Differential Module Analysis in Neuroblastoma Regulatory Networks

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Background and aim

Neuroblastoma (NB) is a tumour of the sympathetic nervous system, which arises from the undifferentiated precursor cells of the neural crest. This tumour afflicts children at an early age and is characterised by its clinical heterogeneity which varies from spontaneous regression to an aggressive phenotype and death. At least 40% of all children with NB are confronted with an advanced stage of this disease [1]. The survival rates of a child with high risk NB is estimated at 50%, despite the intensive and multimodal treatments [2].
The chromosomal characterisation of NB with arrayCGH has resulted in significant contributions in constructing a classification model. This model consists of three genomic subtypes [3]: low risk subtype 1 and high risk subtypes 2A and 2B. The analysis gave a detailed insight into the recurring chromosomal abnormalities, as shown in Figure 1.

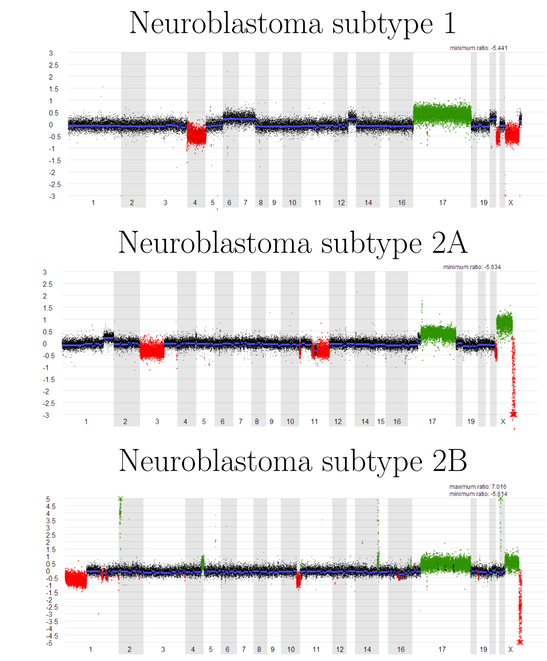

Figure 1: Overview of the chromosomal abnormalities for neuroblastoma subtypes 1, 2A and 2B.

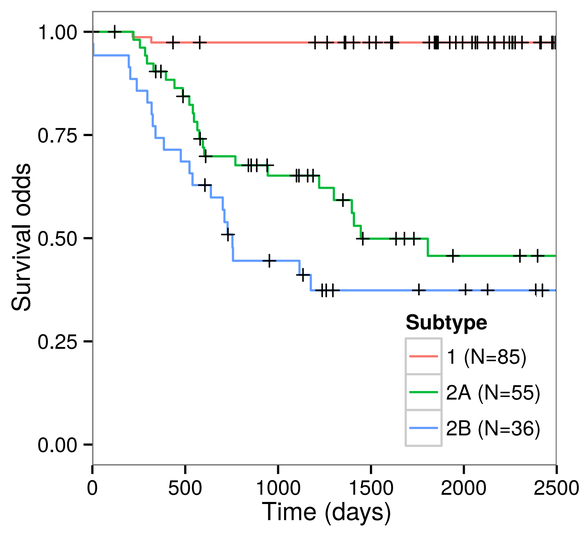

Figure 2: Survival plot of 176 neuroblastoma patients.

The MYCN oncogene is highly amplified in NB subtype 2B tumors, in combination with a high MYCN overexpression. The identification of MYCN as driver gene for NB subtype 2B tumors led to the identification of some of the most abberated interactions [4]. Currently, there are no known drivers for NB subtype 2A.

Aim

To develop a method based on differential networks (DN) designed for unravelling the biological processes which lead to tumour growth in specific NB subtypes.

Differential networks aim to compare groups of patients – or more generally, organisms – by construction interaction networks for each of the groups. A subnetwork which is very characteristic and specific to one network in comparison to the other networks, might be causal for the formation of tumours of that subtype. Such condition-specific subnetworks might contain interesting targets for the development of more personalised drug treatments. An example of a simple DN method is shown in Figure 3.

