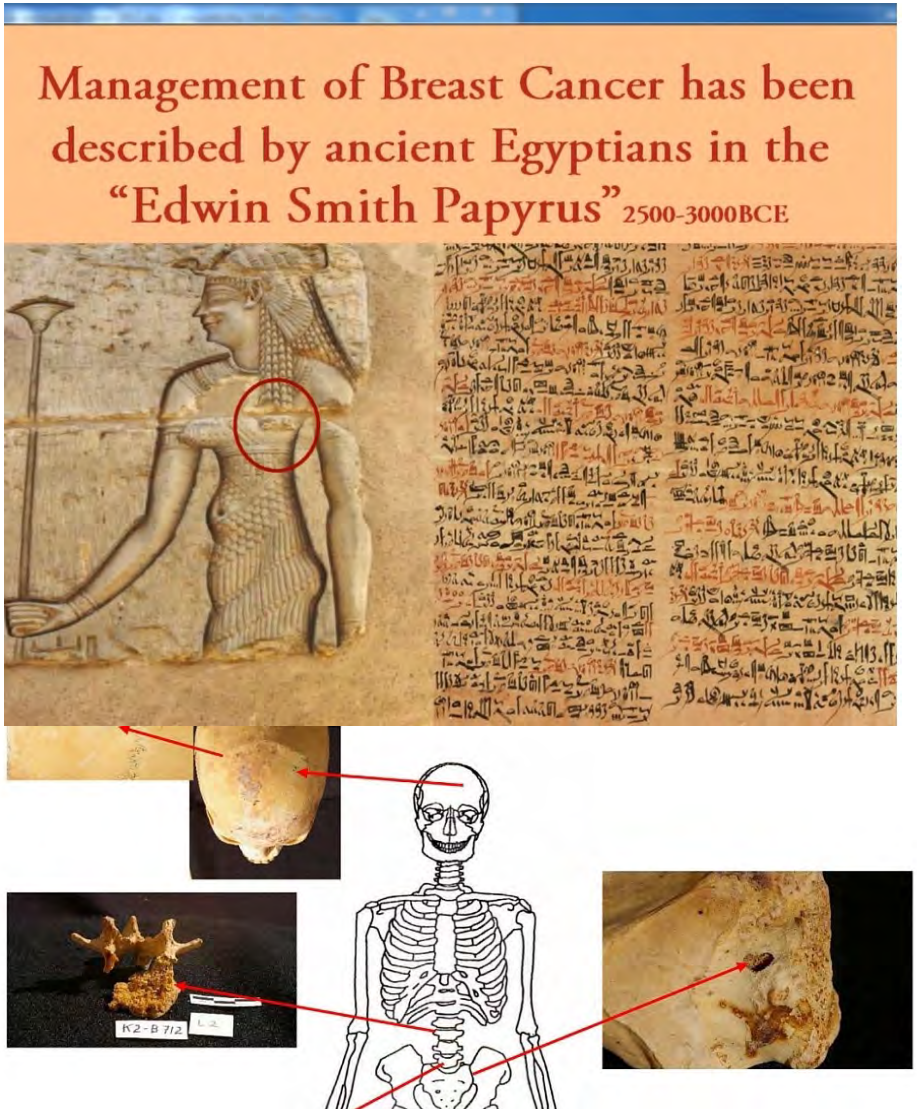
Cancer Biology Course - Introduction

Human beings and other animals have had cancer throughout history

Some of the earliest evidence of cancer is found among fossilized bone tumors, human mummies in ancient Egypt, and ancient manuscripts.

Edwin Smith Papyrus from 3000 B.C. is oldest description of cancer, is part of an Egyptian textbook on trauma surgery. It describes 8 cases of tumors or ulcers of the breast that were removed by cauterization with a tool called the fire drill. The writing says about the disease, “There is no treatment.”



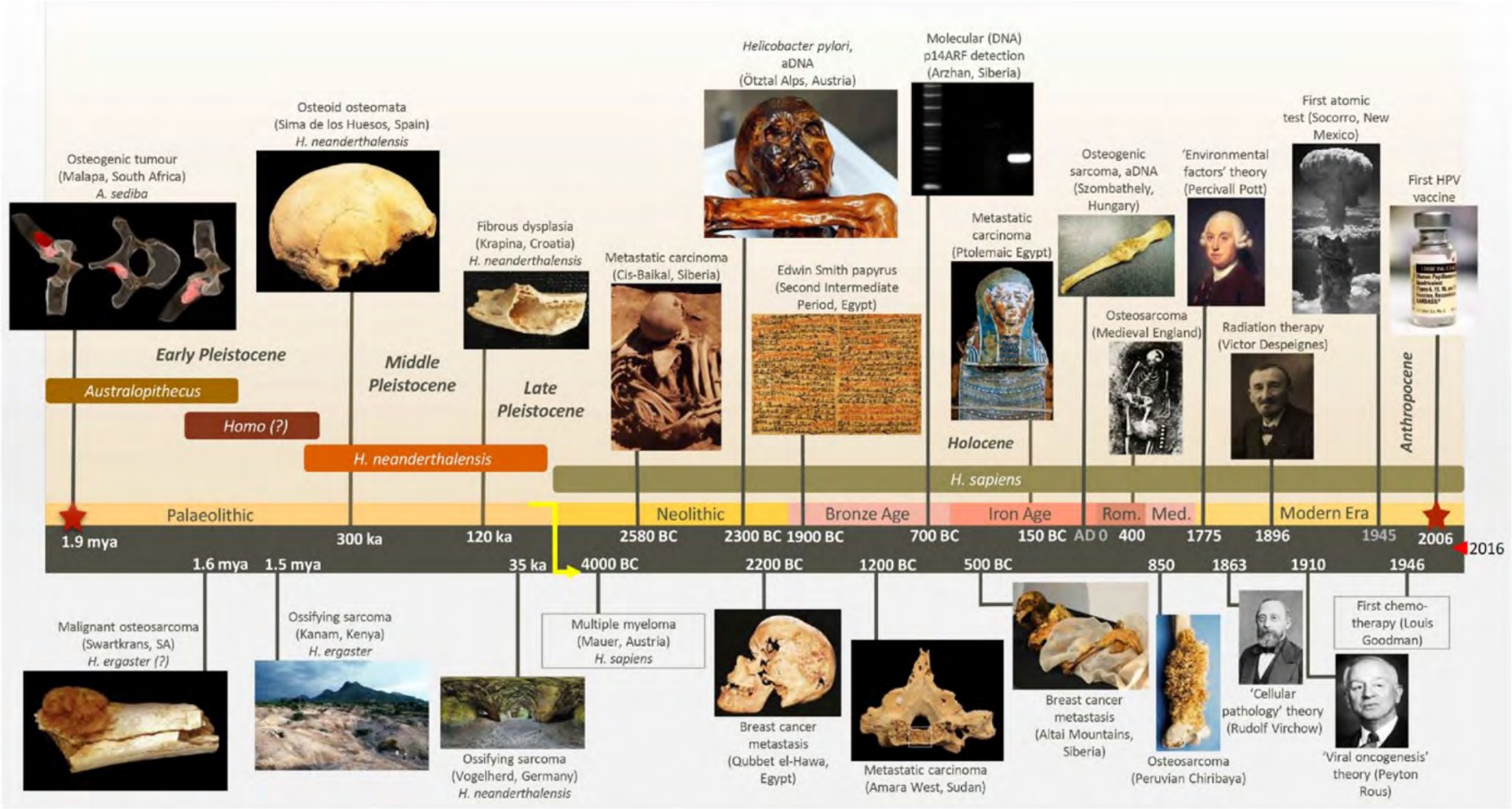


FIGURE 1 Chronological incidence of prehistoric oncogenic tumours and important milestones concerning cancer aetiology and treatment (Binder et al., 2014; Bona et al., 2014; Monge et al., 2013; Odes et al., 2016; Phelan et al., 2007; Randolph-Quinney et al., 2016) ('Rom.' and 'Med.' referes to Roman and Medieval Periods, respectively).

Case Reports > Lancet Oncol. 2020 Aug;21(8):1021-1022.
doi: 10.1016/S1470-2045(20)30171-6.

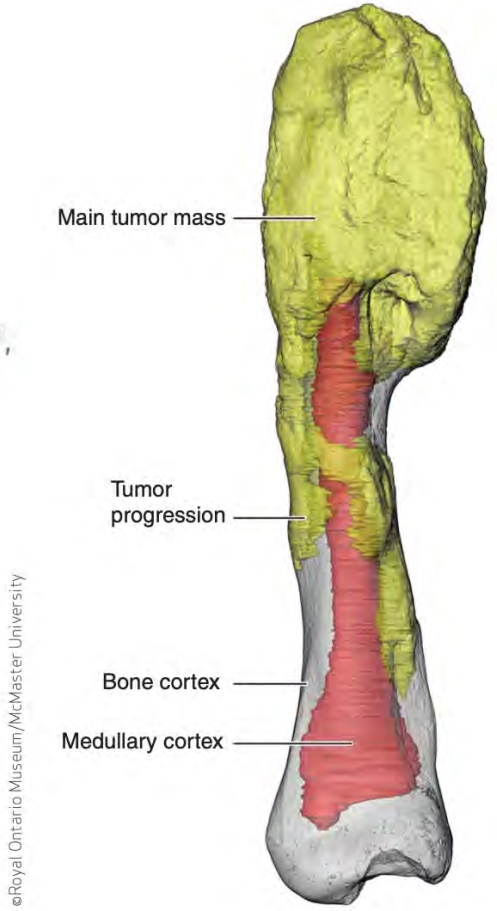
First case of osteosarcoma in a dinosaur: a multimodal diagnosis

Seper Ekhtiari [1], Kentaro Chiba [2], Snezana Popovic [3], Rhianne Crowther [4], Gregory Wohl [5], Andy Kin On Wong [6], Darren H Tanke [7], Danielle M Dufault [8], Olivia D Geen [3], Naveen Parasu [9], Mark A Crowther [3], David C Evans [10]

Affiliations + expand

PMID: 32758461 DOI: 10.1016/S1470-2045(20)30171-6

Researchers discovered osteosarcoma in the fibula (shown in red) of a horned dinosaur, *Centrosaurus apertus*, estimated to be 76 million years old.



In this CT-based 3-D reconstruction of the cancerous fibula, the main tumor mass (yellow) is at the top of the bone. Gray indicates normal bone, and red denotes the medullary cavity.

Classical Cell Theory: Cancer

1665 Discovery of Cells

In 1665, Robert Hooke published *Micrographia*, a book filled with drawings and descriptions of the organisms he viewed under the recently invented microscope. While looking at cork, Hooke observed box-shaped structures, which he called “cells” as they reminded him of the cells, or rooms, in monasteries. This discovery ultimately led to the development of the classical cell theory.

1839 Cell Theory

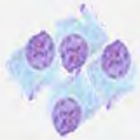
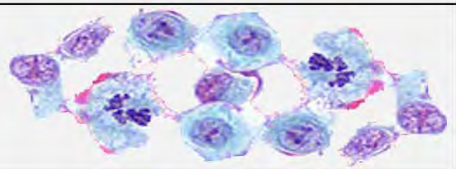
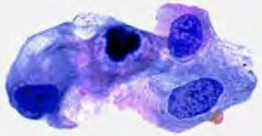
The classical cell theory is proposed by Theodor Schwann (and others):

1. All organisms are made of cells.
2. Cells are the basic units of life.
3. Every cell arises from a preexisting cell that has multiplied.

Rudolf Virchow 1821–1902

Famous German Pathologist. His first scientific paper included the pathological description of leukemia, a term he invented. Contributed to cell theory by proposing that all cells, including cancer cells, are derived from other cells.

