

The MYCN onco-protein as a therapeutic target for neuroblastoma

Marie Arsenian Henriksson
Department of Microbiology
Tumor and Cell Biology (MTC)



Karolinska
Institutet

Myc rearrangements in human tumors

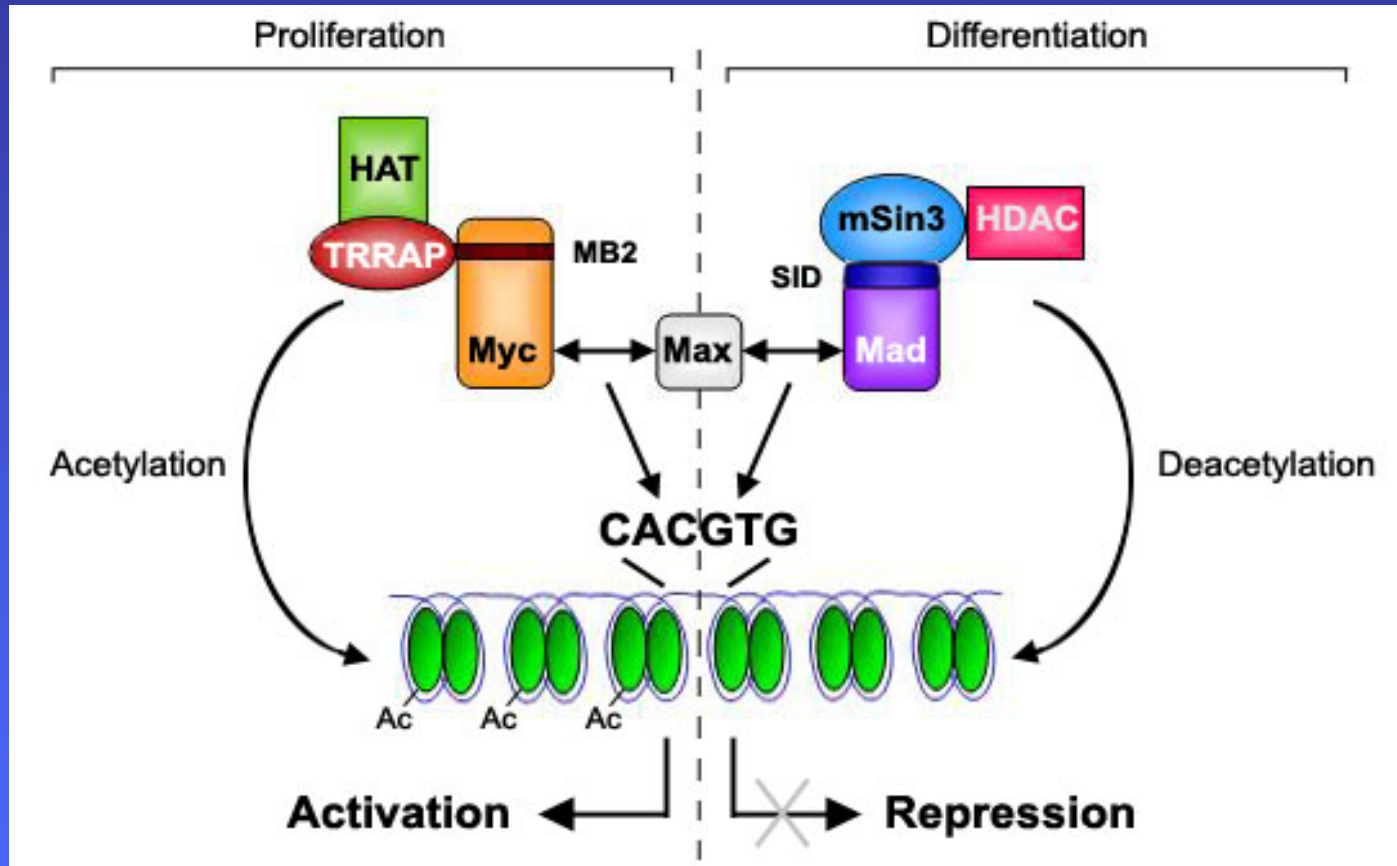
Translocation

AIDS-related non-Hodgkin's lymphoma (NHL) 80% *c-Myc*
Burkitt's lymphoma (BL) 100% *c-Myc*
B-Acute lymphocytic leukemia (ALL) 47-52% *c-Myc*
Diffuse Large Cell Lymphoma 6-16% *c-Myc*
Primary plasma cell leukemia 13% *c-Myc*
Multiple myeloma (MM) 15% *c-Myc*

Amplification

Bladder cancer 33% <i>c-Myc</i>	Breast cancer 9-48% <i>c-Myc</i>
Cervix cancer 29% <i>c-Myc</i>	Colon carcinoma 17% <i>c-Myc</i>
Gastric cancer 15-30% <i>c-Myc</i>	Glioblastoma 5% <i>MYCN</i>
Hepatocellular carcinoma 33% <i>c-Myc</i>	Medulloblastoma 5-15% <i>c-Myc/MYCN</i>
Melanoma 28-61% <i>c-Myc</i>	Neuroblastoma stage IV 25-30% <i>MYCN</i>
Osteosarcoma 7-78% <i>c-Myc</i>	Ovarian cancer 40% <i>c-Myc</i> , 15% <i>L-Myc</i>
Prostate cancer 30-50% <i>c-Myc</i>	Rhabdomyosarcoma 43-67% <i>MYCN</i>
Retinoblastoma 10-20% <i>MYCN</i>	
Small cell lung carcinoma 10% <i>MYCN</i> , 13% <i>L-Myc</i> , 20% <i>c-Myc</i>	

