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Functions of TRPM4 through Bioinformatics Analyses and the Cytotoxic Effects of its Inhibitor 9-phenanthrol in Diffuse Large B-cell Lymphoma

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Abstract— Transient receptor potential cation channel subfamily M member 4 (TRPM4) is overexpressed in activated B-cell-like subtype of diffuse large B-cell lymphoma (ABC-DLBCL) associated with poor survival. In this study, its functions in the disease and the potency of its inhibitor 9-phenanthrol were investigated. The biological functions associated with *TRPM4* mRNA expression were examined through Gene Set Enrichment Analysis (GSEA) in ABC-DLBCL cases (n=15). The cytotoxicity of 9-phenanthrol in three ABC-DLBCL cell lines (SUDHL2, OCI-LY3, OCI-LY10) was tested at six different concentrations (0.01nM, 0.1nM, 1nM, 10nM, 25nM, 50nM). GSEA results showed that cell cycle gene sets conferred the highest number of gene sets representing 42% (n=21/50) of the top 50 most significantly enriched gene sets ranked according to false discovery rate (FDR; all 50 gene sets had FDR<0.01), followed by DNA replication (n=8/50; 16%) and RNA processing (n=8/50; 16%), suggesting the roles of TRPM4 in cell cycle progression and cellular division of ABC-DLBCL. In terms of the cytotoxicity effects of 9-phenanthrol, the resulting GI_{50} for all ABC-DLBCL cell lines ranged from 19nM-41.88nM. In conclusion, TRPM4 is potentially involved in the cell cycle progression and cellular division of ABC-DLBCL cells, and the TRPM4 inhibitor 9-phenanthrol was cytotoxic against ABC-DLBCL cells.

Key words: Cytotoxicity, diffuse large B-cell lymphoma, Gene Set Enrichment Analysis, TRPM4.

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Overview of Presentation

- Introduction (Lymphomas, TRPM4)
- Objectives & Results
- Conclusions & Limitations

Introduction to Lymphoma

- **Lymphoma:** tumour of white blood cells arising from lymph node, tonsil, thymus, bone marrow etc

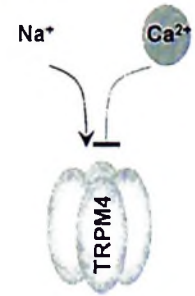
- Divided into two major groups: **Non-Hodgkin (NHL) and Hodgkin lymphoma (HL)**

- NHL is divided into **more than 30 subtypes** derived from B cell, T cell or NK cell

- **Diffuse large B-cell lymphoma (DLBCL)** is the most common NHL subtype, and an aggressive form of malignancy

TRPM4

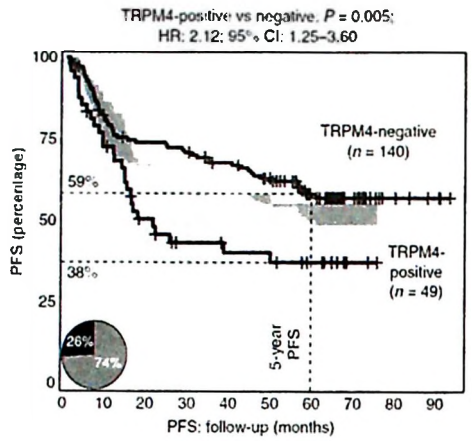
- TRPM4 is a Ca^{2+}-activated, Ca^{2+}-impermeable cation channel that mediates transport of **monovalent cations**

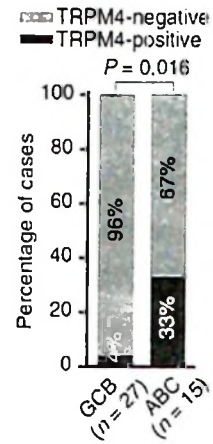


- It has been shown to be upregulated at the mRNA level in DLBCL
- In our previous study (Loo et al. *Histopathology* 2017), we showed its **upregulated protein expression in DLBCL, particularly ABC-DLBCL,** for the first time
- Its **function in ABC-DLBCL is unknown**