Characteristics of cancer cells and tissues

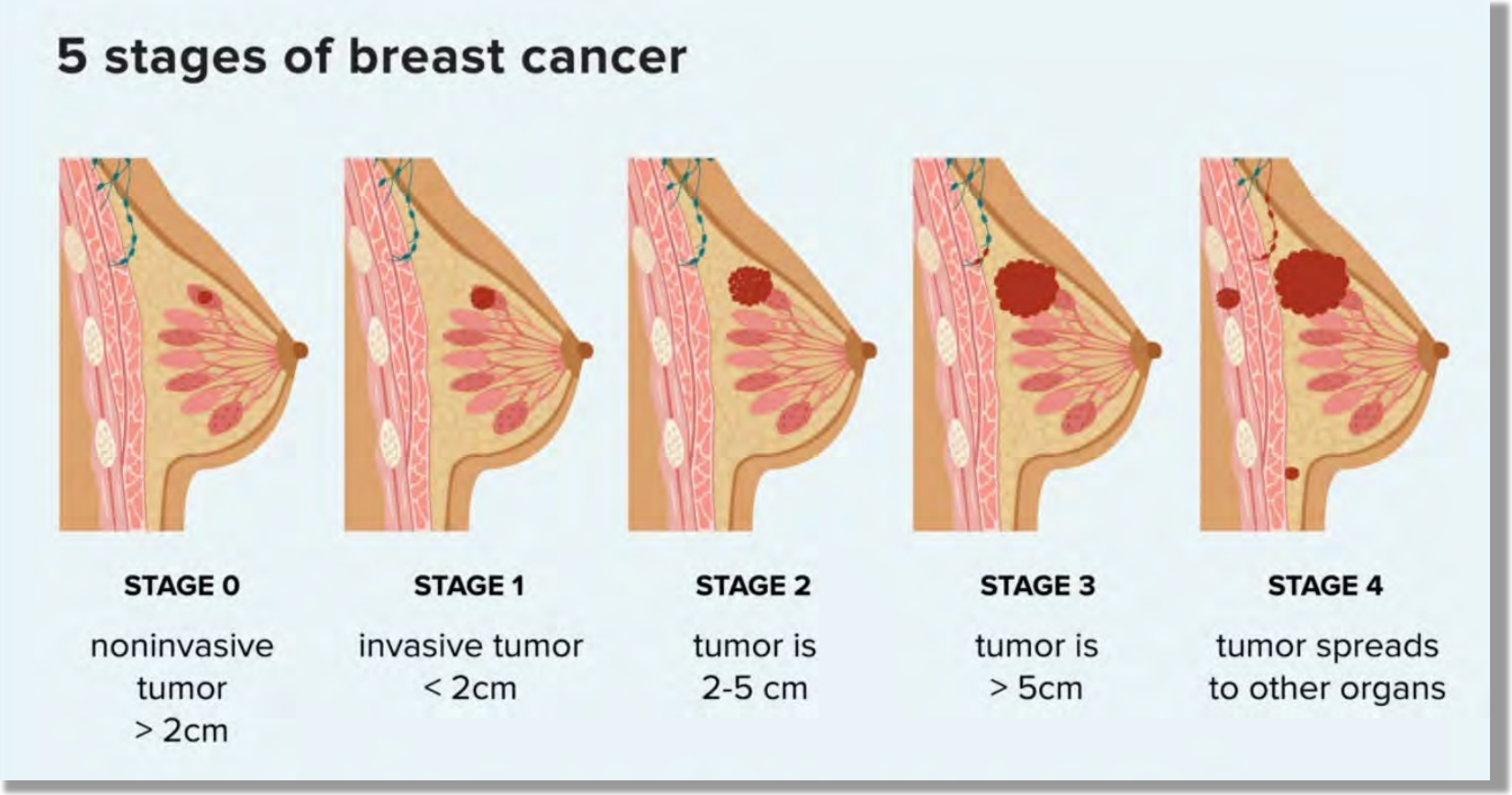
Normal	Cancer	
		Large, variably shaped nuclei
		Many dividing cells; Disorganized arrangement
		Variation in size and shape
		Loss of normal features

Used by pathologists to determine relative aggressiveness of disease

<u>Histological staining:</u>

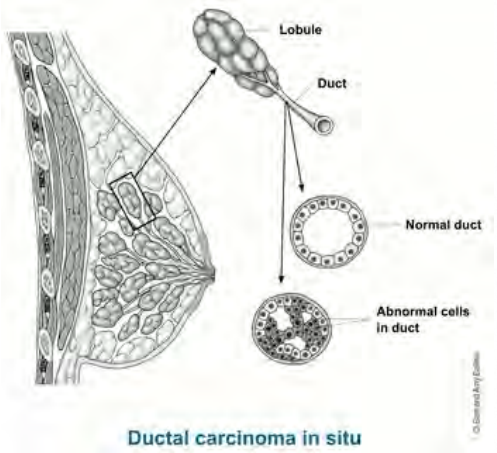
- Hematoxylin (nucleic acids, purple)
- Eosin (proteins, pink)

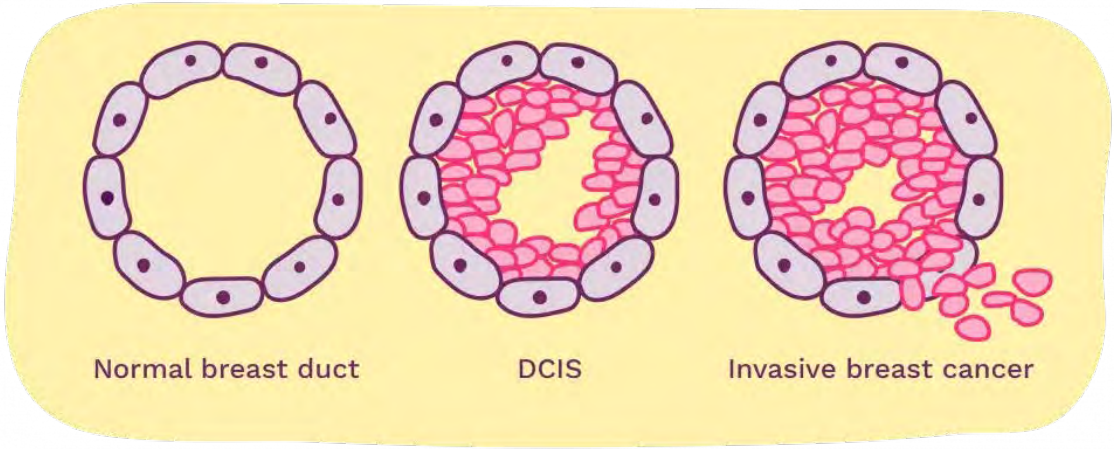
Breast Cancer:



Breast Cancer:

- Ductal Carcinoma In Situ (DCIS) represents 20 - 25% of newly diagnosed breast cancers in the United States
- Diagnosis is made by histologic examination of tissue obtained via needle core biopsy, lumpectomy or mastectomy





Breast Cancer Grading

Pathologists examine:

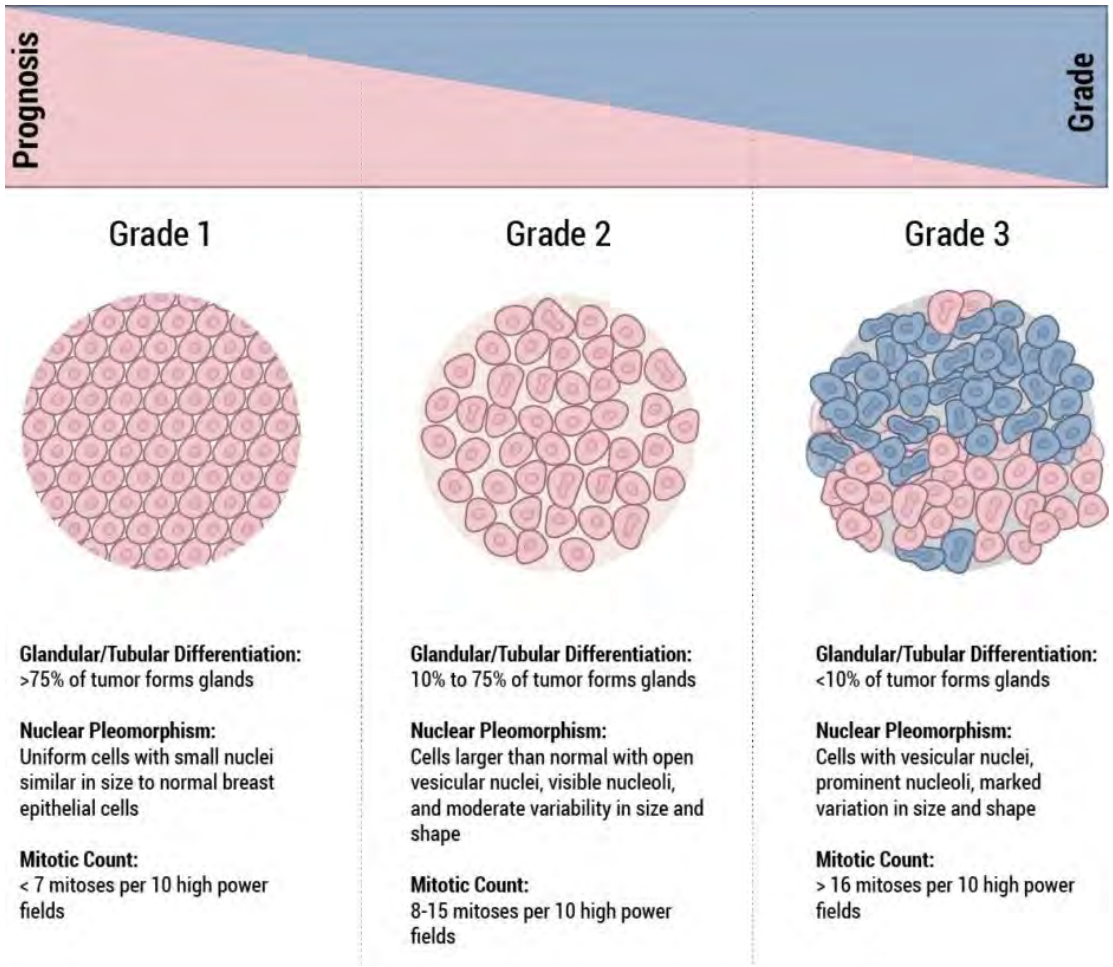
- gland formation
- nuclear grade
- mitotic index

Scores are applied to each category are added up to assign grade:

•If the numbers add up to 3-5, the cancer is grade 1 (well differentiated).

•If they add up to 6 or 7, it means the cancer is grade 2 (moderately differentiated).

•If they add up to 8 or 9, it means the cancer is grade 3 (poorly differentiated).



Breast Cancer Grading

Pathologists examine:

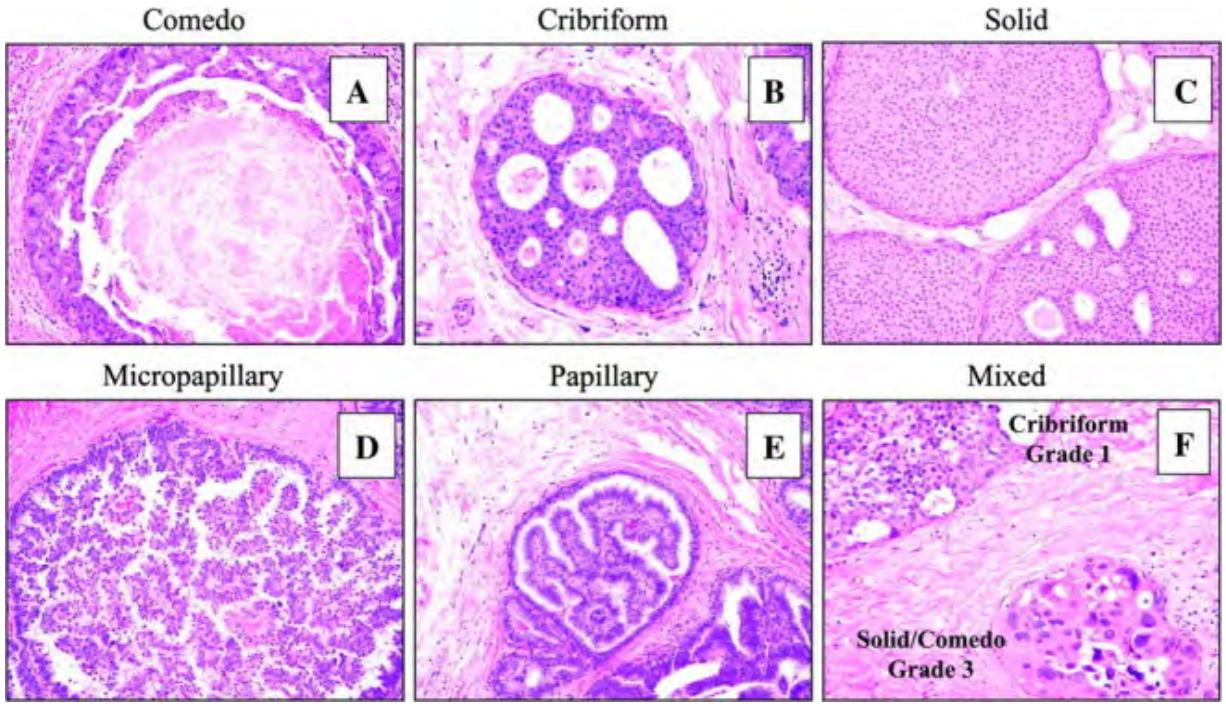
- gland formation
- nuclear grade
- mitotic index

Scores are applied to each category are added up to assign grade:

•If the numbers add up to 3-5, the cancer is grade 1 (well differentiated).

