Don’t count your hedgehogs before they’ve Patched:

Hedgehog signalling in tumorigenesis

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Abstract

Hedgehog signalling is involved in embryonic development and cell proliferation. Unsurprisingly, it’s dysregulation has been **implicated in cancer**. This investigation aimed to elucidate **functions of non-canonical hedgehog signalling** through examining the effects of **PTCH-1 truncation** in colorectal cancer cells, specifically on long non-coding RNAs (lncRNA). RNA sequencing identified 26 lncRNAs, many previously implicated in cancer. The effects on the predicted lncRNA targets however indicated an anti-cancer effect. Characterising hedgehog signalling is key for understanding how it can contribute to tumorigenesis.

Introduction

- Hedgehog (Hh) signalling is a paracrine signal transduction pathway in **embryonic development**, remaining active in progenitor cells to **regulate proliferation**[1].
- Canonical Hh signalling refers to initiation of GLI transcription through Hh mediated PTCH inhibition and Smo activation (figure 1).
- Non-canonical Hh signalling uses only some components of the canonical pathway:
  - Type 1 routes function through PTCH independently of Smo.
  - Type 2 routes require Smo but not the GLI transcription factors [3].
- Type 1 pathways regulate apoptosis and cell cycle progression via actions of the PTCH-1 C-terminal domain (CTD)[3].
- Using CRISPR-Cas9 technology, the PTCH-1 CTD was truncated, inhibiting all actions of the CTD in **colorectal adenocarcinoma** cells.
- 2 clones, 9 and 15, were analysed through RNA sequencing.
- The **aim was to identify roles of non-canonical Hh signalling** through examining the effects of the PTCH-1 truncation on the global transcriptome, specifically focussing on **lncRNAs.**

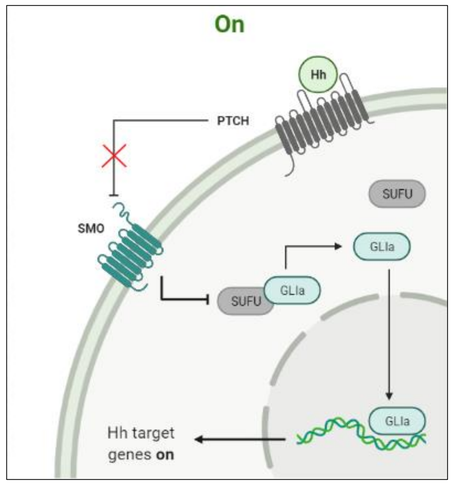


Figure 1: Canonical Hedgehog signalling [2]

Methods

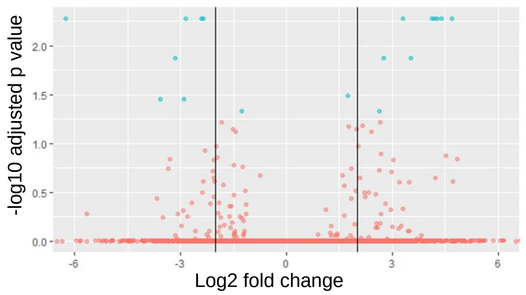
- Statistical analysis was completed on the **RNA sequencing** data. LncRNAs identified as differentially expressed (DE) in both clones 9 and 15 were selected.
- A literature search for data on the DE lncRNAs was conducted to **identify experimentally verified roles and targets.**
- Gene ontology enrichment was completed using the identified targets.
- Expression levels of the predicted targets were checked against the RNA sequencing data.

Results

Distribution of lncRNA expression

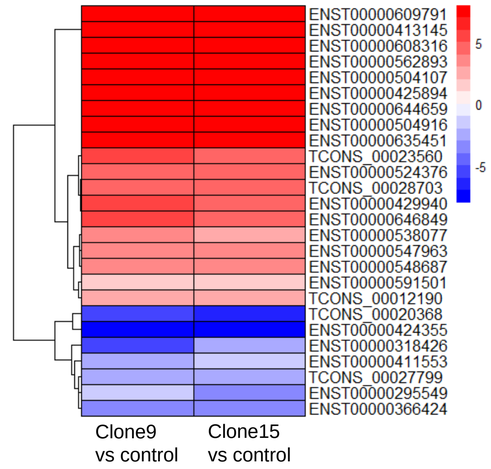
Figure 2A: Volcano plot clone9 vs control

Figure 2B: Volcano plot clone15 vs control



Comparison of the shared differentially expressed lncRNAs of clones 9 and 15

Figure 3: Heatmap - Log2 fold changes of DE lncRNAs in clone9 vs clone15



Roles & target predictions of the differentially expressed lncRNAs

Figure 4A: LncRNA expression in clones9/15 compared to other cancer types

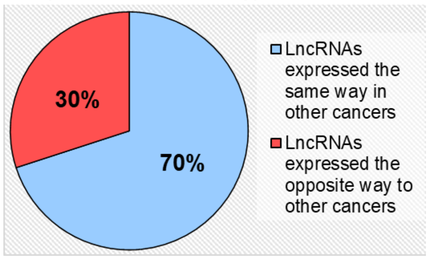
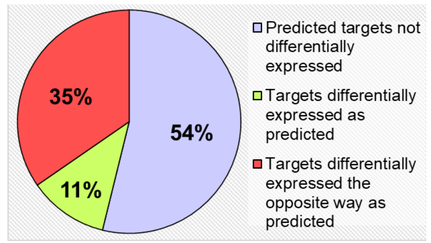


Figure 4B: Expression of the predicted downstream lncRNA targets



- Fewer lncRNAs were found to be differentially expressed in clone15, likely due to better accuracy (figure 2a/b).
- The 26 differentially expressed lncRNAs identified were similarly affected in both clones (figure 3).
- The altered expression of the lncRNAs **coincided well with research of other cancers**, indicating a **pro-tumorigenic** effect.
- The predicted lncRNA targets however, determined through research literature, showed **contradictory results**.
- Most of the targets were not significantly affected at all or **affected in an alternative manner than hypothesised** i.e. upregulated when predicted to be downregulated (figure 4a/b).

Discussion & Conclusions

- The DE lncRNAs indicated the PTCH-1 truncation produced a pro-tumorigenic effect, however the expression of the predicted lncRNA targets strongly suggested the **predictions were inaccurate**.
- The lncRNA roles/ target predictions were based on published research, which was often limited to only 1-2 cell types.
- The **lncRNAs roles evidently vary with cell type and context**.
- In summary**, t**he **precise lncRNA roles in colorectal cells need defining** to reliably determine PTCH-1 truncation effects.
- Combining this data with the differentially expressed coding RNA transcripts will assist in clarifying the ‘bigger picture’ effects of PTCH-1 truncation, and thus the roles of the PTCH-1 CTD.

References & acknowledgements

References

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3.Robbins, D.J., Fei, D.L. and Riobo, N.A. 2012. The Hedgehog Signal Transduction Network. *Science Signaling.* **5**(246), pp.re6-re6.

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