TRPM4 with Survival and DLBCL Subtype

- TRPM4 confers **poorer prognosis** and also expressed in the **poor prognosis ABC-DLBCL** subtype





Objectives

- To elucidate the **potential functions of TRPM4 through Gene Set Enrichment Analysis (GSEA)** of ABC-DLBCL cases
  - ❖ *i.e.* GSEA of TRPM4-positive cases vs TRPM4-negative cases to obtain the **gene sets differentially represented in these two population** of cases

- To **determine the GI50 (50% growth inhibition) of 9-phenanthrol**, inhibitor of TRPM4, in ABC-DLBCL cells

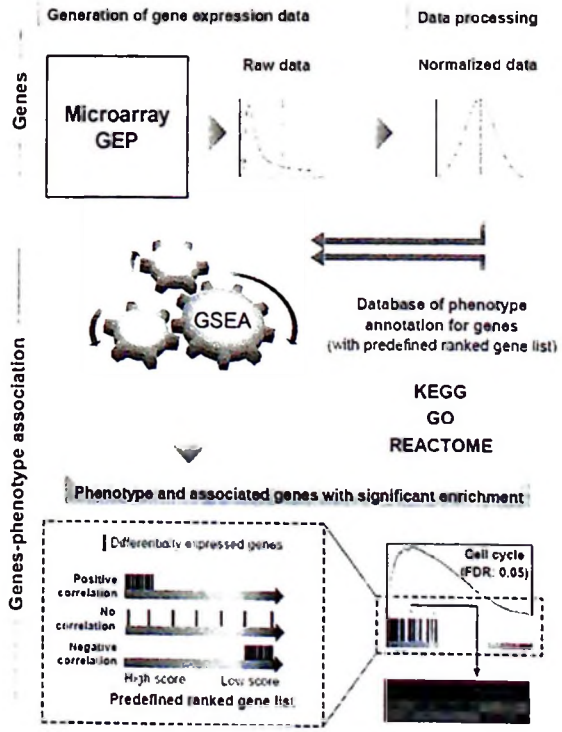
Gene Set Enrichment Analysis (GSEA)

- GSEA is a bioinformatics method to **identify the biological pathways most significantly upregulated in one population vs another** from GEP (e.g. microarrays) data

GSEA
Gene Set Enrichment Analysis

- GSEA is available from the Broad Institute database: http://software.broadinstitute.org/gsea/index.jsp

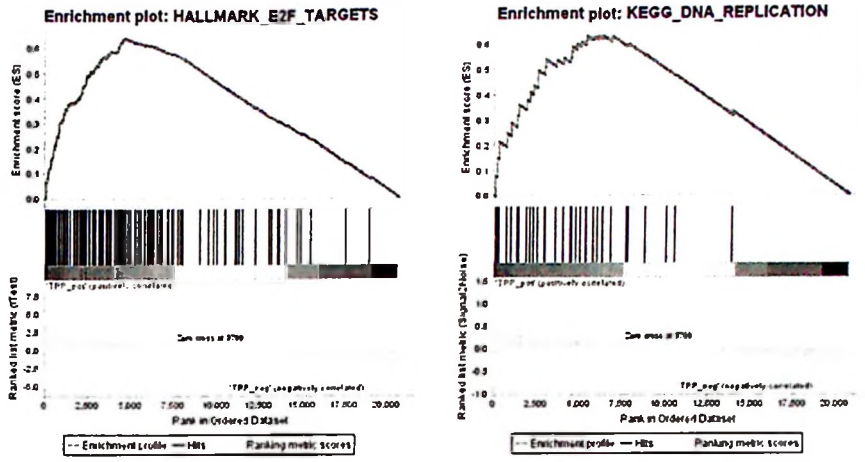
- From microarrays data, **GSEA ranks biological pathways most significantly upregulated** (or downregulated) based on **False-discovery Rate (FDR)**