Effects of Myc network proteins on transcription



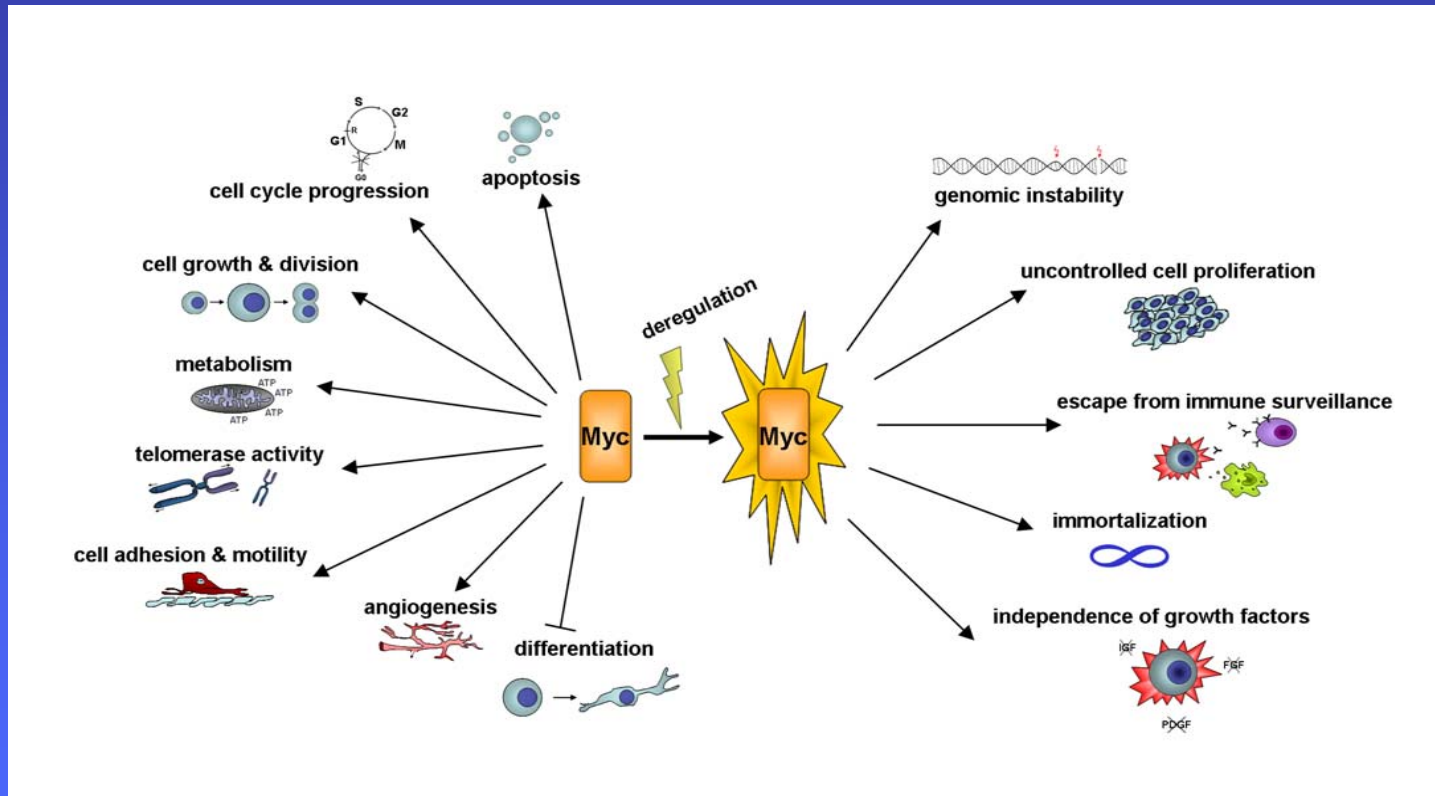
Cell cycle

Cell growth

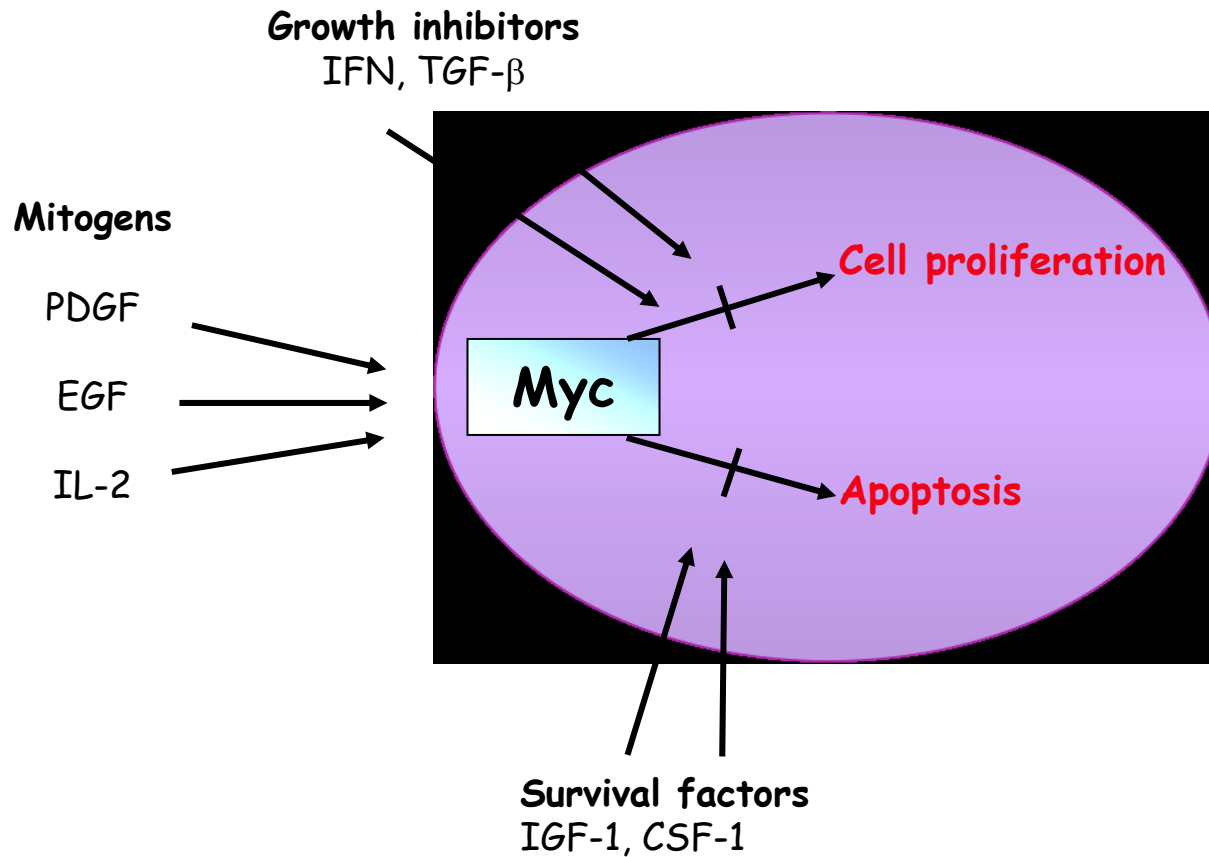
Apoptosis

Differentiation

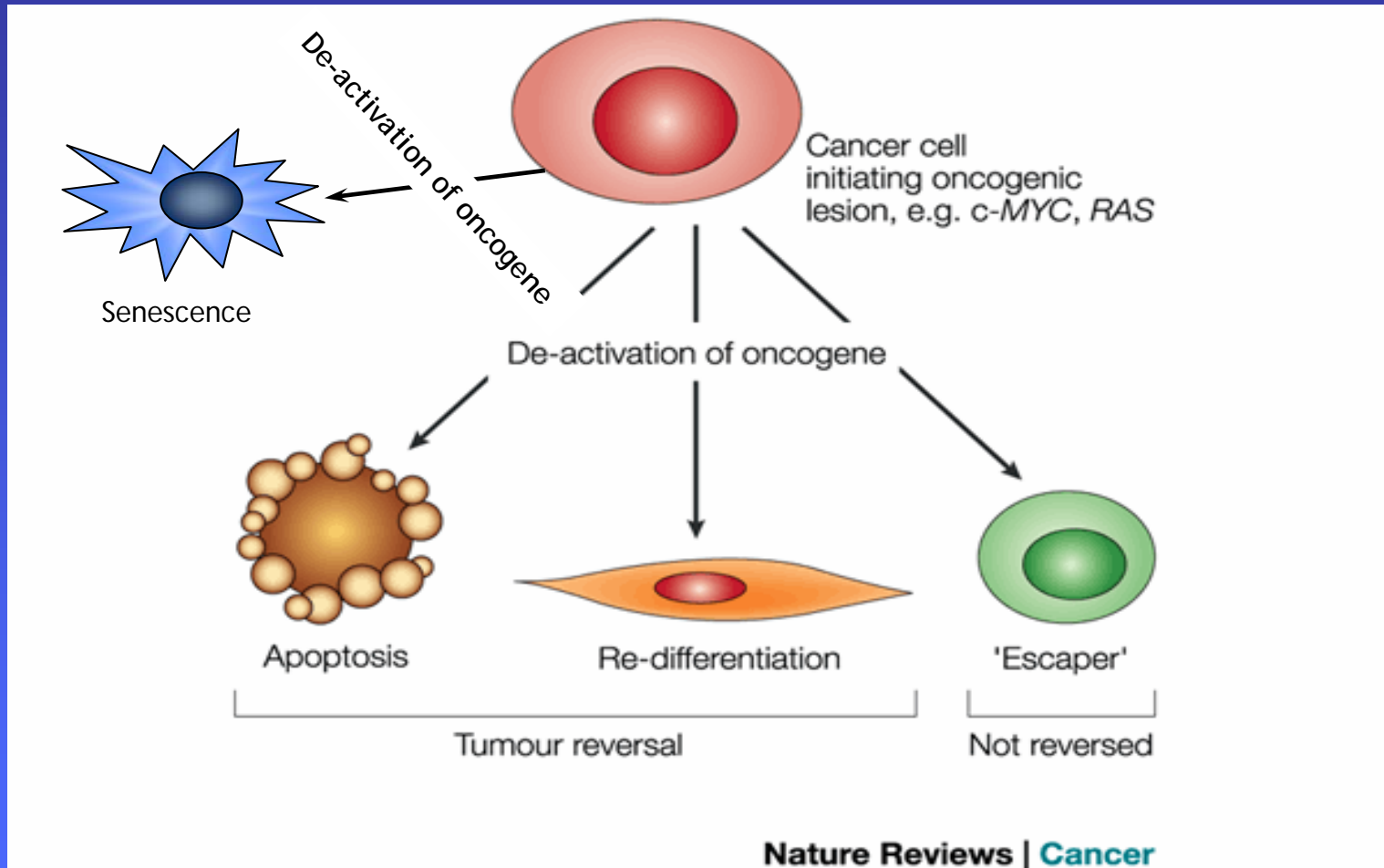
Cellular processes controlled by Myc during normal conditions and tumorigenesis