•If they add up to 6 or 7, it means the cancer is grade 2 (moderately differentiated).

•If they add up to 8 or 9, it means the cancer is grade 3 (poorly differentiated).



Also staining (IHC) for molecular markers ER/PR, Her2

Breast Cancer Grading

Pathologists examine:

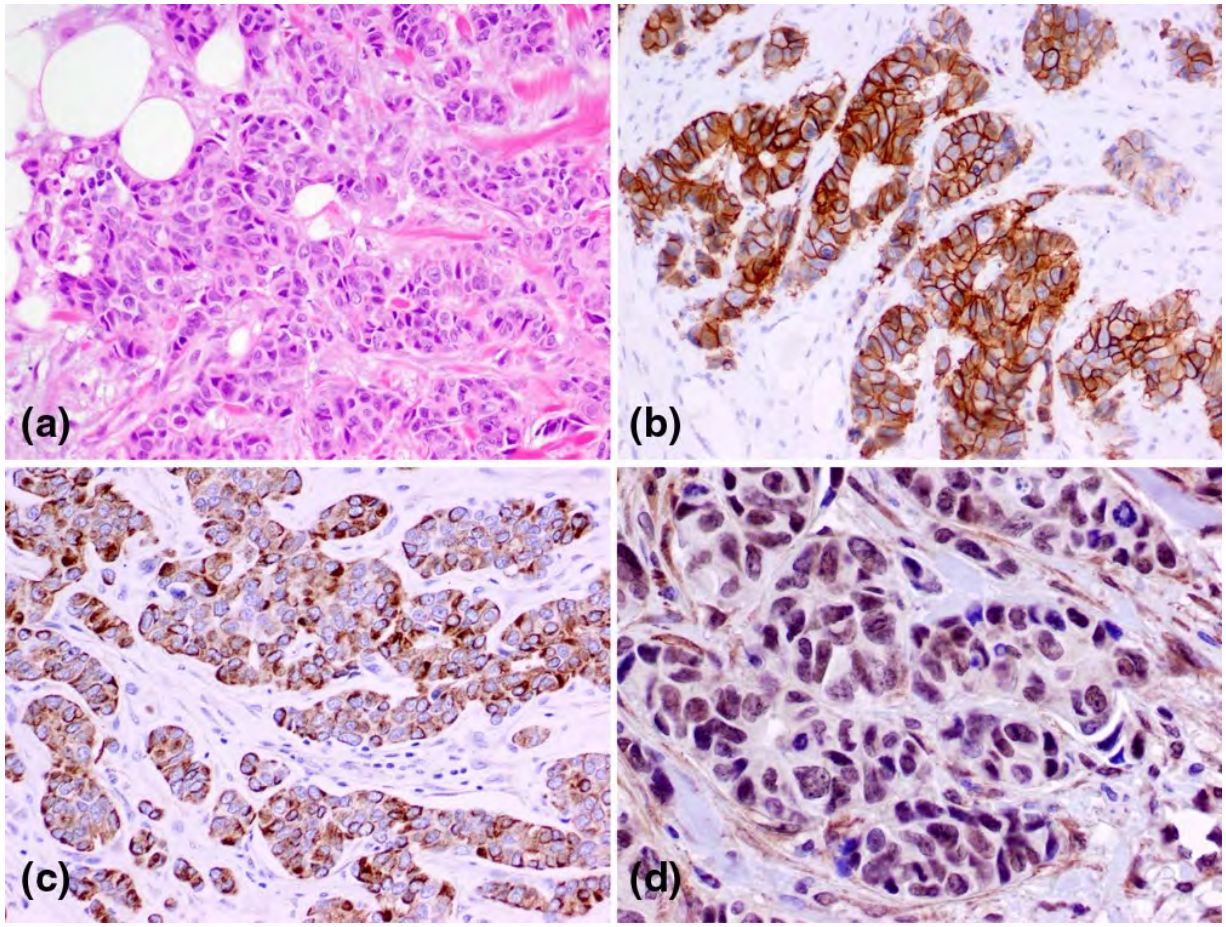
- gland formation
- nuclear grade
- mitotic index

Scores are applied to each category are added up to assign grade:

•If the numbers add up to 3-5, the cancer is grade 1 (well differentiated).

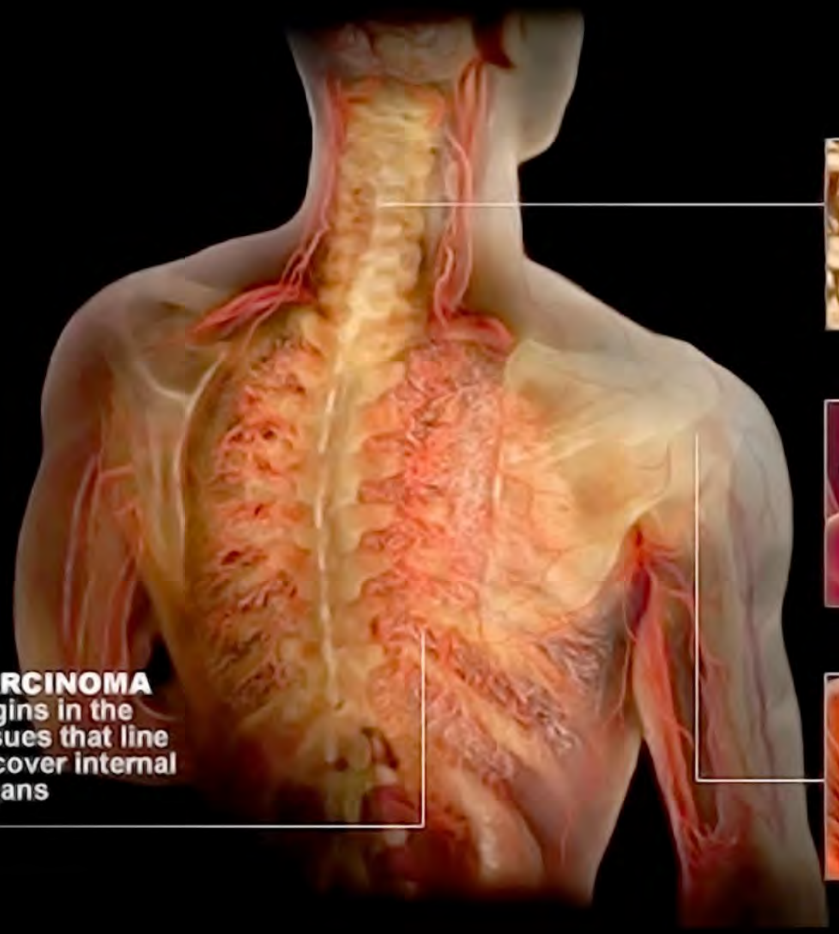
•If they add up to 6 or 7, it means the cancer is grade 2 (moderately differentiated).

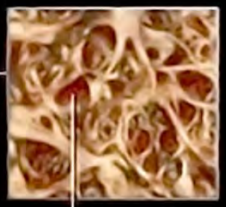
•If they add up to 8 or 9, it means the cancer is grade 3 (poorly differentiated).



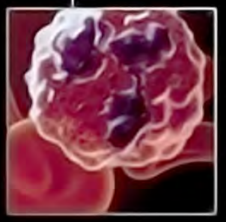
Types of Cancer

Cancer is not one, but over one hundred different diseases. They are grouped into four main categories: carcinoma, leukemia, lymphoma and myeloma, and sarcoma. Over 80% of all cancer cases are carcinomas.

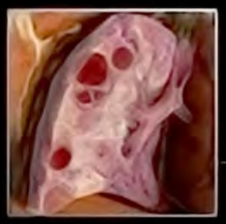




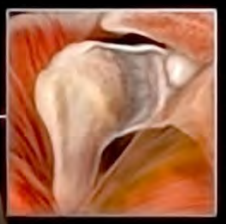
LEUKEMIA
Starts in blood-forming tissue, such as bone marrow, and sends abnormal blood cells into the bloodstream



LYMPHOMA & MYELOMA
Originates in the cells of the immune system



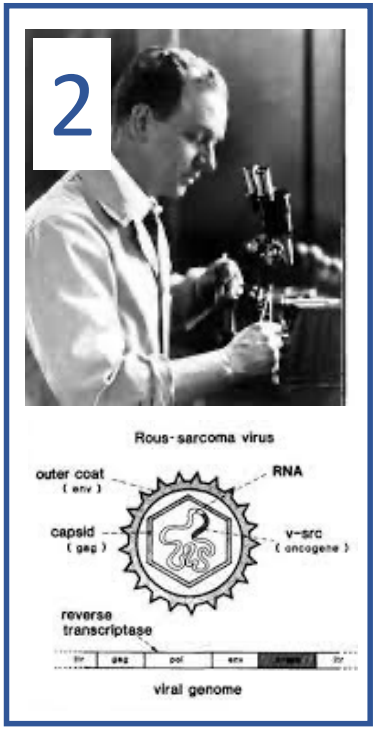
CARCINOMA
Begins in the tissues that line or cover internal organs

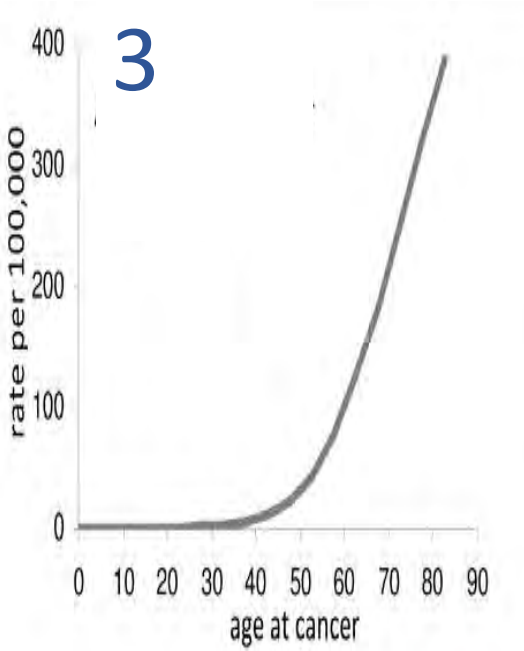


SARCOMA
Starts in bone, muscle, cartilage, fibrous tissue, or fat

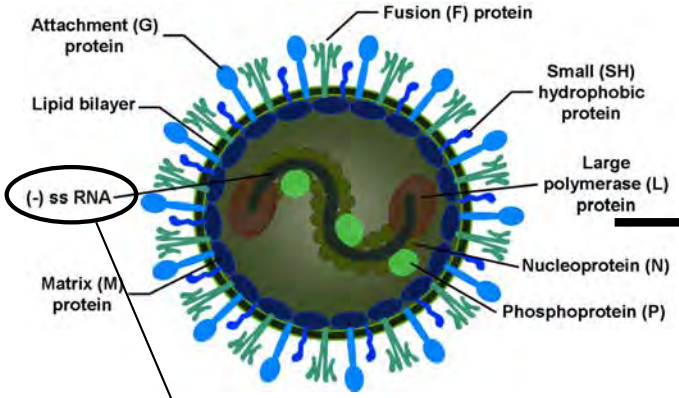
Development of modern knowledge about cancer causes

