Gene Dysregulation Group Honours and PhD projects

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Project 1. Genome-wide analysis of novel long noncoding RNAs critical for tumorigenesis

INTRODUCTION

Neuroblastoma is the most common solid tumour in early childhood, and more than 60% of patients with advanced neuroblastoma die of the disease. Despite great efforts in neuroblastoma research in the last decades, the causes of neuroblastoma in 70% of patients are still unknown, because no genetic abnormality has been found in protein coding RNAs in whole genome analysis of human neuroblastoma tissue samples.

At least 90% of the human genome is transcribed to generate an extraordinary range of long non-protein-coding RNAs (lncRNAs). While protein-coding RNAs have been the main focus of cancer research in the last several decades, lncRNAs have most recently emerged as critical factors in tumour initiation, progression and metastasis.

AIMS

- To identify novel oncogenic lncRNAs which are over-expressed in human neuroblastoma tissues and novel tumour suppressor lncRNAs which are silenced in human neuroblastoma tissues
- To experimentally demonstrate that the oncogenic lncRNAs induce neuroblastoma cell proliferation and resistance to programmed cell death, and that the tumour suppressor lncRNAs block neuroblastoma cell proliferation and causes neuroblastoma cell death.

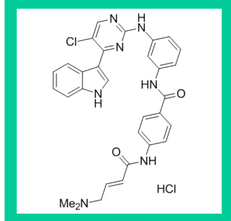
METHODS & TECHNIQUES

(1) Bioinformatics analysis of whole genome data from human neuroblastoma and normal control samples to identify lncRNAs which are aberrantly over-expressed or silenced in tumour tissues, compared with normal tissues.
(2) Design small interfering RNAs (siRNAs) specifically targeting the lncRNAs using siRNA design tools (siRNAs knock down the expression of target RNAs).
(3) Transfect neuroblastoma cells with the siRNAs. Real-time RT-PCR analysis to confirm that transfection with the siRNAs knocks down lncRNA expression.
(4) Cell proliferation and apoptosis assays to demonstrate that knocking down the expression of the lncRNAs modulates neuroblastoma cell proliferation and/or survival/apoptosis.
(5) Affymetrix microarray gene expression study to identify the target genes of the lncRNA.
(6) Real-time RT-PCR and immunoblot studies to confirm the microarray data.

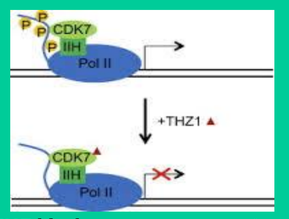
Project 2. Enhancing the anticancer efficacy of transcriptional super-enhancer inhibitors

INTRODUCTION

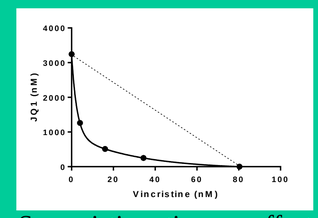
Myc and TERT oncoproteins are the most important oncoproteins in cancer patients. Transcriptional super-enhancers are selectively associated with oncogenes including MYC, MYCN and TERT. Transcriptional super-enhancer inhibitors are among the most promising novel anticancer agents, reduce cancer cell proliferation, induce programmed cell death and block tumour progression in mouse models of cancer. However, single therapy with super-enhancer inhibitors does not cause complete cancer remission.



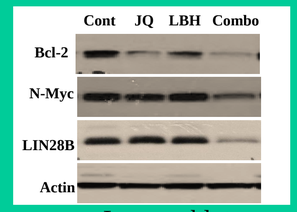
THZ



THZ blocks oncogene expression



Synergistic anticancer effect



Immunoblot

AIMS: To identify anticancer agents which exert the best synergistic anticancer effects with super-enhancer inhibitors, and to identify the mechanisms of action.

METHODS

1. Treatment of neuroblastoma cells with vehicle control, super-enhancer inhibitors, 121 anticancer agents, or combination of super-enhancer inhibitors and the 121 anticancer agents, followed by cell viability assays.
2. Combination index analyses to identify which anticancer agents exert the best synergistic anticancer effects with super-enhancer inhibitors.
3. Treatment of neuroblastoma cells with vehicle control, multiple doses of super-enhancer inhibitors, multiple doses of anticancer agents identified above, or combination of super-enhancer inhibitors and the anticancer agents.
4. Combination index analyses to identify which anticancer agents exert the best synergistic anticancer effects with super-enhancer inhibitors.
5. Flow cytometry analyses of neuroblastoma cell death after the cells are treated with vehicle control, super-enhancer inhibitors, the anticancer agent identified above, or combination of super-enhancer inhibitors and the anticancer agent identified above.
6. RNA sequencing, microarray, real-time RT-PCR and immunoblot analysis of oncogenes.

TECHNIQUES: Cell culture, cell viability assays, RNA extraction for sequencing or microarray, protein extraction, real-time RT-PCR, immunoblot, flow cytometry analysis of cell death, drug synergy analysis, microarray or RNA sequencing data analysis.