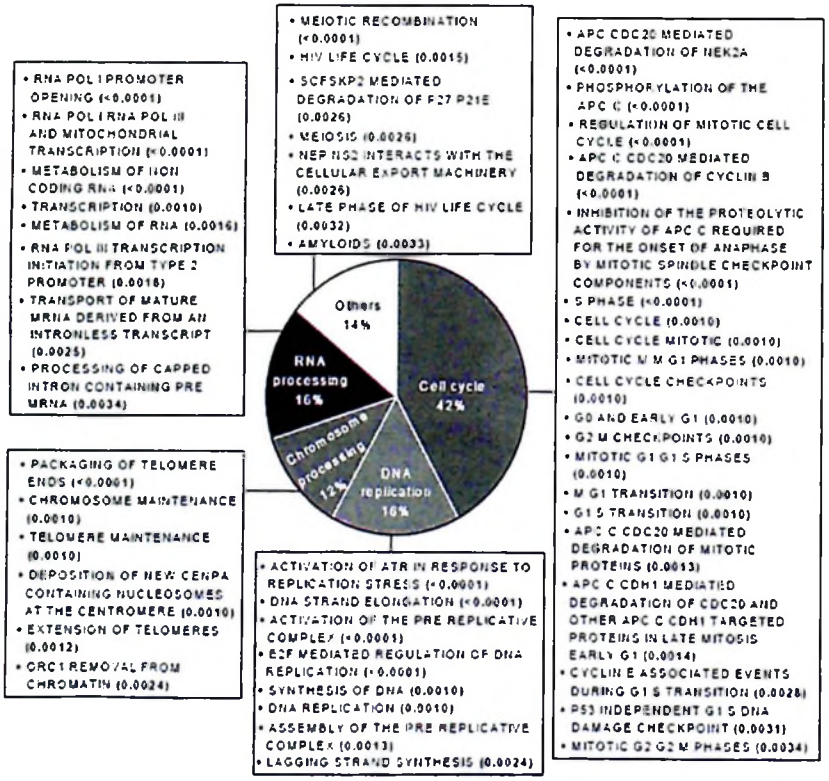


Gene Sets Associated with TRPM4

- Of our previously **15 ABC-DLBCL cases profiled by microarrays**, 5 cases were TRPM4-positive
- Thus, the potential **biological functions of TRPM4** were assessed through **GSEA** on the microarrays data of **TRPM4-positive versus TRPM4-negative** ABC-DLBCL cases
- **Cell cycle gene sets** conferred the **highest number of gene sets representing 42% (n=21/50) of the top 50 most significantly enriched gene sets** ranked according to false discovery rate (FDR; all 50 gene sets had FDR<0.01)

Gene Sets Associated with TRPM4 in ABC-DLBCL





Gene Sets Associated with TRPM4 in ABC-DLBCL

- Other gene sets include:
  - DNA replication (n=8/50; 16%)
  - RNA processing (n=8/50; 16%)
  - Chromosome processing (n=6/50; 12%) and other gene sets (n=7/50; 14%)

- Suggesting the **roles of TRPM4 in cell cycle progression and cellular division** of ABC-DLBCL.

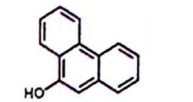
TRPM4 Inhibitor 9-phenanthrol

- Several TRPM4 inhibitors have been identified but **none could specifically inhibit TRPM4** including flufenamic acid (Ullrich et al., 2005; Guinamard et al., 2013), quinine, quinidine (White, 2007; Talavera et al., 2008), glibenclamide (Demion et al., 2007; Alexander et al., 2013; Woo et al., 2013b) and MPB-104 (Grand et al., 2008)
- **9-phenanthrol** is thought to be the most specific inhibitor of TRPM4 identified which is ineffective on TRPM5, the closest relative of TRPM4 (Grand et al., 2008)
- The backbone structure of 9-phenanthrol is **phenanthrene *i.e.* three fused benzene rings**

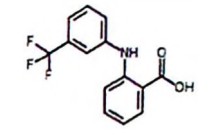
TRPM4 Inhibitor 9-phenanthrol

- Its specificity potentiates its usage in many TRPM4-related studies as **a specific TRPM4 inhibitor** (Caceres et al., 2015; Sarmiento et al., 2015; Kurland et al., 2016)
- 9-phenanthrol **inhibits TRPM4 channel from both sides of the channel**, either from part of channel exposed on the cell surface or part of channel exposed in the cytosol (Grand et al., 2008; Woo et al., 2013a; Guinamard et al., 2014).