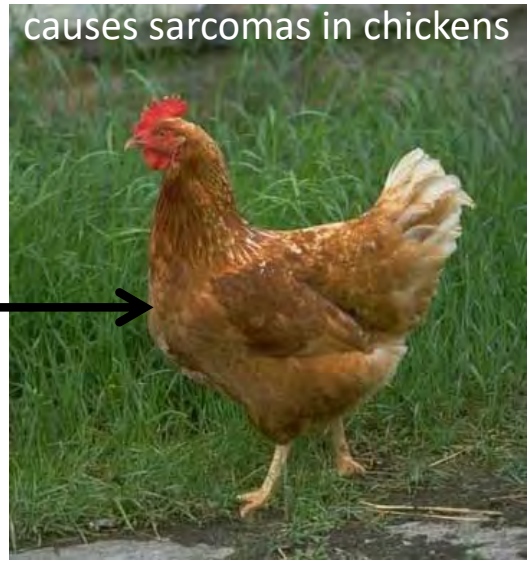
1. 1700's – toxic exposures (chimney sweeps)
2. 1900's – viruses
3. 1900's – aging

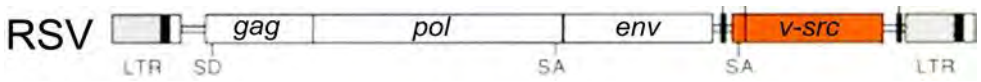




Rous sarcoma virus (RSV) – RNA tumor virus







gag – capsid proteins

pol – reverse transcriptase

env – envelope proteins

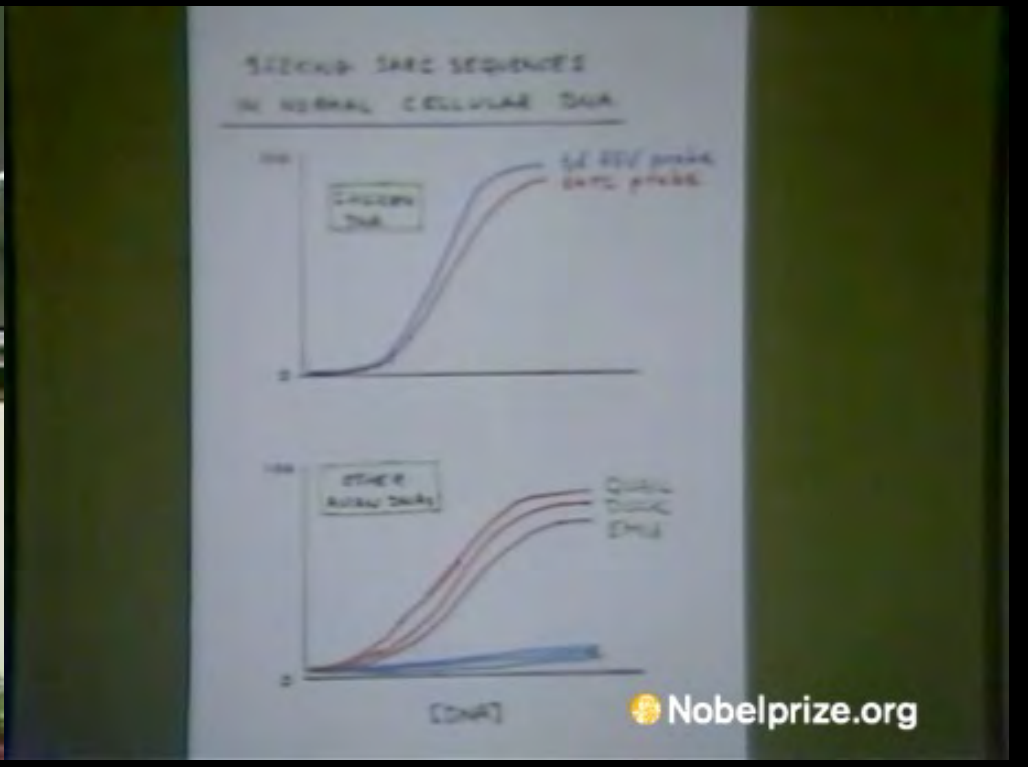
v-src – transforming gene – const/act tyrosine kinase

**v-src is mutated form of cellular proto-oncogene c-src

oncogenes cause cancer in animals

http://www.kuleuven.be/rega/mvr/images/RSV-1.jpg

Harold E. Varmus Nobel Lecture on 8 December 1989 at Karolinska Institutet, Stockholm



Nobelprize.org

nature

Explore content ~ About the journal ~ Publish with us ~ Subscribe

nature > letters > article

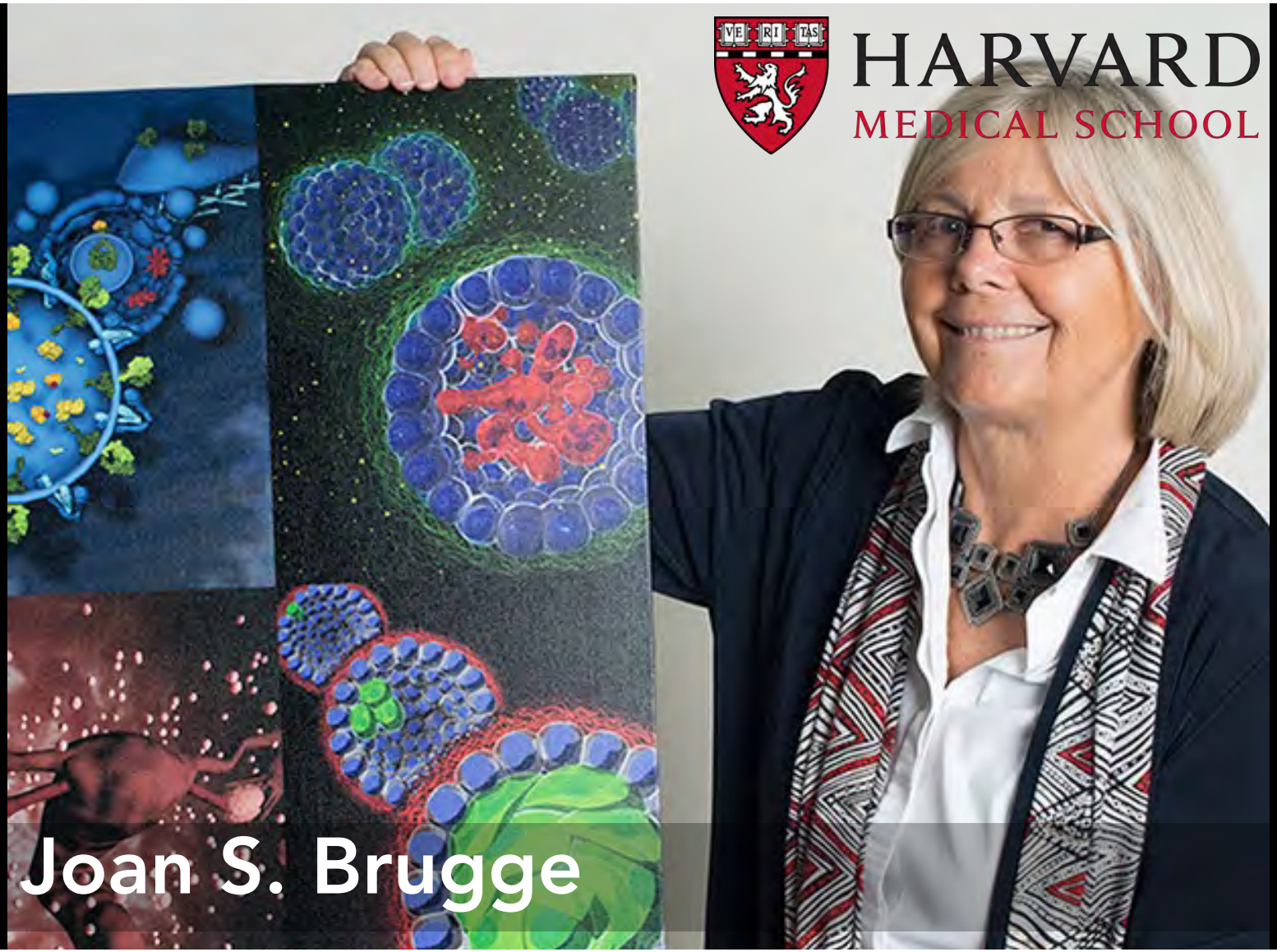
Published: 01 September 1977

Identification of a transformation-specific antigen induced by an avian sarcoma virus

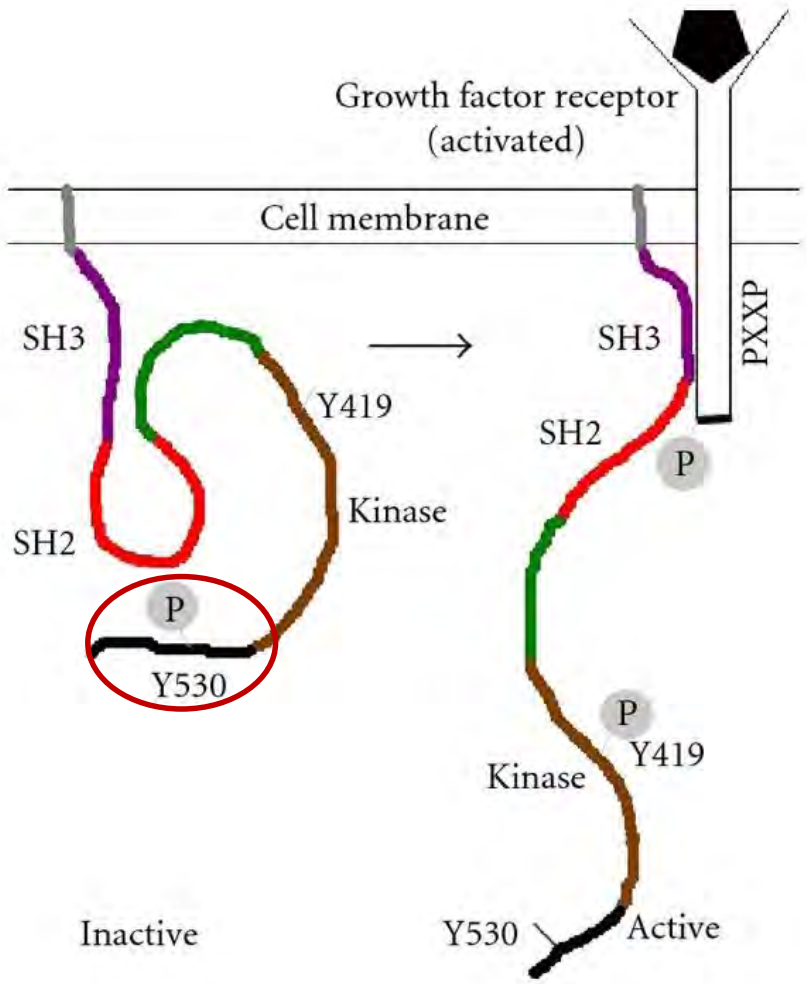
JOAN S. BRUGGE & R. L. ERIKSON

Abstract

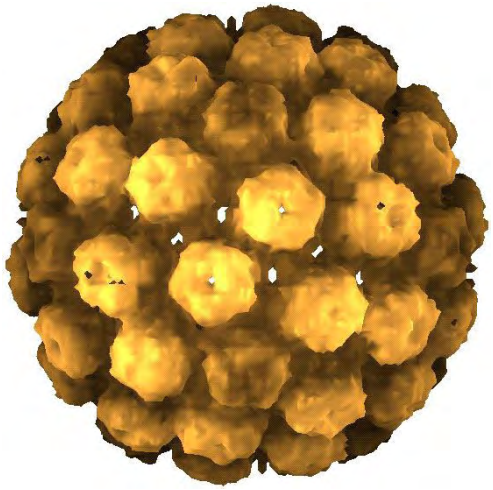
GENETIC analyses of avian sarcoma viruses (ASV) have led to the identification of a gene, designated *src*, which encodes a product required for the initiation and maintenance of neoplastic transformation in infected fibroblasts[1–5]. Because the *src* gene product has not been identified biochemically, this study was initiated to detect a transformation-specific protein, using serum from rabbits bearing ASV-induced tumours. We describe here the identification of a 60,000-MW transformation-specific antigen detectable in ASV-transformed chicken cells and ASV-induced hamster tumour cells by immunoprecipitation of radiolabelled cell extracts with serum from tumour-bearing rabbits. Moreover, the expression of this antigen is temperature dependent in chicken cells transformed by an ASV temperature-sensitive mutant in the *src* gene. The use of this antiserum may lead to the unequivocal identification and characterisation of the ASV *src* gene product and this, in turn, may lead to the elucidation of the mechanism of ASV-induced oncogenesis.