Myc and the dual signaling model

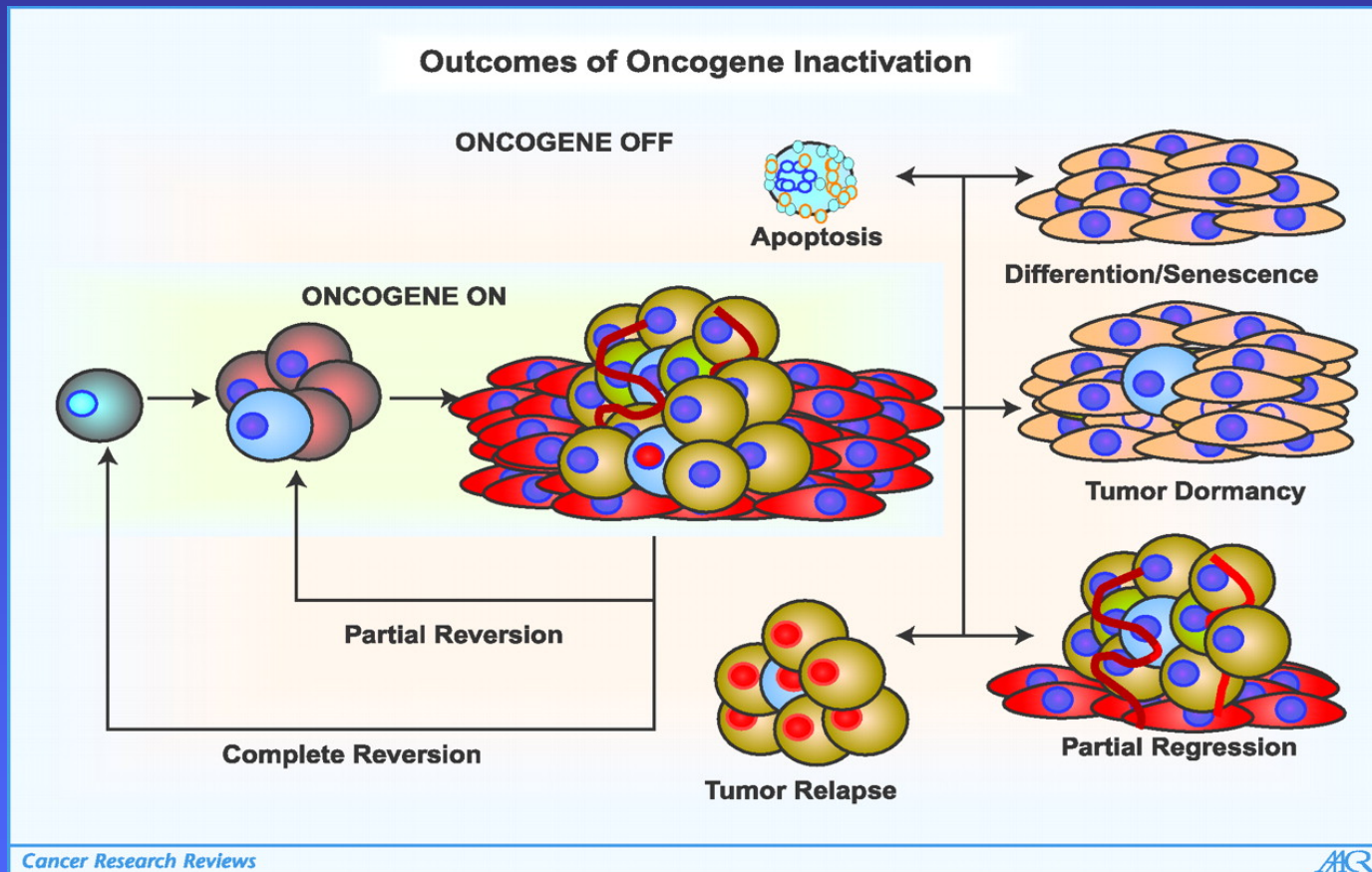


Tumor regression following de-activation of the initiating oncogenic lesion

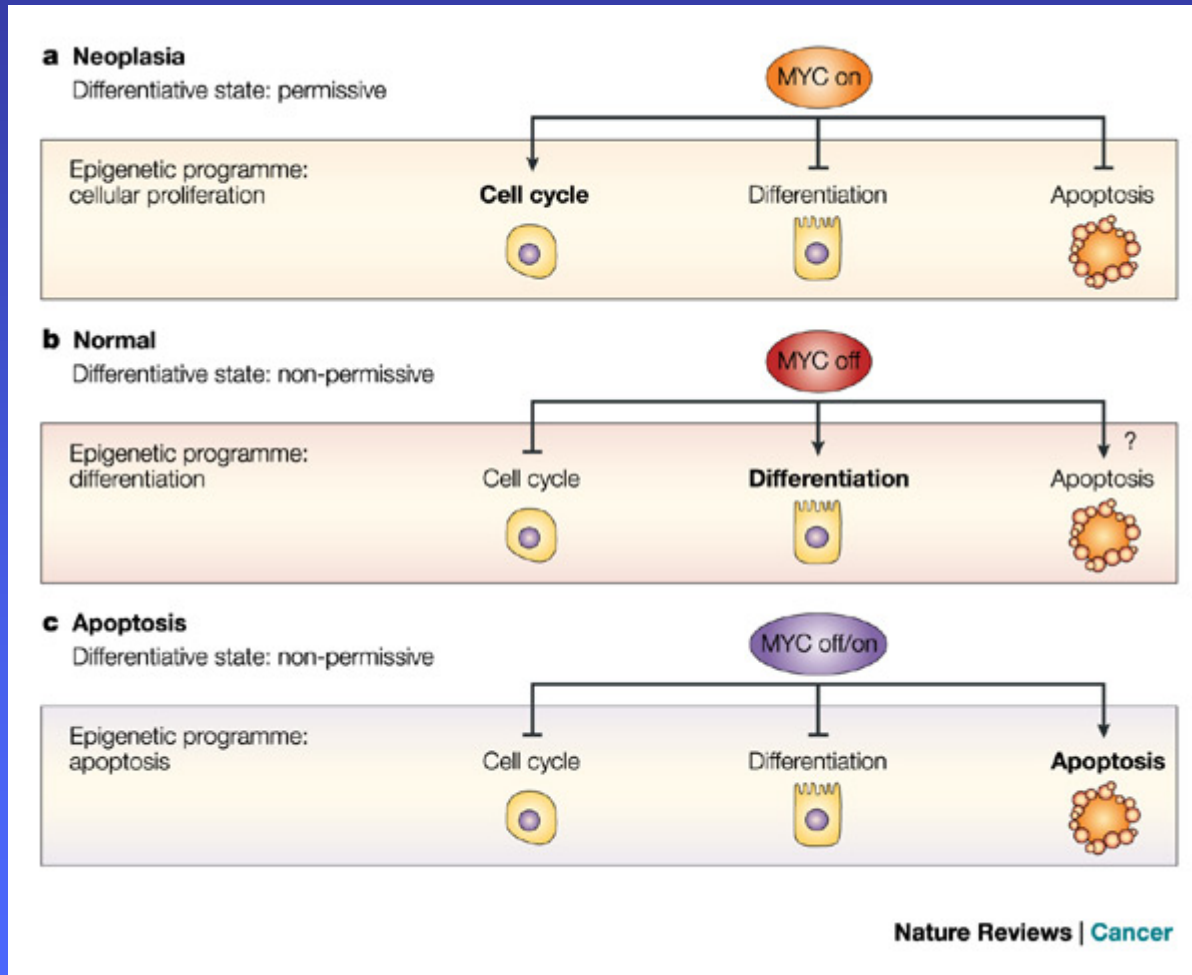


Adapted from Nature Reviews Cancer 2, 764-776, 2002

Many possible outcomes to oncogene inactivation: no effect, complete, or partial tumor reversion



Oncogene inactivation might revoke tumorigenesis by changing the epigenetic programme

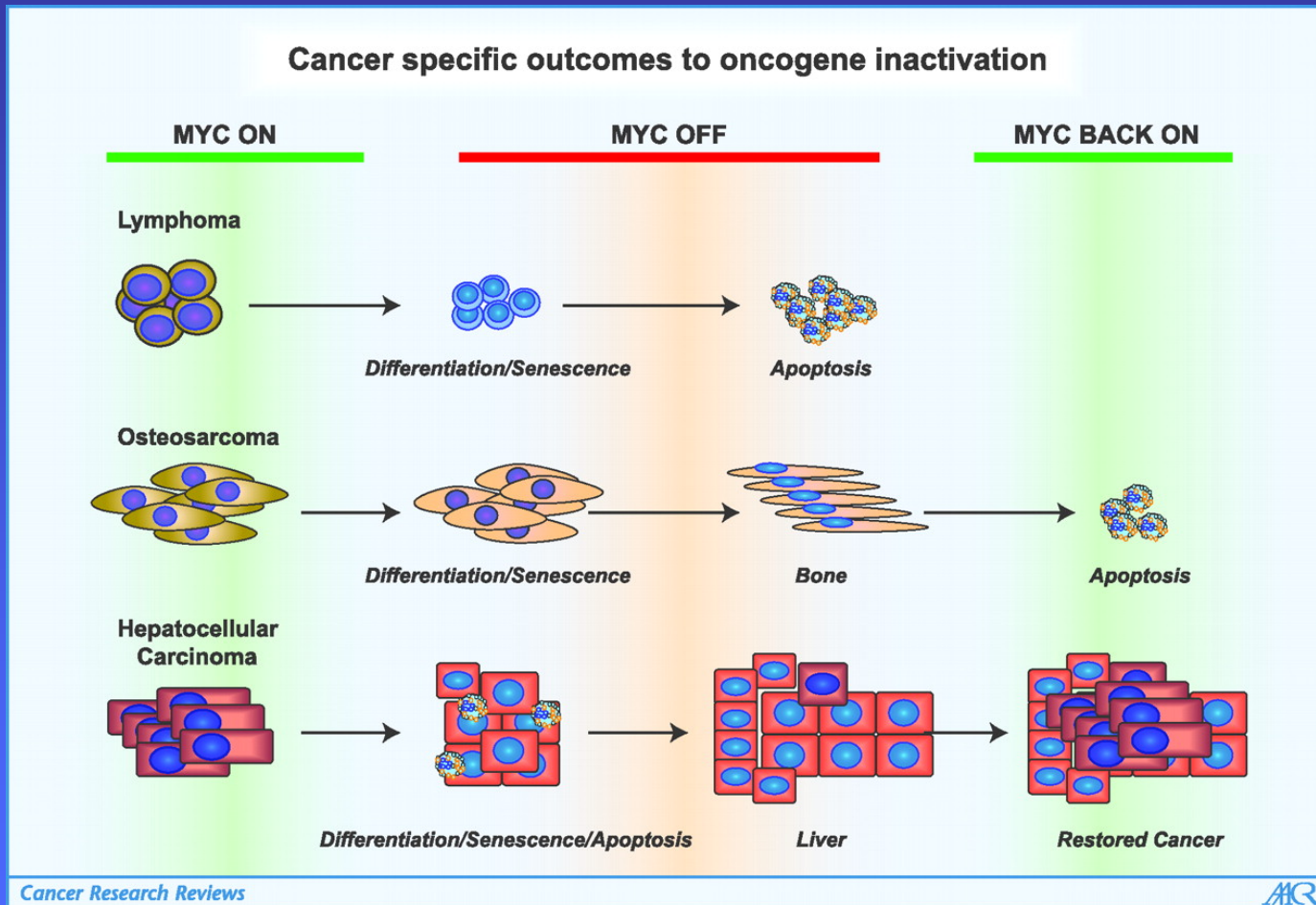


What happens if Myc is reactivated?

- In some cases, the cells are dormant tumor cells that recover their neoplastic properties upon Myc reactivation
- In other cases, the tumor does not reappear; brief inactivation of Myc -> sustained regression of tumors and differentiation of osteogenic sarcoma cells into mature osteocytes
Subsequent reactivation of Myc did not restore the cells' malignant properties (Felsher)

- ➔ Brief Myc inactivation may cause epigenetic changes in tumor cells that render them insensitive to Myc-induced tumorigenesis
- ➔ Raise the possibility that transient inactivation of Myc may be an effective therapy for certain cancers

Oncogene inactivation have different outcomes in different types of tumors including proliferative arrest, differentiation, apoptosis, and/or cellular senescence



Effects on normal tissues on Myc inactivation

- Modeling Myc inhibition using a Myc mutant, Omomyc, that homodimerize with Myc
- No effect on adult organs with low proliferation
- No effect on animal well being
- Profound effects on normal regenerating tissues
 - basal layer of skin epidermis
 - small intestinereduction in proliferation but no apoptosis
- These effects were well tolerated over extended periods and completely reversible
- Why viable arrest in normal tissues but apoptosis in tumors?

