation factor 5A–like 1

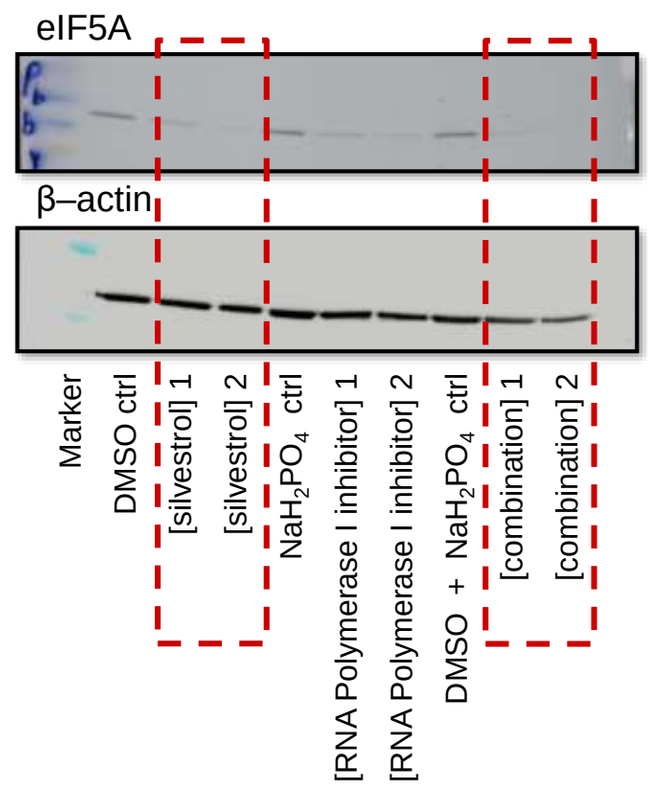
Proteomics & RNA microarray

RNA microarray investigation of HK1 cells unfolded 32 downregulated (fold change < 0.625; p < 0.05) entities mutually overlapping with the proteomics dataset of HK1.



Downregulation, inclusive of *EIF3B* and *EIF5A*, was detected in combination–treatment only, and not in single–agent treatment. Interaction between silvestrol at the translation level and an RNA Poly I inhibitor at the transcription level possibly explains the synergism observed in NPC cells.

Western Blot



Combination–treatment notably decreased eIF5A protein level in HK1 cells at 2 days post–treatment; corroborating the proteomics result.

A protein synthesis–promoting factor

Functions in translation elongation

eIF5A

Plays a role in cell cycle progression, cell growth and differentiation

Presently, no known drug approved or in clinical trials for eIF5A

EIF5A expression levels increased by more than 2–fold in all NPC tissue (without silvestrol–mediation) analysed by RNA sequencing, compared to non–NPC control. Stage IVC NPC had the highest *EIF5A* expression level contrasted to other stages.

Conclusion

Silvestrol + an RNA Polymerase I inhibitor combination downregulated eIF5A in NPC cell lines at the proteome and transcriptome level.

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