VE RI TAS
HARVARD
MEDICAL SCHOOL
Joan S. Brugge

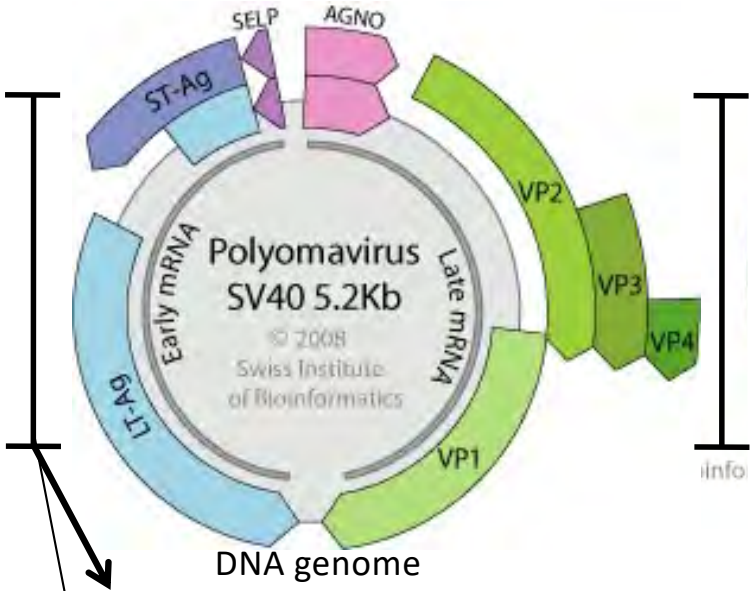
The v-Src mutation relieves autoinhibition of kinase activity



Simian Virus 40 (SV40) – DNA tumor virus



Simian Virus 40 (SV40) – DNA tumor virus



Late gene products – viral structural proteins

Early gene products – activate replication

- SV40 virus encodes no variants of *cellular* genes – must produce *viral* proteins that activate cellular proteins
- Host animals infected with SV40 produce antibodies to viral proteins, these antibodies can be used to interrogate viral – host protein complexes
- Viral proteins recognized by host antibodies called *tumor antigens*
  - Large Tumor Antigen (LT-Ag), **Small Tumor Antigen (ST-Ag)**

Genetic analyses showed LT-Ag required for initiation and maintenance of transformation

1979 *Immunoprecipitation of LT-Ag from cells infected with SV40*

53kD protein co-immunoprecipitated with LT-Ag

Following excision of both bands from the gel, and protein extraction and renaturation, LT-Ag antibody bound to LT-Ag protein (T) but not 53K –

This shows that the 53K protein bound to LT-Ag and not the antibody

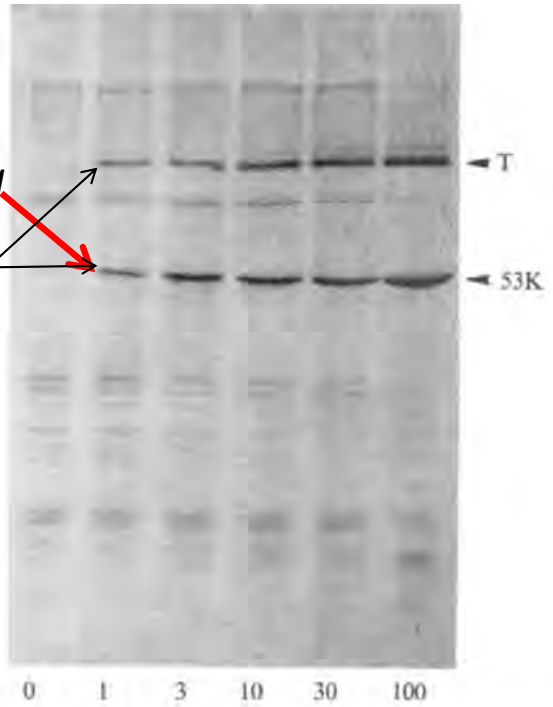


Fig. 1 Quantitation of T and 53K immunoprecipitation by rabbit anti-T serum. An equivalent aliquot of an NP40 cell extract of ^{35}S-methionine-labelled SVA31E7 cells was added to each of 6 tubes followed by normal rabbit serum and rabbit anti-T serum in the following respective amounts: track 0, 30 µl, 0 µl; track 1, 29 µl, 1 µl; track 3, 27 µl, 3 µl; track 10, 20 µl, 10 µl; track 30, 0 µl, 30 µl; track 100, 0 µl, 100 µl. After 3 h incubation at 4 °C, 100 µl of a 10% suspension of fixed *Staphylococcus aureus*[20] was added to each tube and following a further 10 min incubation the bacteria were washed 3 times in NET buffer[20] and collected by centrifugation. Bound proteins were eluted in 55 µl of sample buffer and 10 µl of each eluate loaded on a 7–20% linear gradient acrylamide gel. The dried gel was autoradiographed for 24 h.

T antigen is bound to a host protein in SV40-transformed cells

D. P. LANE*

Department of Zoology, Imperial College London SW7, UK

L. V. CRAWFORD

Department of Molecular Virology, Imperial Cancer Research Fund, London WC2, UK

Nature Vol. 278 15 March 1979

The expectation was that
p53 might be an oncogene:

- Retroviruses were known to promote tumor formation by expressing “hijacked” cellular genes.
- p53 expression levels correlated with tumorigenicity
  - temperature-sensitive alleles of LT-Ag supported high p53 expression only at the permissive temperature
  - tumors harboring no SV40 expressed higher levels of p53 than normal tissues

1984 *p53 cDNAs promoted tumorigenesis*

Table 1 Transforming of REFs and Rat-1 cells after co-transfection of the p53-encoding gene and the EJ-c-Ha-*ras* oncogene

Transfected gene	No of foci per 10^5 cells REFs	RAT-1		Tumorigenicity of REFs nude mice (No. of tumours/no. of injections)
REF/DNA	0	0		0/10
pEJ6.6	0	2,000–2,500	Focus	0/20
psvcmyc-1	0	0		0/15
ras + myc	200–300	2,000–2,500		†29/29
pL8R6	0	0		0/5
pL8R20	0	0		ND
p53 clone1 + ras	20–30	2,000–2,500		‡6/6
p53 clone2 + ras	10–20	2,000–2,500		§3/3
pL8R6 + pSVcmyc-1	0	0		0/6
pL8R20 + pSVcmyc-1	0	0		ND
*pL8R6 (*Bam*HI/*Hin*dIII + pEJ6.6)	0	ND		0/3

Cooperation between gene encoding p53 tumour antigen and *ras* in cellular transformation

Luis F. Parada, Hartmut Land, Robert A. Weinberg, David Wolf* & Varda Rotter*

Whitehead Institute for Biomedical Research, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 20139, USA
* Department of Cell Biology, Weizmann Institute, Rehovoth 76100, Israel
NATURE VOL. 312 13 DECEMBER 1984

Conclusion: p53 is an oncogene

1984

p53 cDNAs promoted tumorigenesis

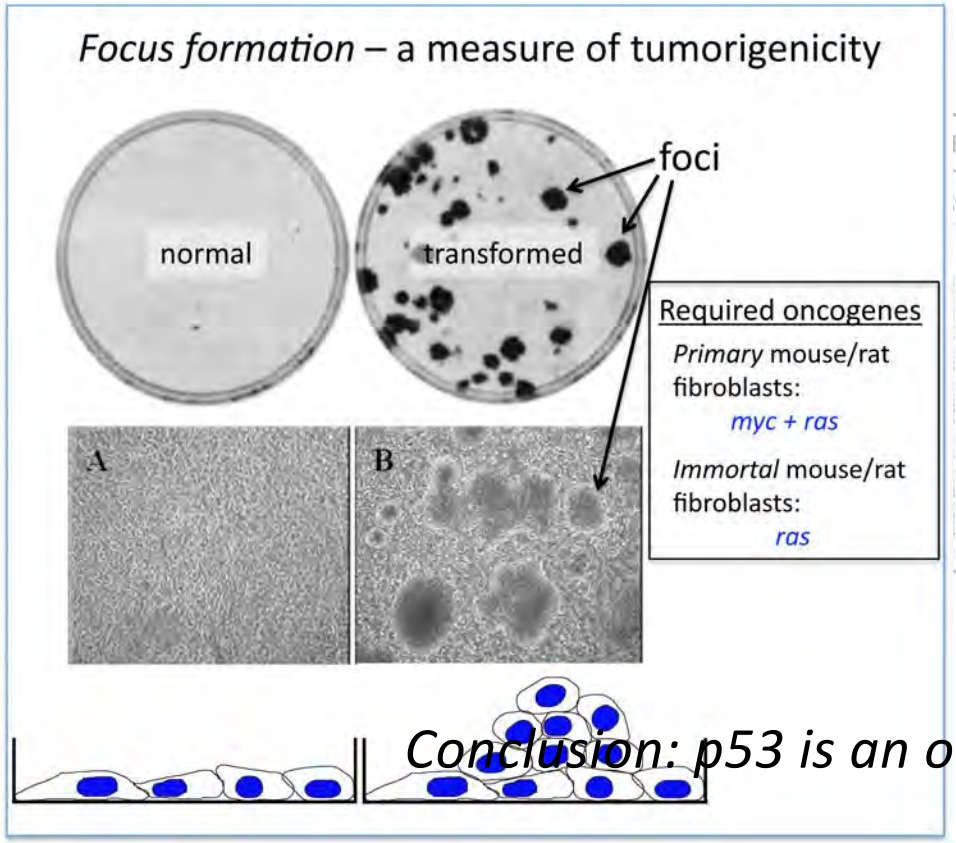


Table 1 Transfor

Transfected gen

REF/DNA
pEJ6.6
psvcmyc-1
ras + myc
pL8R6
pL8R20
p53 clone1 + ras
p53 clone2 + ras
pL8R6 + pSVcmyc-1
pL8R20 + pSVcmyc-1
*pL8R6 (*BamHI/*Hind*

EJ-c-Ha-*ras* oncogene

enicity of REFs nude mice
tumours/no. of injections)

0/10
0/20
0/15
†29/29
0/5
ND
‡6/6
§3/3
0/6
ND
0/3

Cooperation bet
p53 tumour anti
ras in cellular t

Luis F. Parada, Hartm
David Wolf* & Varda

Whitehead Institute for Biomedical Research, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 20139, USA
* Department of Cell Biology, Weizmann Institute, Rehovoth 76100,

Conclusion: p53 is an oncogene

PNAS **April 29, 2008 vol. 105 no. 17 6215-622**
Journal of Undergraduate Research Volume 4, Issue 4 - December 2002Retroviral Transfer of cDNA Libraries to NIH3T3 Cells; Cloning of Novel Oncogenes from the Leukemic Cell Line HL-60 Lorch, D.

› Int J Cancer. 1968 Sep 15;3(5):683-93. doi: 10.1002/ijc.2910030517.

Anchorage and growth regulation in normal and virus-transformed cells

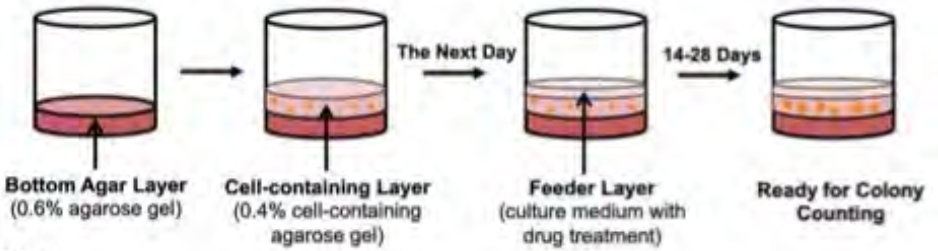
M Stoker, C O'Neill, S Berryman, V Waxman

Abstract

Many cell types will grow when attached to a rigid surface but not in suspension, a phenomenon termed „anchorage dependence". Anchorage dependence can be studied by incorporating solid particles of varying size into gels. It has been found that colonies will form on glass fibrils 500 μ in length, but not in the presence of silica fragments smaller than the cells. This shows that the suspending medium is not itself inhibitory, and confirms the requirement for a rigid surface of adequate size.

