Therapeutic Opportunities From the MYC Target Gene Network

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TOPICS

• Background and Significance of MYC
• Myc Function: Normal versus Pathologic
• Targeting MYC
• Genomic and Functional Analysis of Target Genes: Therapeutic Opportunities
Burkitt’s Lymphoma

- **MYC**
- **IgH**

- karyotype: t(8;14) (q24;q32)
- 14q+
- IgH rearrangement

- Clinical features:
  - Acute onset
  - High fever
  - Abdominal pain
  - Osteolysis
  - Enlarged liver
  - Enlarged spleen
MYC encodes a transcription factor

(From Dang, MCB 1999)
Myc affects gene transcription through chromatin modification and stimulation of RNA Pol
Mechanism of c-Myc Function

- Gene activation through E-Box
- Gene repression (through Inr, Miz-1)

Normal vs. Abnormal Cell Proliferation
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Control of Cell Size

Animal Genetic Studies

• Homozygous deletion of c-myc:
  – embryonic lethality

• Conditional knockout:
  – Diminished growth of primary fibroblasts
  – Virtually absent lymphocytic mitogenesis

• Transgenic mice: neoplasia:
  – Inducible transgenic animals: reversible tumorigenesis with some hit-and-run outcome
Normal Myc Function

protein levels vs. time

Myc
Max
growth-related genes

mitogen
Oncogenic Activation of Myc

protein levels

ONCOGENIC ACTIVATION

Target genes
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Targeting MYC

- Rationale: Inducible Myc transgenes reveal reversible tumorigenesis through withdrawal of Myc activity
Targeting MYC

- Approaches
  - Quadruplex-stabilizing molecules
  - Antisense
  - Ribozymes and RNAi
  - Small molecules: Myc/Max dimerization
  - Targeting downstream events
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Functional c-Myc Target Transcriptomes

Adapted from Dang, MCB 1999
The emergence of complex DNA microarray data on gene expression creates vast new opportunities but also poses serious challenges for information and library sciences. This complexity becomes more challenging as certain genes, such as MYC that encodes a transcription factor, switch on other genes. The purpose of this website is to provide a hub for the integration of information on Myc target genes, the role of Myc in human cancers, and proteins that interact with the Myc transcription factors. Links are provided that connect to PUBMED citations, Unigene database, and in specific cases to original data.

www.myccancergene.org

Funded by the National Library of Medicine
Gene Ontology of 1591 Myc Target Genes

- Metabolism
- DNA repair
- Cell organization and biogenesis
- Transcription
- Transport
- Carbohydrate metabolism
- Stress response
- RNA localization
- Cell cycle
- Transcription
- RNA processing
- Protein dephosphorylation
- Cell growth or maintenance
- Protein degradation
- Protein biosynthesis
- Cell cycle
- Protein transport
- DNA repair
- Unknown
Formaldehyde crosslink chromatin

Sonicate DNA and precipitate chromatin

Reverse crosslinks and PCR amplify precipitated DNA with locus specific primers
Where does Myc bind in a target gene? Scanning Chromatin Immunoprecipitation (SChIP)

Zeller et al., JBC 2001
ChIP-PET

- Ditag sequencing of ChIP DNA
P493: Burkitt’s Lymphoma model cell line

EREB2-5 (parental) → tet-Myc → P493-6

-Time after removal of Tetracycline (Hrs)

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<th>0.6</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
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<th>24</th>
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<td>c-Myc</td>
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<td>Tubulin</td>
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+TET     -TET

P493

(- Myc)    (+ Myc)

Kate O’Donnell
Scanning Chromatin Immunoprecipitation (SChIP)  
Nucleophosmin (NPM1)

Zeller et al., JBC 2001
Haggerty et al., PNAS, 2003
ChIP-PET mapping on NPM1
ChIP-PET mapping on C13orf25/miR cluster

- **C13orf 25**
- **MiR cluster**
- **Myc binding**
Myc Regulation of Glycolysis

Direct Target Genes
The Warburg Effect

- Glucose
- Pyruvate
- Lactate
- LDH
- ATP
- O₂
- TCA cycle
- FDG-PET
Targeting MYC
Targeting downstream events: glycolysis

- **O₂**
- **HIF1**
- **glucose** → **pyruvate** → **lactate**
- **2ATP** ↔ **4ATP**
- **MYC**
- **ROS**
- **H₂O**
- **NADH, FADH₂**
- **O₂**
- **PDH**
- **TCA cycle**
- **acetyl-CoA**
- **Complex I-IV**
Meta-signature of neoplastic transformation

12 tumor types
67 genes up T/N
>3,700 cancer samples
40 data sets
Sixty-seven genes overexpressed in cancer relative to normal tissue counterpart in at least 12 of 39 "cancer vs. normal" signatures

Reduction of LDHA Diminishes Tumorigenicity

Shim et al. PNAS 1997
Lactate, H⁺, glucose, growth factors

150 µm
Targeting Hexokinase

Robey & Hay DDT, 2005
Disruption of hexokinase–mitochondrion interaction (all preclinical)
- Hexokinase mitochondrial-binding domain peptides (HKmtBD).
- Clotrimazole (CTZ) Unknown Specificity
- \(N,N\)-Dicyclohexylcarbodiimide (DCCD). Covalent modification of hexokinase-binding site of VDAC. Not specific for hexokinase–VDAC interaction.
- TH-070 (Lonidamine) Unknown Specificity
- Glc-6-P analogs
Targeting MYC
Targeting downstream events: glycolysis

- 2-deoxyglucose: Threshold Therapeutics–TH-CR-101 is a randomized Phase 1 dose-escalation trial that will evaluate efficacy and safety of 2-DG in combination with docetaxel in patients with advanced solid tumors.
- 3-bromopyruvate: xenografts–toxicity profile pending
Therapeutic Window

• Model of B-cell lymphoma with inducible MYC
• High MYC – B-cell lymphoma model
• Low MYC – EBV-driven, similar to EBV-immortalized B cells
• Microarray analysis
• ChIP-PET; encode arrays
Normal Myc-target Gene Network

Diagram showing the interaction network of Myc-target genes, with nodes for p53 and Myc.
Pathological Myc-target Gene Network

$p53$  

$Myc$
P493: Burkitt’s Lymphoma model cell line

primary B cells

EBV genome EBNA2-ER

EREB2-5 (parental)

tet-Myc

P493-6

High Myc

-No Myc

Low Myc

+Tet +estradiol
Growth of P493-6 cells with High, Low, or No Myc levels

![Graph showing cell number growth over days with different Myc levels.](image)

- **High Myc** (solid blue line with diamonds)
- **Low Myc** (dashed red line with squares)
- **No Myc** (dotted green line with triangles)

**c-MYC and Tubulin Western Blots**

- **c-MYC**
- **Tubulin**

*J. Yustein*
High MYC State is Tumorigenic

![Graph showing estimated tumor volume over weeks for untreated, doxy posttreated, and doxy pretreated groups.]

- Untreated
- Doxy Posttreated
- Doxy Pretreated

**Dinavahi & Dang**

![Microscopic images of Ramos and P493 cells]
“Physiologic vs Pathologic”

Targets

<table>
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<tr>
<th>No</th>
<th>Low MYC</th>
<th>High</th>
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Use of $\gamma$-secretase inhibitors

- Jagged 2 is a Notch receptor ligand
- Notch, WNT, and Sonic-Hedgehog pathways as putative cancer stem-cell signal-transduction pathways
- $\gamma$-secretase inhibitors are more inhibitory on the high MYC than the low MYC state
Pathological Myc-target Gene Network

$p53$
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