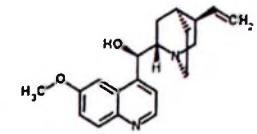
9-phenanthrol



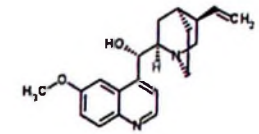
Flufenamic acid



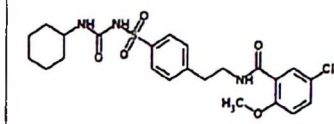
Quinine



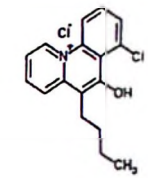
Quinidine



Glibenclamide

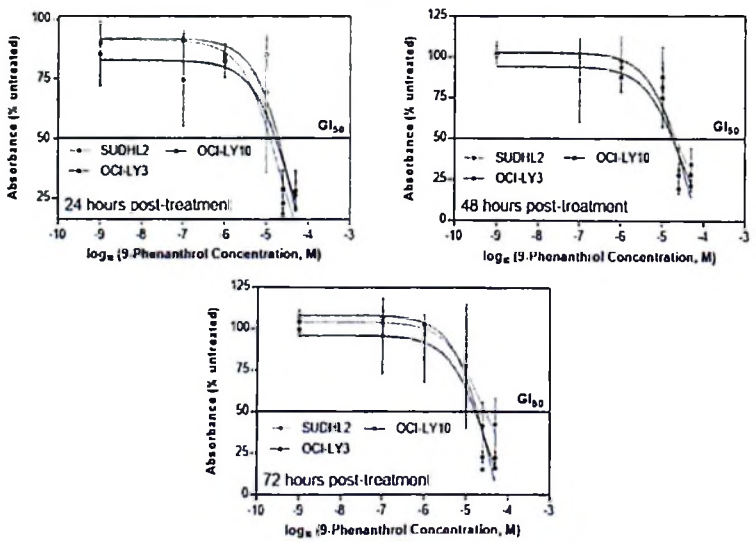


MPB-104



9-phenanthrol Cytotoxicity in ABC-DLBCL Cells

- MTS assay was performed at 24h, 48h & 72h for 3 ABC-DLBCL cell lines, 6 concentrations, triplicate & 3 independent experiments, to obtain **9-phenanthrol's GI_{50} (50% growth inhibition)**



GI_{50} of 9-phenanthrol in ABC-DLBCL cells

- **Cell death was apparent at 24h** where the **GI_{50} ranged 19-41.88 nM** in all three ACB-DLBCL cell lines
- Similar GI_{50} values were obtained for 48h and 72h

Cell line	24 hours (GI_{50} in nm)	48 hours (GI_{50} in nm)	72 hours (GI_{50} in nm)	Average GI_{50} (nm)
SUDHL2	19.00	23.34	22.40	21.58
OCI-LY3	41.88	35.31	36.17	37.79
OCI-LY10	32.45	26.17	28.48	29.03

Conclusions & Limitations

Conclusions:

- TRPM4 is potentially involved in the **cell cycle progression and cellular division** of **ABC-DLBCL cells**
- The TRPM4 inhibitor **9-phenanthrol was potent against the viability of ABC-DLBCL** cells at nanomolar concentrations *in vitro*

Limitations:

- Requirement of TRPM4 for DLBCL survival needs to be elucidated through **knockdown experiments**
- ***In vivo* experiments** required for 9-phenanthrol against ABC-DLBCL xenografts

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