Integrated Analysis of GBM using Boolean Network

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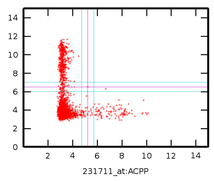
Introduction

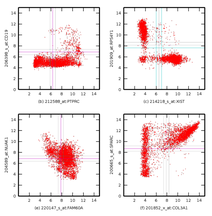
- **Boolean Network**

Discovering Boolean Relationships



Analyze pairs of genes.
Analyze the four different quadrants.
Identify sparse quadrants.
Record the Boolean relationships.
ACPP high ⇒ GABRB1 low
GABRB1 high ⇒ ACPP low

Four Asymmetric Boolean Relationships



- A low ⇒ B low
- A low ⇒ B high
- A high ⇒ B low
- A high ⇒ B high

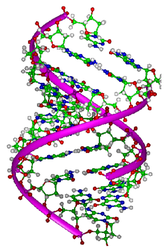
(Based on 4,787 Affymetrix U133 plus 2.0 human arrays, various cell types, from GEO as of 2006.)

- **GBM**
  - ✓ Glioblastoma multiforme (GBM) is a cancer that grows in the supporting tissue of the brain and spinal chord. Its tumors are usually made up of many different kinds of cells, making the disease very difficult to treat. For most adults with GBM the median survival rate is 2-3 years.

- **TCGA**
  - ✓ The Cancer Genome Atlas is a joint project between the National Human Genome Research Institute and the National Cancer Institute to make cancer data from major labs readily available with the goal of furthering cancer research and treatment. GBM is just one of 20 cancers represented.

Objectives

We used Boolean Networks to investigate TCGA GBM data on a large scale with integrative analysis of gene expression, mutation, methylation and copy number alteration.



Methods

- We mainly used Boolean relations to perform integrative analysis of cancer.
- We built the Boolean network using different types of data for the same samples such as expression, copy number, mutation and methylation.
- We systematically analyzed the Boolean network using the following methods:
  - ✓ Look for relations between expression, methylation and copy number/mutation.
  - ✓ Look for relations between copy number and mutation.
  - ✓ Look for relations between known categories of TCGA GBM and mutation, expression and copy number.
  - ✓ Look for chains of Boolean implications.

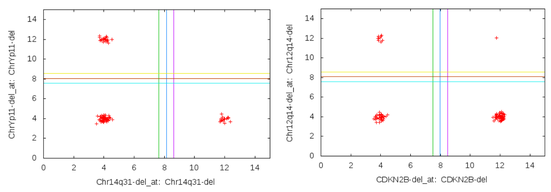
Results

Boolean Relations between genomic alterations

- Boolean relations between mutations and/or chromosomal alterations are interesting and could represent various events important to cancer:
  - ✓ Mutually Exclusive Hits on the Same Pathway
  - ✓ Group-specific Events
  - ✓ Loss of Heterozygosity
  - ✓ Temporal Ordering of genomic events
- Since chromosomal segments tend to be deleted or copied in chunks, the copy number data is organized according to cytobands.
  - ✓ A virtual gene was added to the Boolean network for each cytoband by merging CNA information of genes in the same cytoband.

HILO Relations - Mutual Exclusion

- A high → B low represents a mutual exclusion between A and B
  - ✓ Could represent group-specific events or hits that are alternative ways of achieving the same effect, thus suggesting that they are on the same pathway

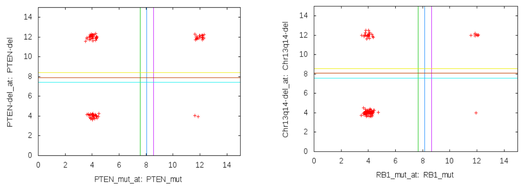


This is a strong HILO relation between Chr14q31-deletion and ChrYp11-deletion, which may suggest Chr14q31 deletion in GBM only occurs in women.

CDKN2B-del --> Chr12q14-del low (Chr12q-14-del has CDK4 which has a protein-protein interaction with CDKN2B in STRING - possibly implying they are on the same pathway. Also, CDKN2B-del and CDK4-del are shown to be mutually exclusive in literature)

HIHI relations on the same gene -Loss of Heterozygosity (LOH)

- A_mut high → A_del_high implies all samples where the A is mutated also have A deleted
  - ✓ Represents double hits to gene A, which means LOH, ie loss of normal function of one allele of a gene in which the other allele was already inactivated

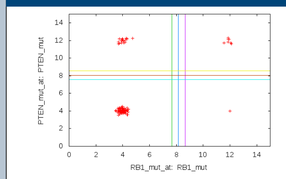


The figures above show a strong LOLO relations between PTEN mutation and PTEN deletion , a strong HIHI relation between RB1 mutation and deletion in Cyto-Band where RB1 is located, suggesting loss of heterozygosity (LOH) in GBM.

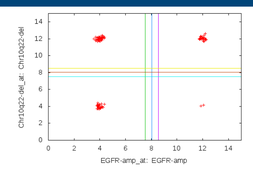
HIHI relations between different genes/ chromosomal regions

- Cancer progresses in a sequence of mutations that cause increased proliferation, defeat checkpoints in the cell cycle, turn off programmed cell death (apoptosis), and cause cells to migrate (metastasis).
- A high -> B high where A and B are genomic alterations could represent a temporal ordering where B occurs first followed by A

Results

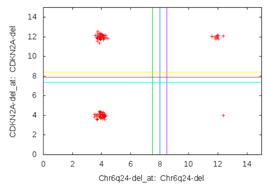


This is a strong HIHI relation between RB1 mutation and PTEN mutation. Literature study reveals there is a temporal ordering between these two events.