The state of inhibited cells in suspension culture was examined by dispersing them in a methyl cellulose gel, in vessels lined with agar. In this system aggregation is prevented and the cells may be recovered quantitatively. Normal, as well as transformed, cells increase in size, and a proportion synthetize DNA during the first 24 hours in suspension culture. Growth and DNA synthesis in normal cells then virtually cease, while transformed cells continue to grow into colonies. The stationary normal cells remain competent for further growth for at least a week in suspension. When such cells are allowed to attach to a rigid surface in the presence of colchicine, DNA synthesis occurs and is followed by mitosis. These results indicate that suspended cells are blocked between mitosis and the end of the S phase of the cycle.

Soft agar assay



p53 is an oncogene

1984 **Cell immortalization and transformation by the p53 gene.**
Nature. 1984 Dec13-19;312(5995):596-7.

1984 **Participation of p53 cellular tumour antigen in transformation of normal embryonic cells.**
Nature. 1984 Dec13-19;312(5995):646-9.

1984 **Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein p53.**
Nature. 1984 Dec13-19;312(5995):651-4

1985 **The cellular oncogene p53 can be activated by mutagenesis.**
Nature. 1985 Oct 31-Nov 6;317(6040):816-8

1986 **Expression of the p53 oncogene in acute myeloblastic leukemia.**
J Exp Med. 1986 Sep 1;164(3):751-61

1987 **p53 in Paris, an oncogene comes of age.**
Oncogene. 1987;1(3):241-2.

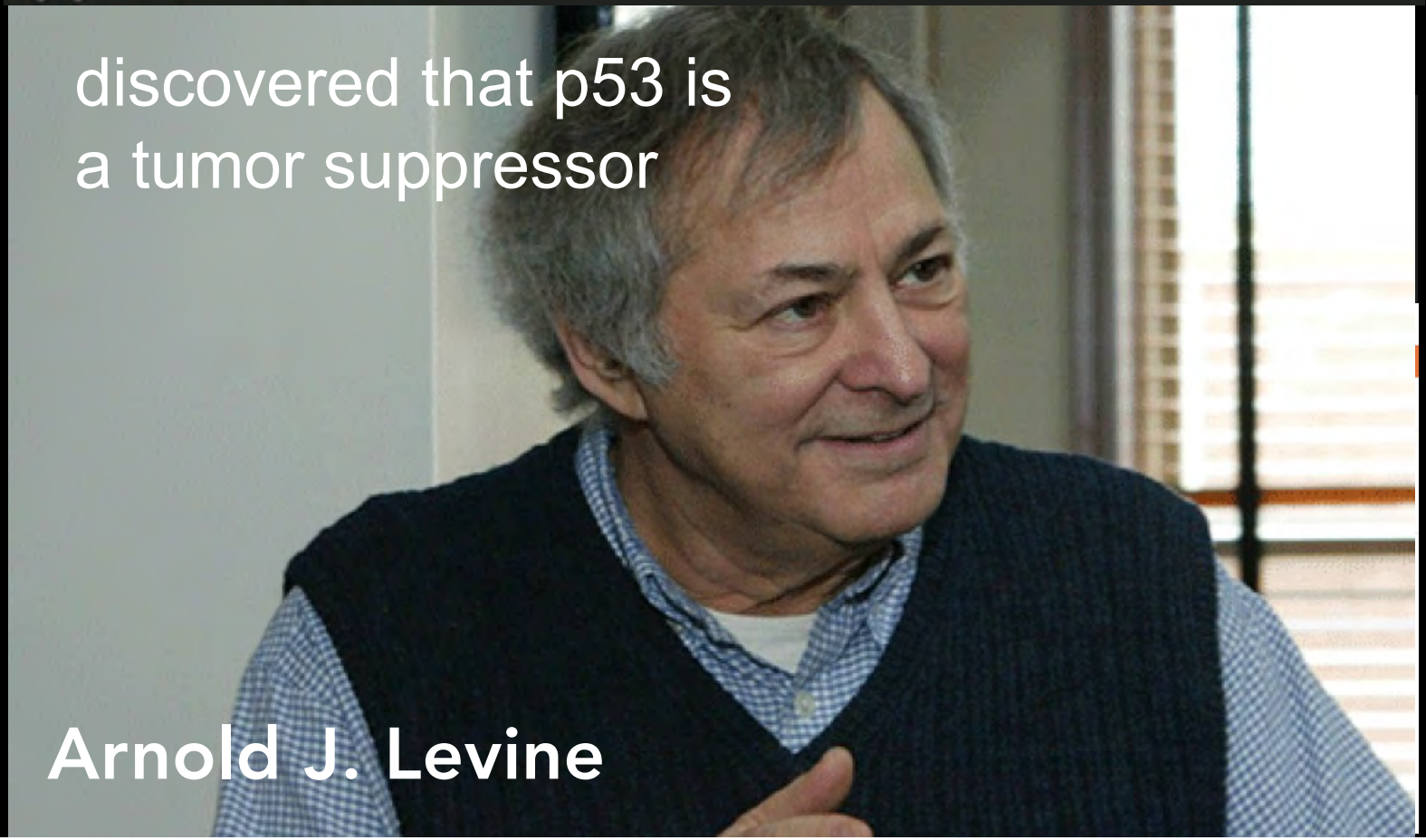
There was just one problem -

Cell, Vol. 57, 1083–1093, June 30, 1989,

The p53 Proto-Oncogene Can Act as a Suppressor of Transformation

Cathy A. Finlay, Philip W. Hinds, and Arnold J. Levine
Princeton University
Department of Biology
Princeton, New Jersey 08540-1014

Department of
MOLECULAR
BIOLOGY



1989 *The p53 gene is inactivated in human cancers*

Earlier that year, the Vogelstein group published that both p53 alleles were disrupted in colorectal carcinomas, one by mutation and one by deletion, fitting the two-hit model for tumor suppressors put forward by Knudson studying retinoblastoma

Chromosome 17 Deletions and p53 Gene Mutations in Colorectal Carcinomas

Suzanne J. Baker, Eric R. Fearon, Janice M. Nigro, Stanley R. Hamilton, Ann C. Preisinger, J. Milburn Jessup, Peter vanTuinen, David H. Ledbetter, David F. Barker, Yusuke Nakamura, Ray White, Bert Vogelstein*

Previous studies have demonstrated that allelic deletions of the short arm of chromosome 17 occur in over 75% of colorectal carcinomas. Twenty chromosome 17p markers were used to localize the common region of deletion in these tumors to a region contained within bands 17p12 to 17p13.3. This region contains the gene for the transformation-associated protein p53. Southern and Northern blot hybridization experiments provided no evidence for gross alterations of the p53 gene or surrounding sequences. As a more rigorous test of the possibility that p53 was a target of the deletions, the p53 coding regions from two tumors were analyzed; these two tumors, like most colorectal carcinomas, had allelic deletions of chromosome 17p and expressed considerable amounts of p53 messenger RNA from the remaining allele. The remaining p53 allele was mutated in both tumors, with an alanine substituted for valine at codon 143 of one tumor and a histidine substituted for arginine at codon 175 of the second tumor. Both mutations occurred in a highly conserved region of the p53 gene that was previously found to be mutated in murine p53 oncogenes. The data suggest that p53 gene mutations may be involved in colorectal neoplasia, perhaps through inactivation of a tumor suppressor function of the wild-type p53 gene.

Science. 1989 Apr 14;244(4901):217-21.

1990 *p53 is a tumor suppressor in humans by genetic criteria*

- *Li-Fraumeni syndrome (LFS) – dominantly inherited cancer predisposition syndrome – childhood and adult tumors*
- *LFS families carry germline mutations in p53*
  - *~1/2 of tumors lose the remaining wtp53 allele*

Germ Line p53 Mutations in a Familial Syndrome of Breast Cancer, Sarcomas, and Other Neoplasms

David Malkin, Frederick P. Li, Louise C. Strong, Joseph F. Fraumeni, Jr., Camille E. Nelson, David H. Kim, Jayne Kassel, Magdalena A. Gryka, Farideh Z. Bischoff, Michael A. Tainsky, Stephen H. Friend*

Science 30 November 1990

1992 *p53 is a tumor suppressor in mice by genetic criteria*

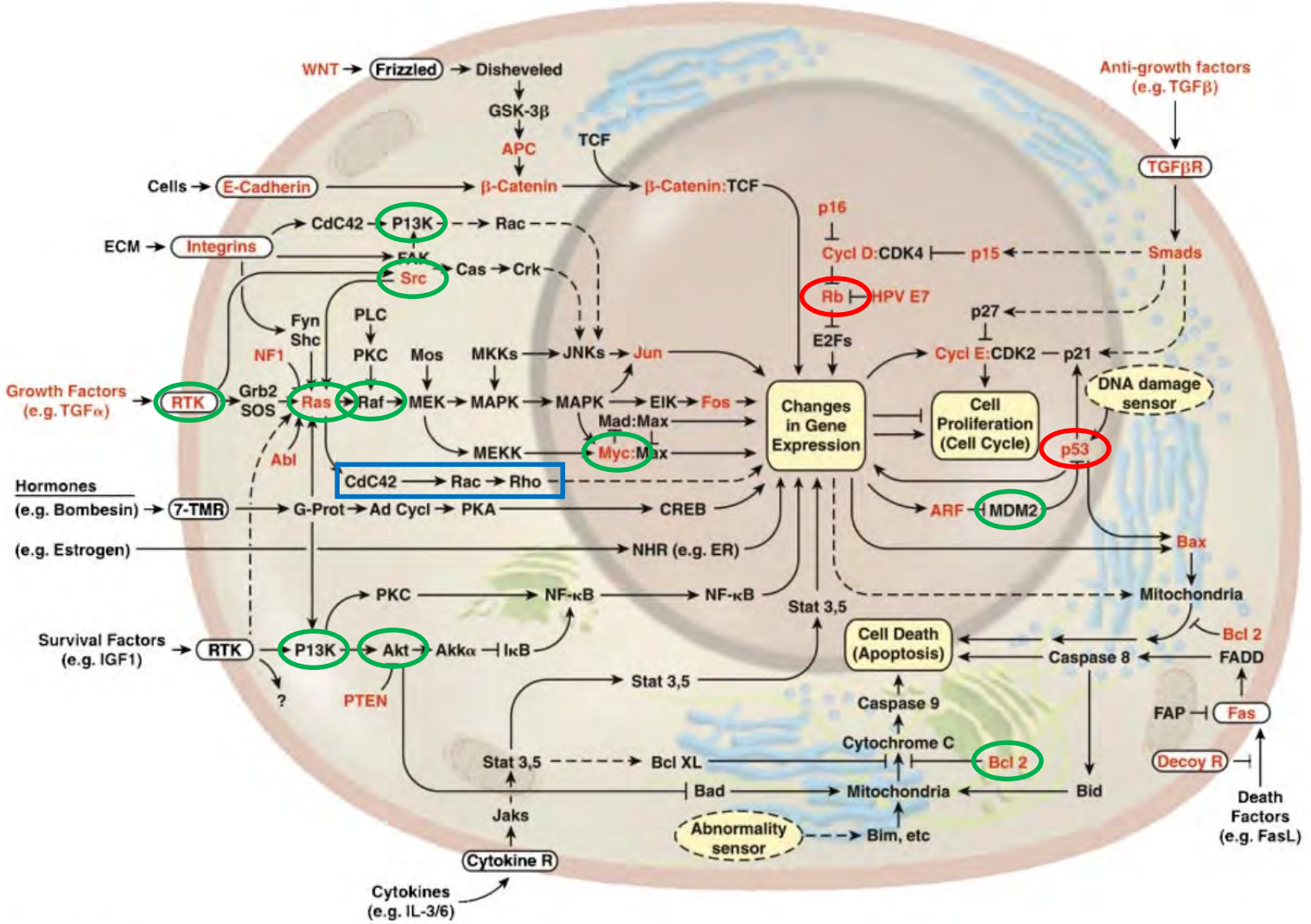
- *~75% of p53-/-mice develop tumors by 6 months of age (mostly thymic lymphoma)*
- *>50% of p53+/-mice develop tumors by 18 months of age with different spectrum: (sarcoma > lymphoma > carcinoma)*
  - *~1/2 of tumors lose the remaining wtp53 allele*