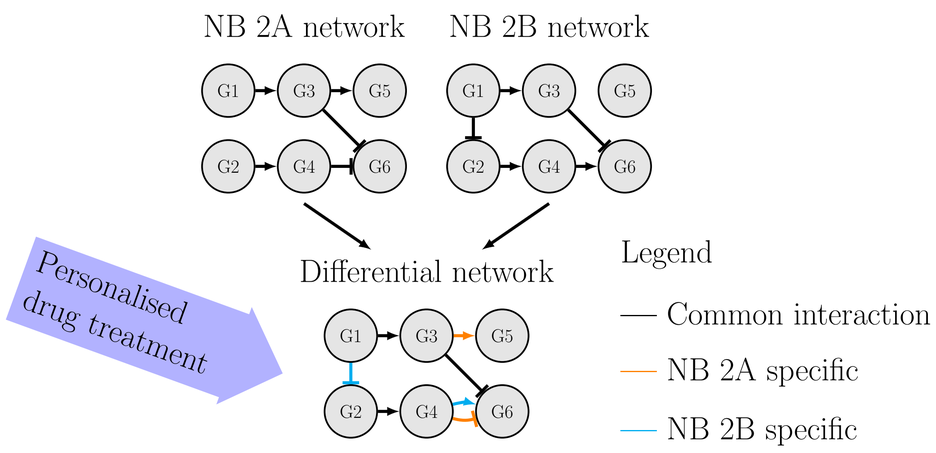

Figure 3: An example of a differential network method.

Method

An outline of the methodology is shown in Figure 4. We used expression data from the Neuroblastoma Research Consortium (NRC, a collaboration between several European NB research groups) in order to infer two regulatory networks, one for NB 2A patients and one for NB 2B patients. We inferred the networks using a variant of the GENIE3 [5] algorithm. By grouping together strongly connected genes using MCL [6], we created a higher-order structure in the graph, which we call a module, each representing a biological mechanism. We gave each module in each of the networks a differentiality score. A score of 0 means this module is not differential at all; that there is a module in the other network which is exactly the same. A score of 1 means this module is very differential; there is no module in the other network which is anything like the current module.

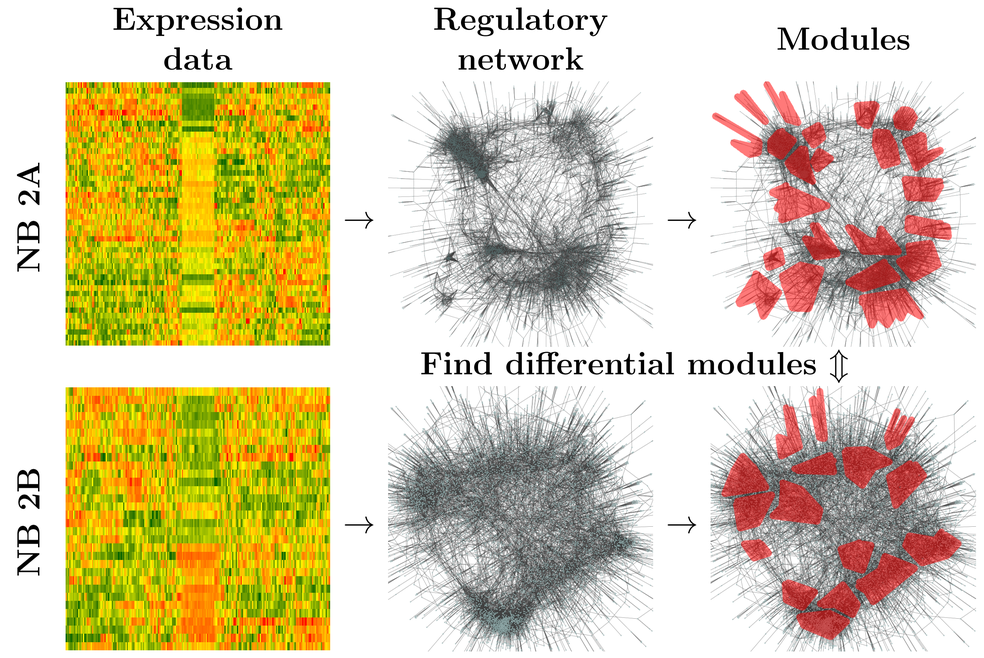

Figure 4: The concept of differential module analysis for NB subtypes 2A and 2B.