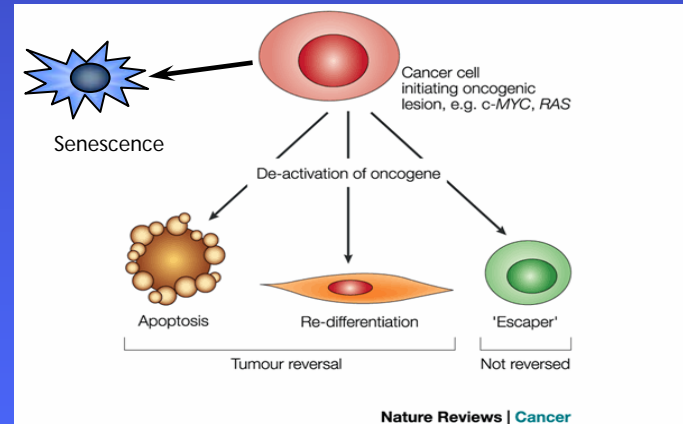
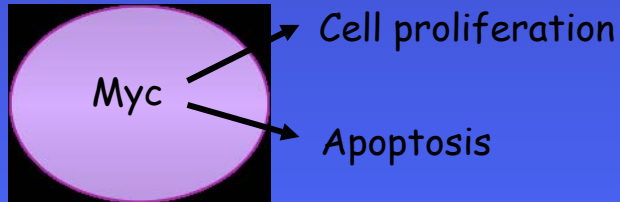
Myc as a target for cancer therapy

Myc genes are deregulated in many human tumors

Myc activates proliferation and apoptosis

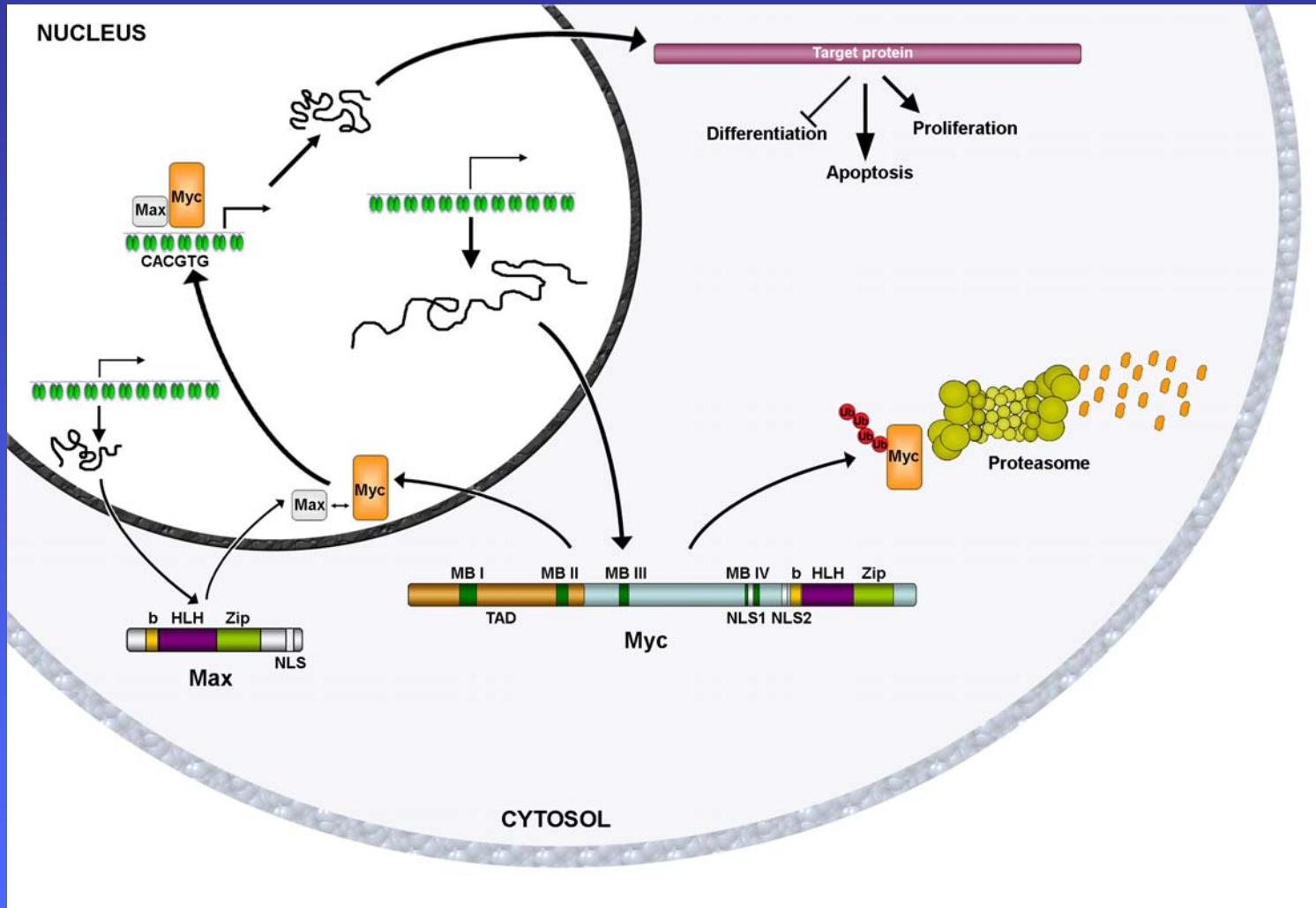
Inactivation of *Myc* in experimental mouse tumor models
→ tumor regression, apoptosis and/or differentiation