Spontaneous and carcinogen-induced tumorigenesis in p53-deficient mice

Michele Harvey[1], Mark J. McArthur[2], Charles A. Montgomery Jr.[2], Janet S. Butel[1], Allan Bradley[3] & Lawrence A. Donehower[1]

nature *genetics* volume 5 november 1993

Cancer Signaling Pathways



Discovered through *viruses*

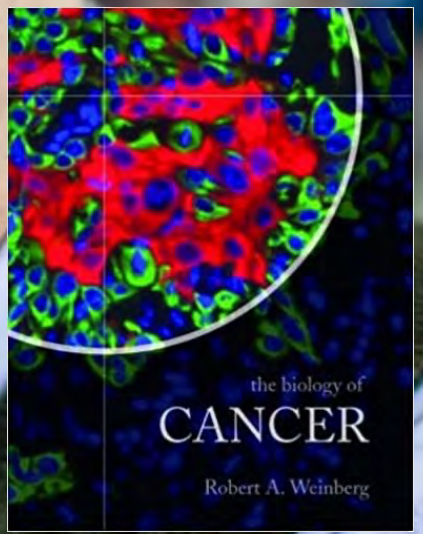
- Akt
- EGFR
- p53
- PI3Kinase
- Raf
- Ras
- Src

Discovered through *cancer genetics*

- Brca1/2
- Bcl-2
- Mdm-2
- Myc
- RB

Oncogenes
Tumor suppressors

discovered of the first human oncogene Ras and the first tumor suppressor gene Rb



BROAD INSTITUTE

Biology

Robert A. Weinberg

nature

Explore content ⌄ About the journal ⌄ Publish with us ⌄ Subscribe

nature › articles › article

Published: 10 June 1982

Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus *ras* gene

Luis F. Parada, Clifford J. Tabin, Chiaho Shih & Robert A. Weinberg

Nature **297**, 474–478 (1982) | Cite this article

Abstract

Examination of homologies between retroviral oncogenes and
defined by transfection reveals that the human bladder carcino
homologous to the Harvey sarcoma virus oncogene (*ras*). Structural analysis limits the region of homology to a 3.0-kilobase *Sac*I fragment of the EJ oncogene. Both EJ and *ras* DNA probes detect similar transcripts in transfectants derived from bladder carcinoma cell lines.

nature

Explore content | About the journal | Publish with us | Subscribe

nature > articles > article

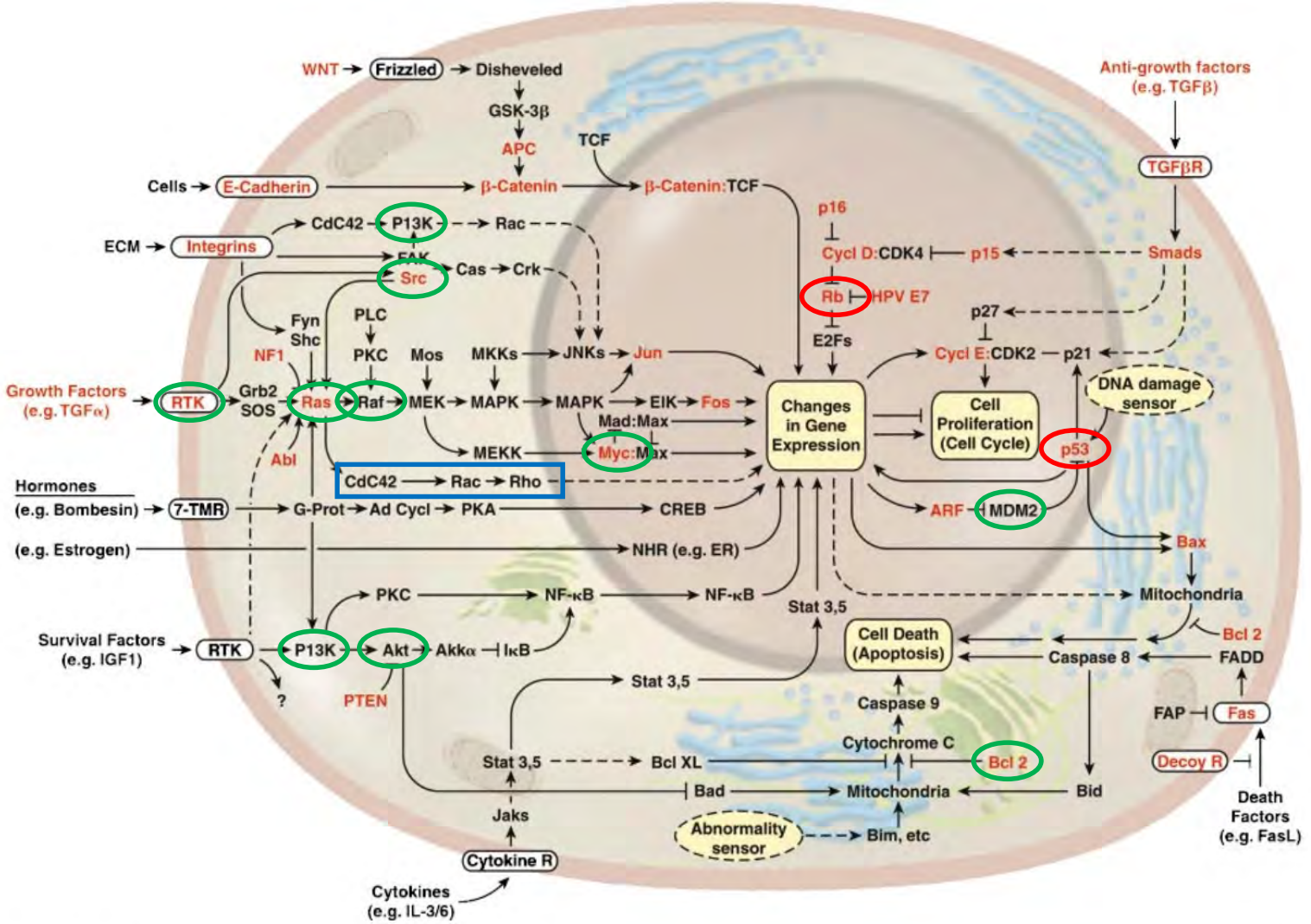
Published: 02 June 1983

Identification of transforming gene in two human sarcoma cell lines as a new member of the *ras* gene family located on chromosome 1

Alan Hall, Christopher J. Marshall, Nigel K. Spurr & Robin A. Weiss

Although we had our methodologies firmly established, the search for novel transforming activities from human tumor DNA was frustrating. I presented the results of screening about 60 tumor DNAs at a laboratory meeting, and Robin was so critical that Alan and I decided we needed to have a meeting to discuss what to do. He suggested I bring my family out to his home on the next Sunday, and so we took our children to the park in the drizzling rain while we talked. In the end, we decided we had a robust assay and would look at another 20 tumor DNAs. If nothing came out, we would have to think about alternatives for our careers. Fortunately, in those 20 DNAs we identified novel transforming activities, and we were able to clone the third member of the RAS family NRAS (Hall et al., 1983).

Cancer Signaling Pathways



Discovered through *viruses*

- Akt
- p53
- PI3Kinase
- Raf
- Ras
- Src

Discovered through *cancer genetics*

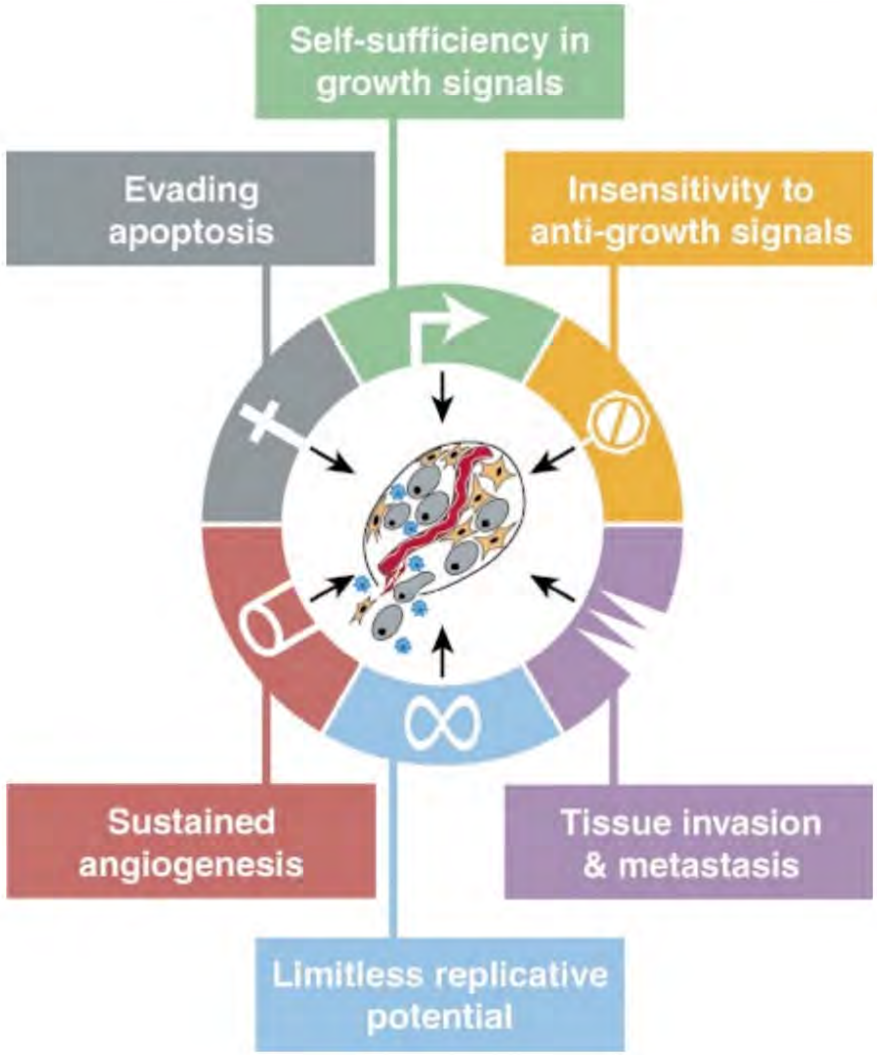
- Bcl-2
- Brca1/2
- Mdm-2
- Myc
- RB

Oncogenes
Tumor suppressors

Cell, Vol. 100, 57–70, January 7, 2000, Copyright ©2000 by Cell Press

The Hallmarks of Cancer

Douglas Hanahan* and Robert A. Weinberg†
*Department of Biochemistry and Biophysics and Hormone Research Institute
University of California at San Francisco
San Francisco, California 94143
†Whitehead Institute for Biomedical Research and Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02142