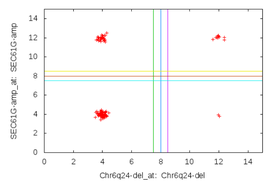


This is a strong HIHI relation between EGFR amplification and Chr10q22 deletion. Previous work shows chromosome 7 and 10 amplifications tend to co-occur.

HIHI relations between well-known GBM genes and chromosomal alterations



This is a strong HIHI relation between Chr6q24 deletion and CDKN2A deletion which is on chromosome 9.



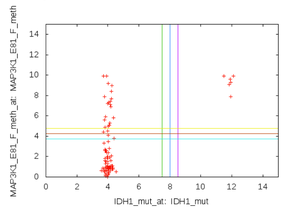
This is a strong HIHI relation between Chr6q24 deletion and SEC61G deletion which is on chromosome 7.

Many Relations that need further investigation

Mutation vs. Mutation/CNA	CNA vs. CNA
Chr13q21-del low -> PTEN_FSD_mut low (temporal sequence between chromosome)	Chr10p15-del low -> Chr22q11-del low
Chr13q21-del low -> RB1_mut low	Chr10q26-del low -> chr13q21 low
EGFR_mut high -> Chr7p11-amp high	Chr10q26-del low -> Chr6q22-del low
FKBP9_mut high -> Chr10q11-del high	Chr10q26-del low -> Chr9p23-del low
FKBP9_mut high -> Chr7p11-amp high	Chr6q23-del high -> SEC61G-amp high
PTEN_mut high -> Chr13q21-del high	KLHL9-del high -> Chr12q15-del low (mutual exclusion)
EGFR_M_mut high ->SEC61G-amp high	Chr14q22-del -> MLLT3-del (MLLT3 is on Chromosome 9).
FKB9_mut high -> MIR605-del high	

Mutation vs. Methylation

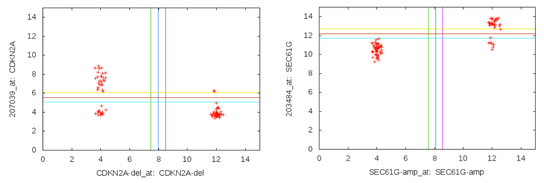
- We investigated the relationship between mutation and methylation. We confirmed the well-known fact that mutated IDH1 is related to hyper-methylation; mutated IDH1 had the most number of HIHI relations with methylation probes.



In the plot above, IDH1_mut high implies MAP3K1_E81_F_meth high.

Copy Number vs. Expression

- We examined the relationship between copy number alterations (amplifications and deletions) and gene expression. We found 121 HILO relations and 6 OPP relations involving deletions. We found 6 LOLO and 10 EQ involving amplifications. Several genes related to cell cycle were in this list.



Above left: deletion of CDK2NA implies low expression of CDKN2A.
Above right: high expression of SEC61G implies SEC61G amplification

Results

GBM Subtype Analysis

- Recent work [1] revealed four subtypes of GBM : Classical, Mesenchymal, Proneural and Neural.
- We made up virtual genes of each subtype by evaluating the table in [1] which maps from each sample to subtype.
- Subtype virtual genes revealed the same signature as in [1], which are selectively show as below.

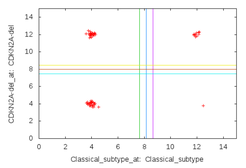


Figure1 shows Classical subtype high -> CDKN2A-del high, which is coherent with the observation of prevalent CDKN2A deletion in Classical subtype in [1].

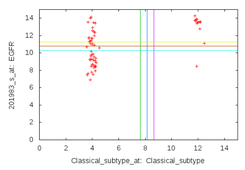
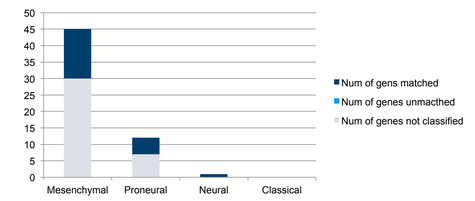


Figure2 shows Classical subtype high -> EGFR expression high, which is coherent with the observation of significant EGFR expression in Classical subtype in [1].

- In Boolean Network, for each subtype virtual gene, we took the genes that have EQUIVALENCE relation with it and checked what subtype [1] assigned these genes to.



- Due to the small number of genes classified in [1], many genes we found were not classified.
- For the genes that have been classified by [1], the match rate is 100%.

Discussion

- This work validated the ability of Boolean Network in finding various relation between genes and chromosomes in genomics in the development of cancer.
- This work confirmed many findings from previous work, including:
  - ➢ Genes associated with the four subtypes of GBM
  - ➢ Important genes in GBM, including RB1, CDKN2A and PTEN.
  - ➢ The role of IDH1 in hyper-methylation
  - ➢ Significant deletion in chromosome 7 and amplification in chromosome 10 and their close relationship.
- This work also found new strong relations that haven't been found before
  - ➢ New Mutual Exclusion Relations
  - ➢ HIHI relations which may suggest temporal sequence of process in the development of GBM
  - ➢ HIHI relations between expression and mutation of the sample gene, which suggest the loss of heterozygosity in GBM patient.
  - ➢ Many other inter-chromosomal relations that are very interesting and require further investigation.

Acknowledgement

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References

[1] Roel G.W. Verhaak, etc. "An integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR and NF1." Cancer Cell 2010, Jan 19, 17(1): 98
[2] D. Sahoo, D. Dill, A. Gentles, R. Tishirani and S. Plevrithis, Boolean implication networks derived from large scale, whole genome microarray datasets, Genome Biology, Oct 2008.

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