Results

For both networks, we selected the top 3 *most* differential modules, and the top 3 *least* differential modules. We performed an Gene Ontology enrichment analysis on each of the modules, listed for each module the top 4 GO enrichments, and found that most modules were enriched for biological processes related to oncogenesis, neural development or the process of growth. This is not unexpected, as the samples were taken from neural tissue of children with high risk NB.
There were two specific enrichments of interest. Firstly, the Wnt/β-catenin program has previously been reported to be deregulated in high-risk NB without MYCN amplification [7], which matches the NB subtype 2A module in which this enrichment was found. Secondly, Trk receptors were also previously reported as a prognostic marker for NB. A high expression of the TrkA neutrophin receptor is associated with a good prognosis, while a high expression of the TrkB neutrophin receptor usually occurs in high risk NB [8].

NB 2A specific (highly differential) module 1
- reg. of morphogenesis of a branching structure (p=2.8e-5)
- reg. of organ morphogenesis (p=6.4e-4)
- bone mineralization (p=1.9e-3)
- **positive reg. of canonical Wnt signaling pathway (p=4.8e-3)**

NB 2A specific (highly differential) module 2
- metal ion transport (p=1.8e-3)
- cation transport (p=6.1e-3)
- in utero embryonic development (p=7.5e-3)
- negative reg. of intestinal cholesterol absorption (p=8.3e-3)

NB 2A specific (highly differential) module 3
- protein activation cascade (p=4.8e-10)
- **wound healing (p=1.4e-5)**
- **coagulation (p=1.7e-5)**
- **blood coagulation (p=1.7e-5)**

NB 2B specific (highly differential) module 1
- cilium morphogenesis (p=5.2e-4)
- mannose biosynthetic process (p=9.5e-3)
- rRNA 5'-end processing (p=9.5e-3)
- septin cytoskeleton organization (p=9.5e-3)

NB 2B specific (highly differential) module 2
- sperm motility (p=1.9e-3)
- hexose biosynthetic process (p=3.4e-3)
- **monosaccharide biosynthetic process (p=4.2e-3)**
- hexitol catabolic process (p=5.5e-3)

NB 2B specific (highly differential) module 3
- fibroblast growth factor receptor signaling pathway (p=1.4e-2)
- transcription initiation from RNA polymerase III promoter (p=1.5e-2)
- cellular response to fibroblast growth factor stimulus (p=1.8e-2)
- response to fibroblast growth factor (p=1.8e-2)

Common (highly similar) module 1
- pos. reg. of transcr. from RNA polymerase II promoter (p=8.1e-10)
- **response to endogenous stimulus (p=7.8e-9)**
- **reg. of apoptotic process (p=8.9e-9)**
- **reg. of programmed cell death (p=1.0e-8)**

Common (highly similar) module 2
- lung growth (p=9.1e-4)
- **reg. of neurotrophin TRK receptor signaling pathway (p=1.3e-3)**
- neg. reg. of peptidyl-threonine phosphorylation (p=2.2e-3)
- neg. reg. of fibroblast growth factor receptor sign. pathway (p=2.7e-3)

Common (highly similar) module 3
- **reg. of immune system process (p=9.1e-47)**
- **immune system process (p=1.1e-40)**
- **leukocyte activation (p=4.5e-36)**
- **lymphocyte activation (p=4.5e-36)**

Common (highly similar) module 4
- response to cAMP (p=2.1e-6)
- response to corticosterone (p=3.4e-6)
- response to organophosphorus (p=6.4e-6)
- muscle organ development (p=1.4e-4)

Common (highly similar) module 5
- single-organism transport (p=2.3e-3)
- **reg. of neurotransmitter secretion (p=9.8e-3)**
- **trochlear nerve formation (p=1.6e-2)**
- **trochlear nerve morphogenesis (p=1.6e-2)**

Common (highly similar) module 6
- **granzyme-mediated apoptotic signaling pathway (p=1.3e-2)**
- reg. of sequestering of zinc ion (p=1.3e-2)
- synapse maturation (p=1.6e-2)
- post-embryonic organ morphogenesis (p=1.9e-2)

Conclusion

We performed a differential network analysis on NB expression data. Modules of interest were analysed for Gene Ontology enrichment, after which NB experts immediately recognised biological processes related to oncogenesis. This analysis serves as a prototype for further differential network analyses for neuroblastoma research. Further research in the development of differential networks is needed, however. Currently, this approach was solely based on expression data, but more of the available data types need to be integrated into the network to get a better understanding of the underlying processes. Furthermore, each step in this analysis needs to be evaluated for robustness and performance, by adding slight perturbations to the dataset and observing the effects of the perturbations downstream.

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