Reversible effects on normal regenerating tissues

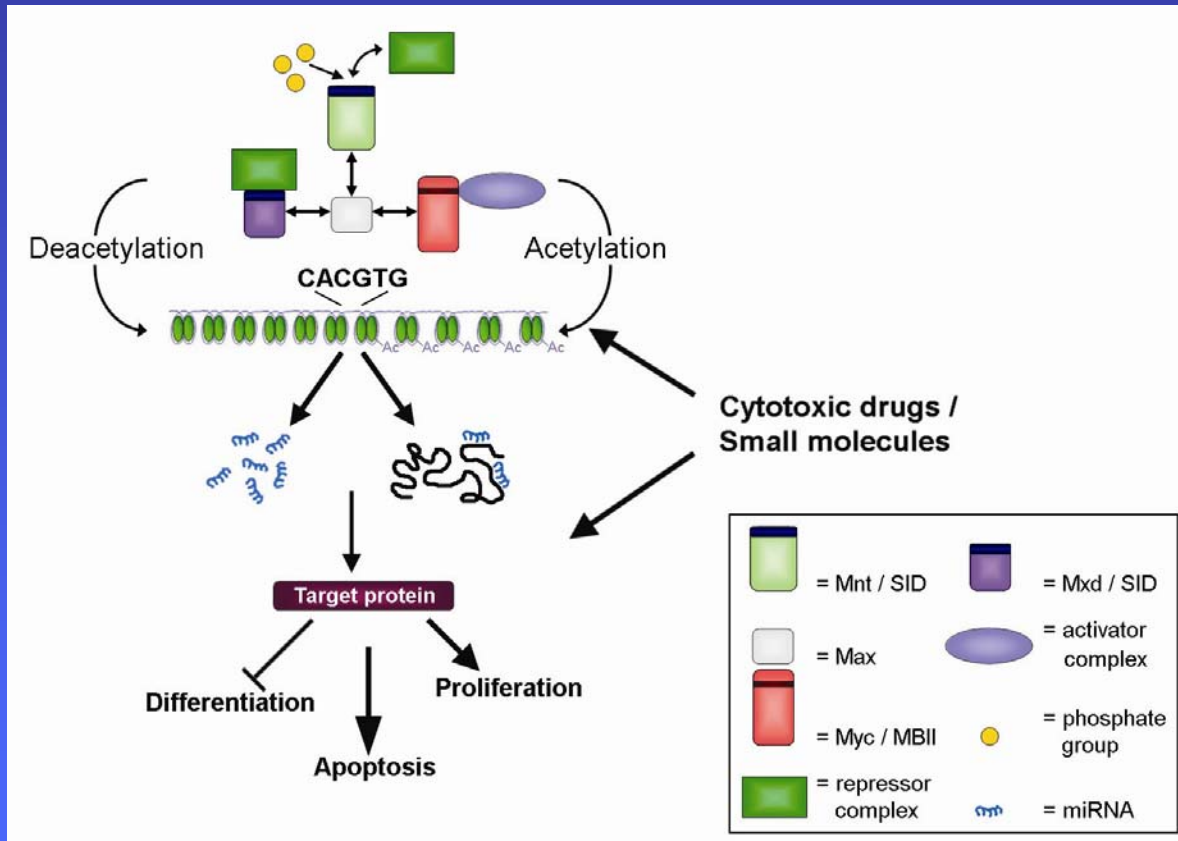


Targeting of *Myc* provides an attractive basis for novel therapies with potential application in a wide variety of human malignancies

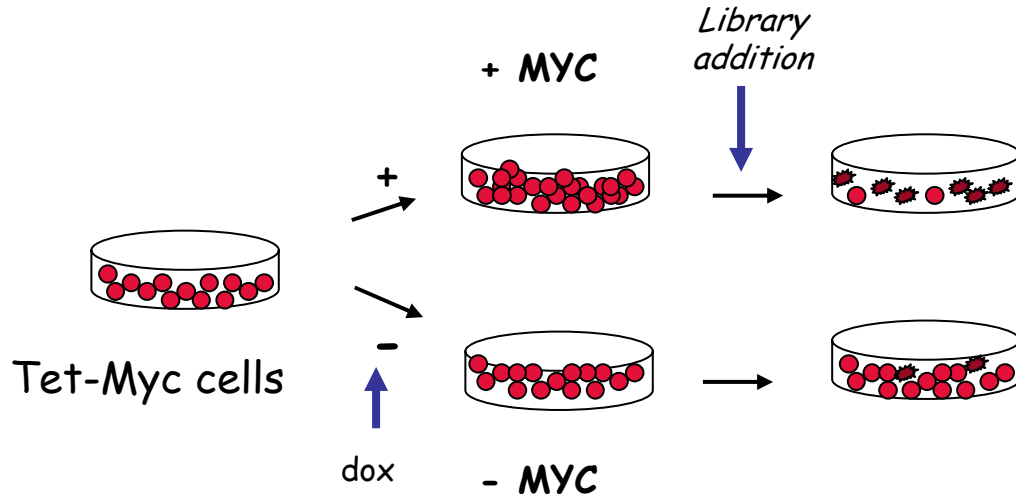
Targeting Myc at different levels



Our Strategies

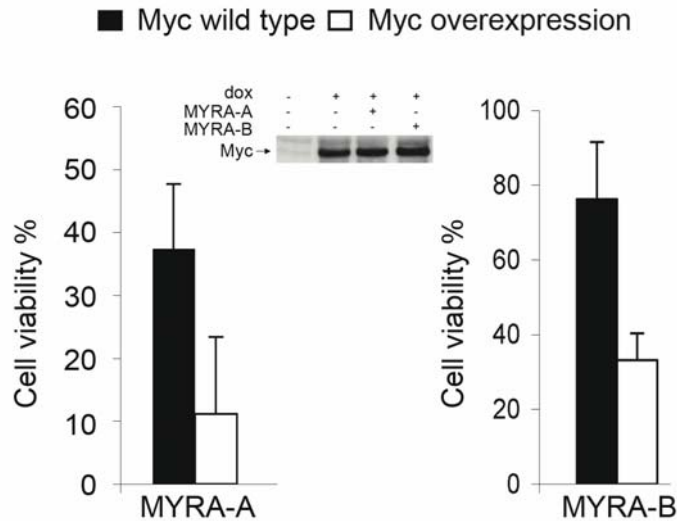


Identification of molecules that inhibit proliferation of c-Myc overexpressing cells



NCI Diversity Library:

- 1990 small molecular compounds representing 140 000 structures
- Selected by low toxicity and permeability to cells

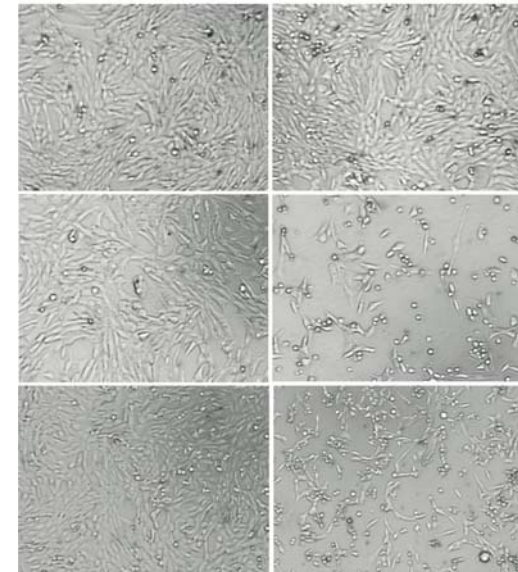


Myc uninduced Myc overexpression

Control

MYRA-A

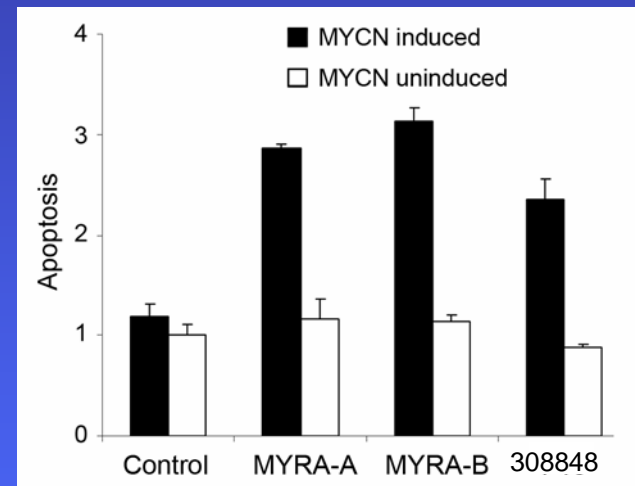
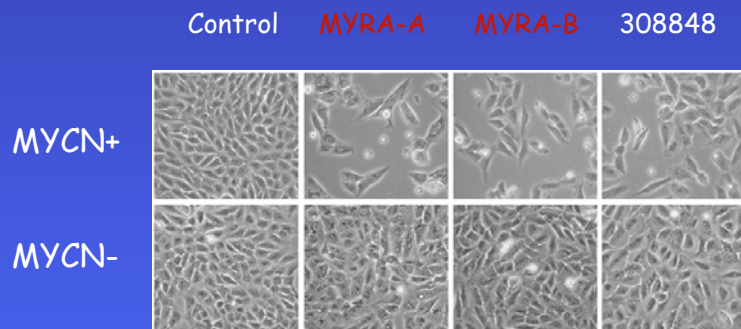
MYRA-B



Myc Pathway Response Agent (MYRA)

Hao Mo

Induction of apoptosis in MYCN overexpressing cells



Apoptosis ELISA

Summary cellular screen

We have identified three structurally different molecules that induce apoptosis in a Myc-dependent manner and that inhibit cellular transformation driven by Myc

These compounds have different mechanisms of action:

- MYRA-A inhibits Myc DNA-binding and Myc transactivation
- The mechanism of MYRA-B is unknown (no effect on Myc transactivation or Myc DNA-binding)
- Treatment with NSC308848 results in reduced Myc protein levels

The further characterization of the mechanism of action of these compounds may help in identifying new key players in the Myc pathway

Myc rearrangements in human tumors

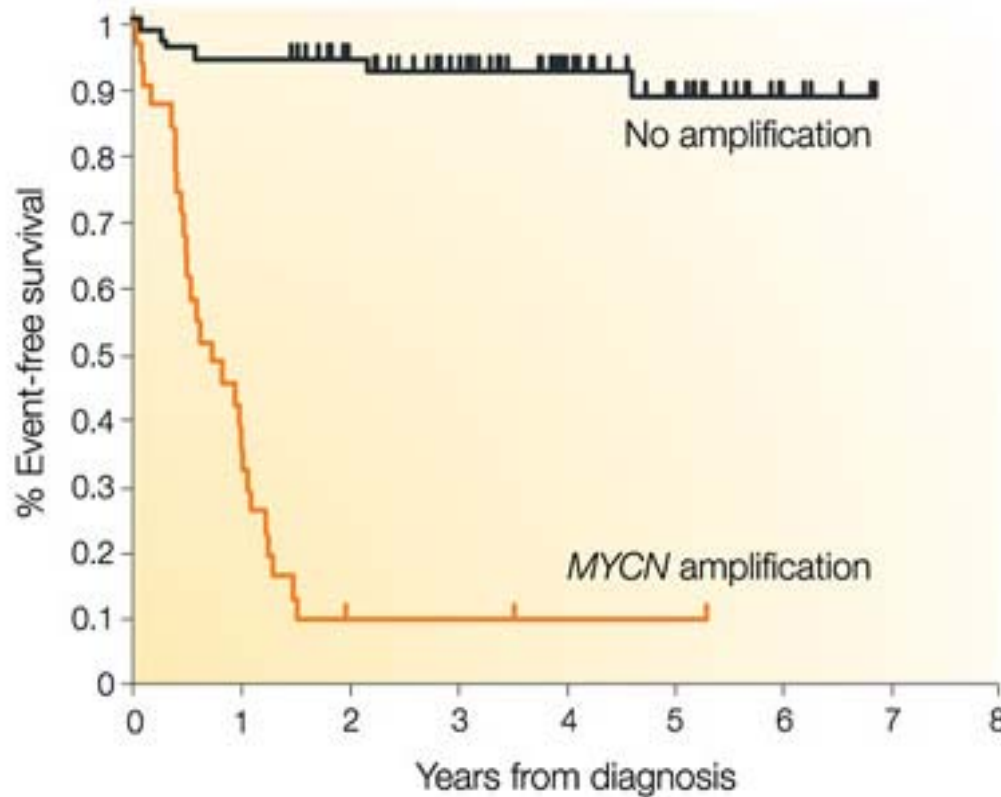
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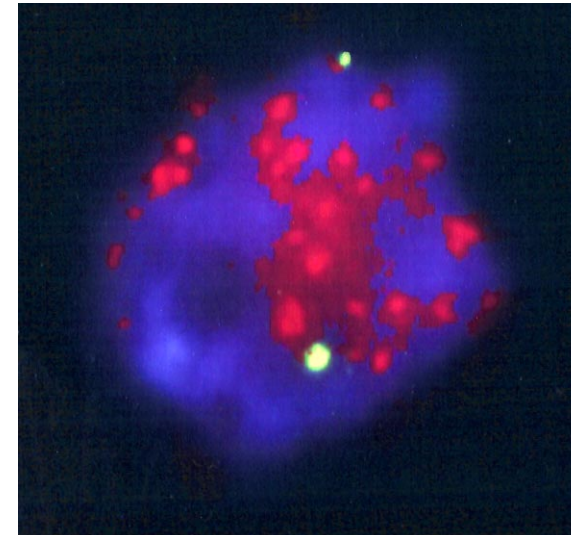
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MYCN-amplification predicts poor survival in neuroblastoma

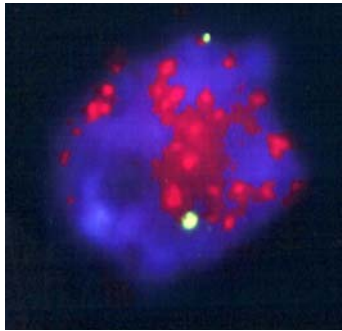


MYCN amplification



Widespread neuroblastoma in infants. Survival depends on MYCN, despite intensive therapy

Approach for tailored neuroblastoma therapy

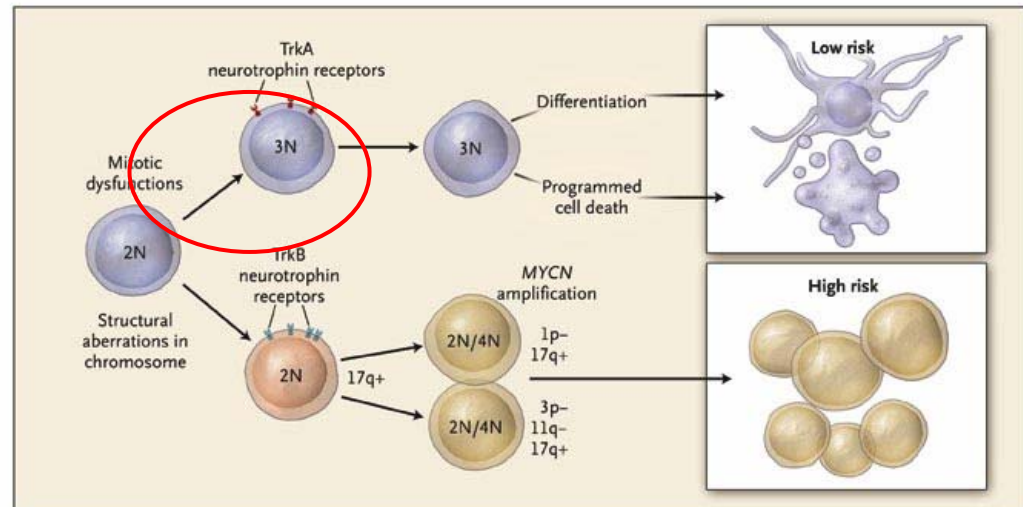


MYCN amplification



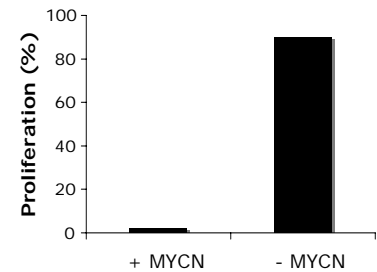
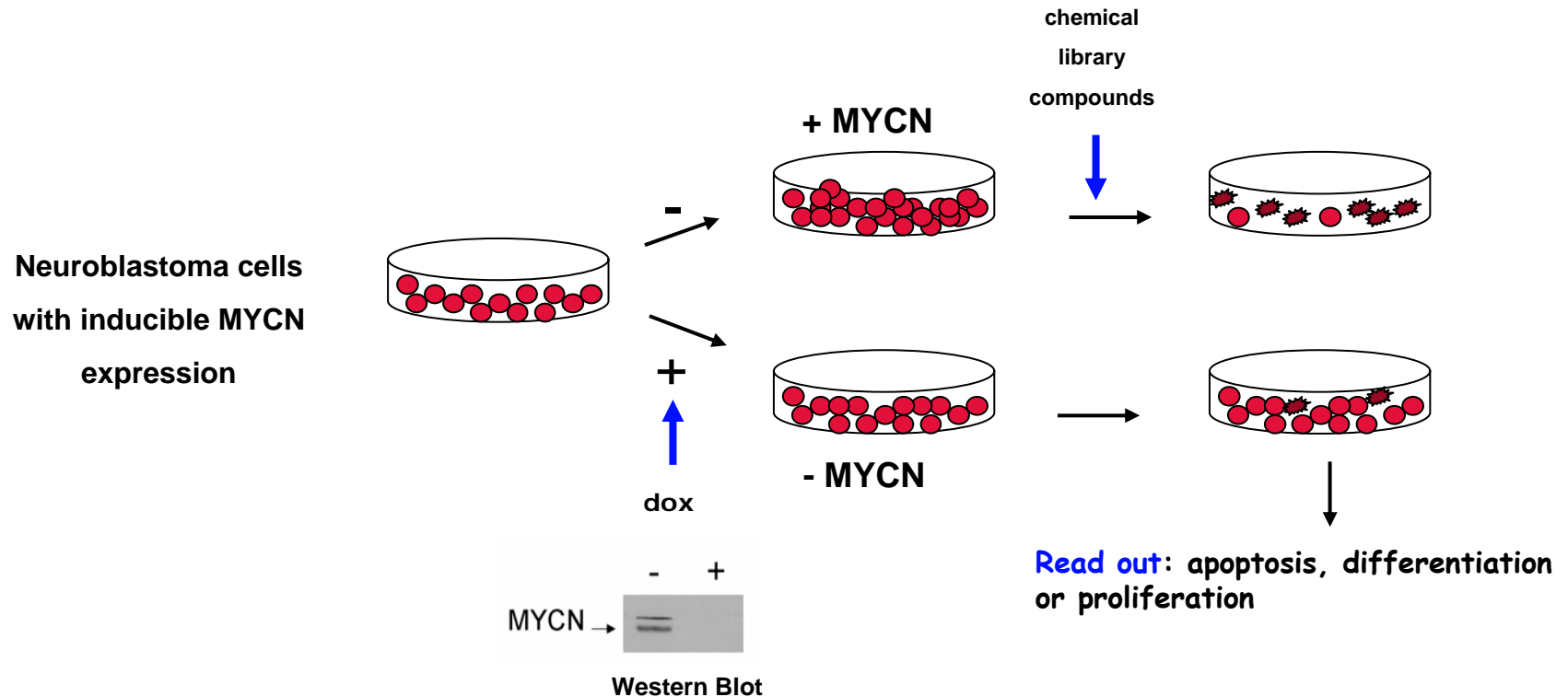
Clinical features: neuroblastoma with *MYCN* amplification

Strategy: *MYCN* as a target for therapy, cellular screening assay



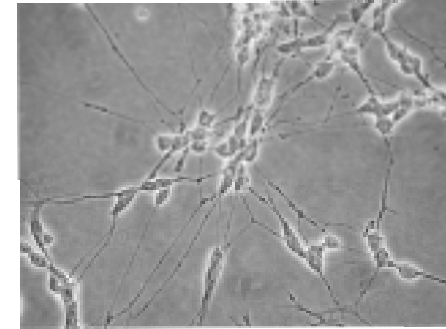
Clinical and biological heterogeneity of neuroblastoma

Library Screening Strategy in Neuroblastoma cells

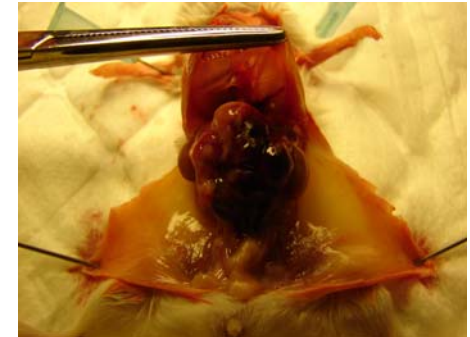
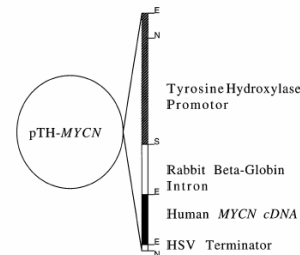
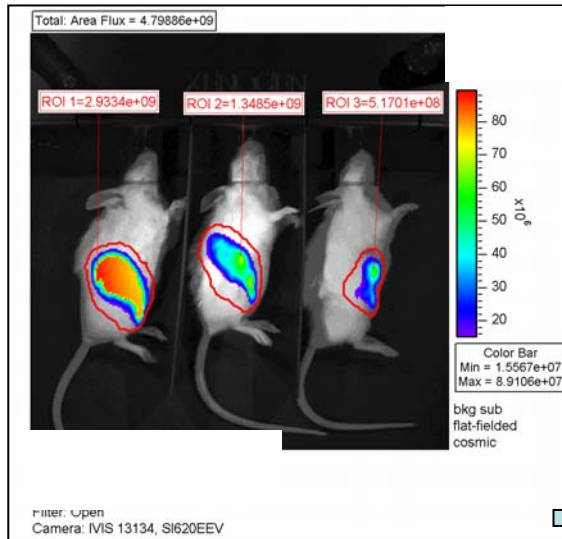


Experimental models

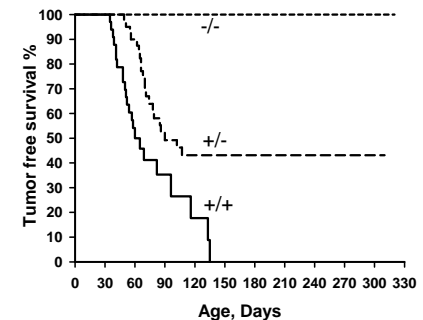
- In vitro* models: panel of cell lines derived from high-risk patients, various phenotypes and genotypes



- In vivo* models:
 - xenografts or metastatic disease after iv/ic injection
 - transgenic model



MYCN transgenic
neuroblastoma model
Weiss *et al*, EMBO J.
16:2985, 1997



Conclusions

Cellular screening assays can be powerful for identification of small molecules eliciting a desired biological response

We have explored a cellular screening assay and identified substances that induce apoptosis in a Myc-dependent manner

The identified compounds can be useful tools for analyzing Myc function and may also be of therapeutic potential as leads for development of novel therapies for human cancer

Summary

- Myc is a key regulator of cell proliferation, apoptosis, and differentiation
 - Deregulation of Myc results in hyper-proliferation -> -> tumorigenesis when the apoptotic program is defective
 - Myc is activated in a wide variety of human cancers
 - Myc sensitizes cells for drug-induced apoptosis
- > Myc is an attractive target for therapy with a potential use in many different tumors

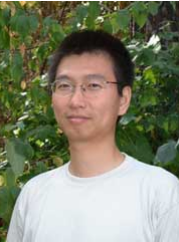
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KICancer

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