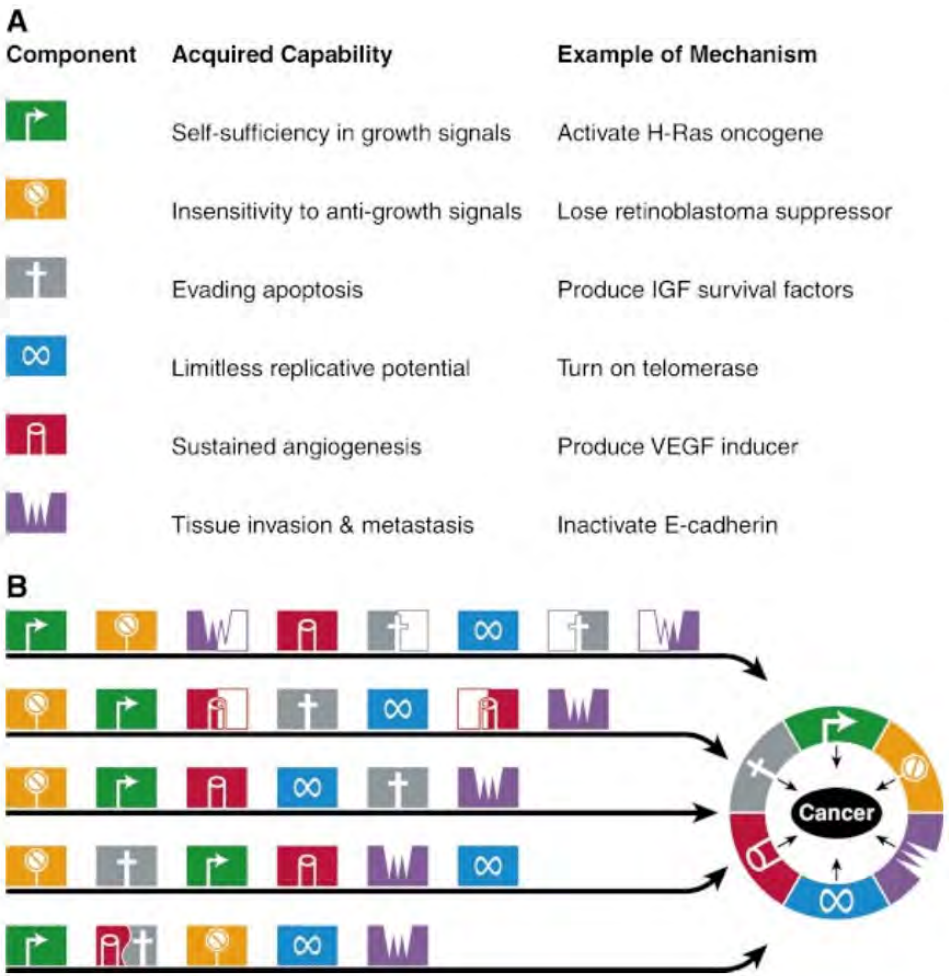


Figure 4. Parallel Pathways of Tumorigenesis

While we believe that virtually all cancers must acquire the same six hallmark capabilities (A), their means of doing so will vary significantly, both mechanistically (see text) and chronologically (B). Thus, the order in which these capabilities are acquired seems likely be quite variable across the spectrum of cancer types and subtypes. Moreover, in some tumors, a particular genetic lesion may confer several capabilities simultaneously, decreasing the number of distinct mutational steps required to complete tumorigenesis. Thus, loss of function of the p53 tumor suppressor can facilitate both angiogenesis and resistance to apoptosis (e.g., in the five-step pathway shown), as well as enabling the characteristic of genomic instability. In other tumors, a capability may only be acquired through the collaboration of two or more distinct genetic changes, thereby increasing the total number necessary for completion of tumor progression. Thus, in the eight-step pathway shown, invasion/metastasis and resistance to apoptosis are each acquired in two steps.

Some cell types may be easier to transform than others?

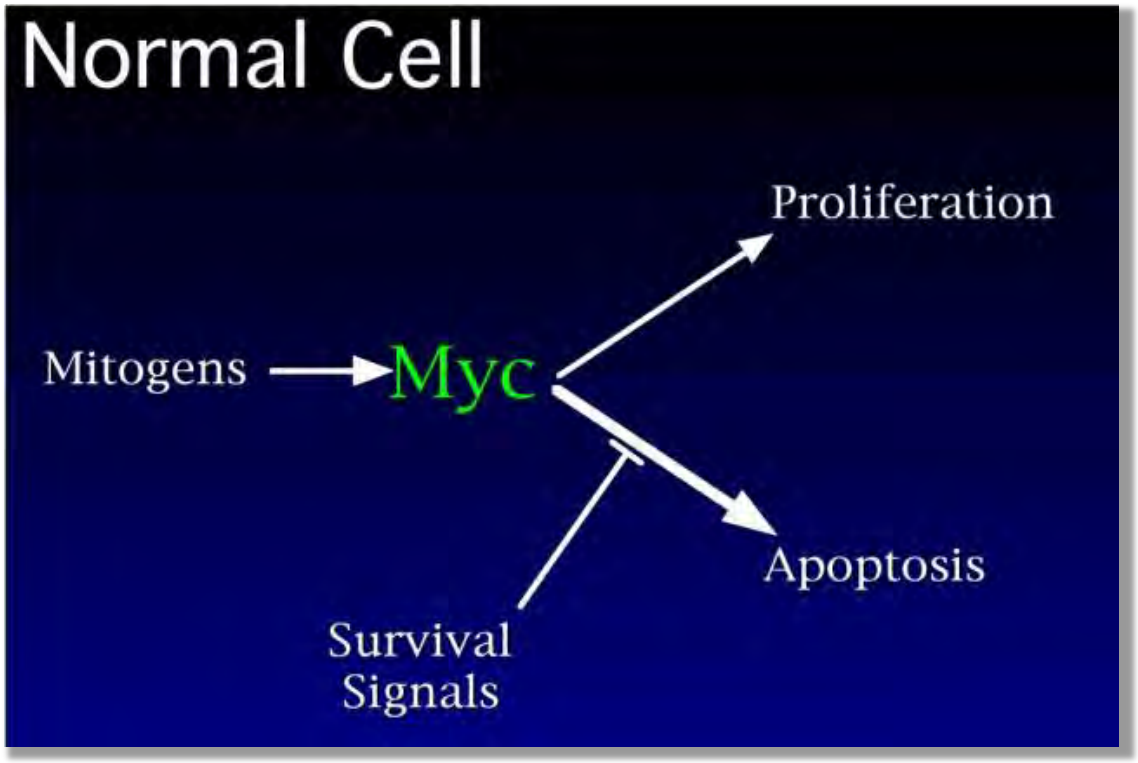
B cell: barriers to tumorigenic transformation may primarily involve proliferation and apoptosis

- Translocations involving MYC are highly characteristic for a type of B cell lymphoma called Burkitt's lymphoma (BL).
- Bcl-2 expression has also been found previously in about 10 to 20% of BL cases, and Bcl-2 translocation is a major mechanism for the deregulation of Bcl-2 expression in non-Hodgkin lymphomas.
- Double-hit lymphomas can also occur at low frequency with MYC/Bcl-2 dysregulation and these form aggressive diffuse B cell lymphomas

Myc + Bcl-2 (or *p53* loss) is sufficient to drive lymphomagenesis

Some cell types may be easier to transform than others?

B cell: barriers to tumorigenic transformation may primarily involve proliferation and apoptosis




www.dnaftb.org

NATURE VOL. 318 12 DECEMBER 1985 ARTICLES 533

The c-*myc* oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice

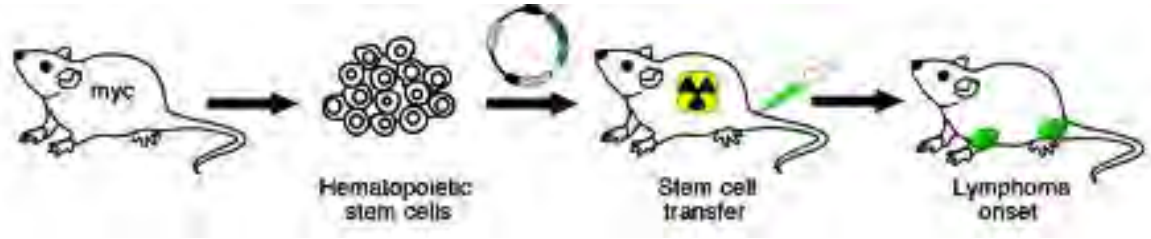
J. M. Adams*, A. W. Harris*, C. A. Pinkert†, L. M. Corcoran*, W. S. Alexander*, S. Cory*, R. D. Palmiter‡ & R. L. Brinster†

* Walter and Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, Victoria 3050, Australia
† School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA
‡ Howard Hughes Medical Institute, University of Washington, Seattle, Washington 98195, USA

Transgenic mice bearing the cellular myc *oncogene coupled to the immunoglobulin μ or κ enhancer frequently develop a fatal lymphoma within a few months of birth. Since the tumours represent both immature and mature B lymphocytes, constitutive c-*myc *expression appears to be highly leukaemogenic at several stages of B-cell maturation. These* myc *mice should aid study of lymphoma development, B-cell ontogeny and immunoglobulin regulation.*

Myc-driven B cell lymphoma:

- *Eμ (immunoglobulin heavy chain enhancer)-myc mice – mice overexpress myc oncogene in B cell lineage*
- *Mice develop B cell lymphoma by several months of age, resemble human Non-Hodgin's lymphoma*



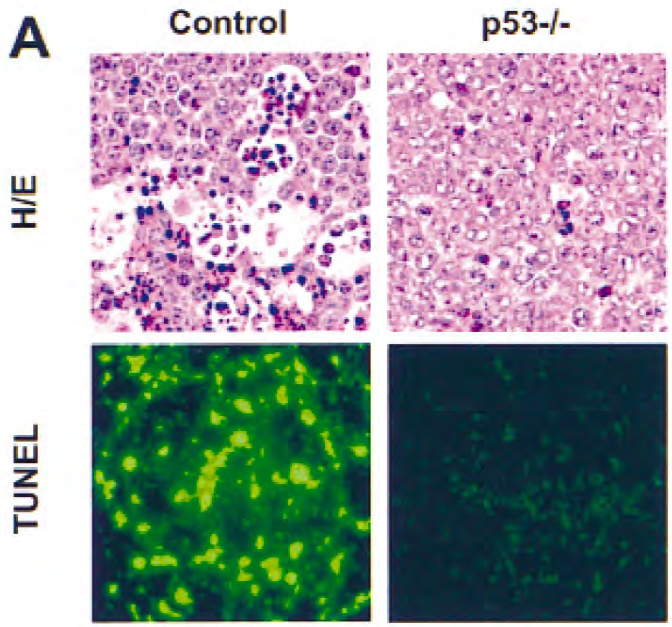
Can isolate hematopoietic stem cells, introduce genes, shRNAs, etc. of interest, transfer cells in vivo and monitor lymphoma onset – animals develop systemic lymphomas…

Can also perform competition assays by mixing GFP+ cells at defined ratios

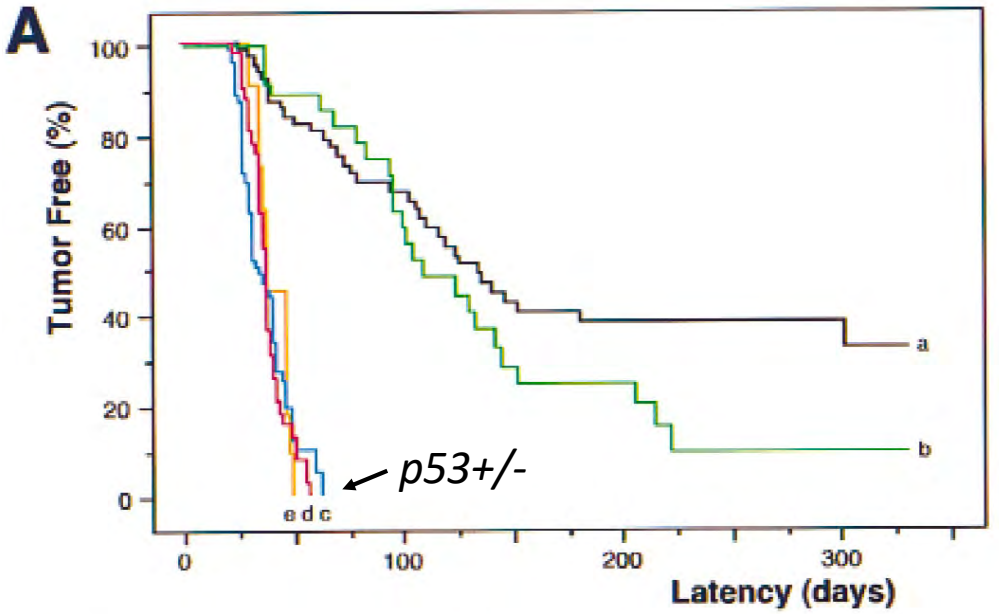
INK4a/ARF mutations accelerate lymphomagenesis and promote chemoresistance by disabling p53

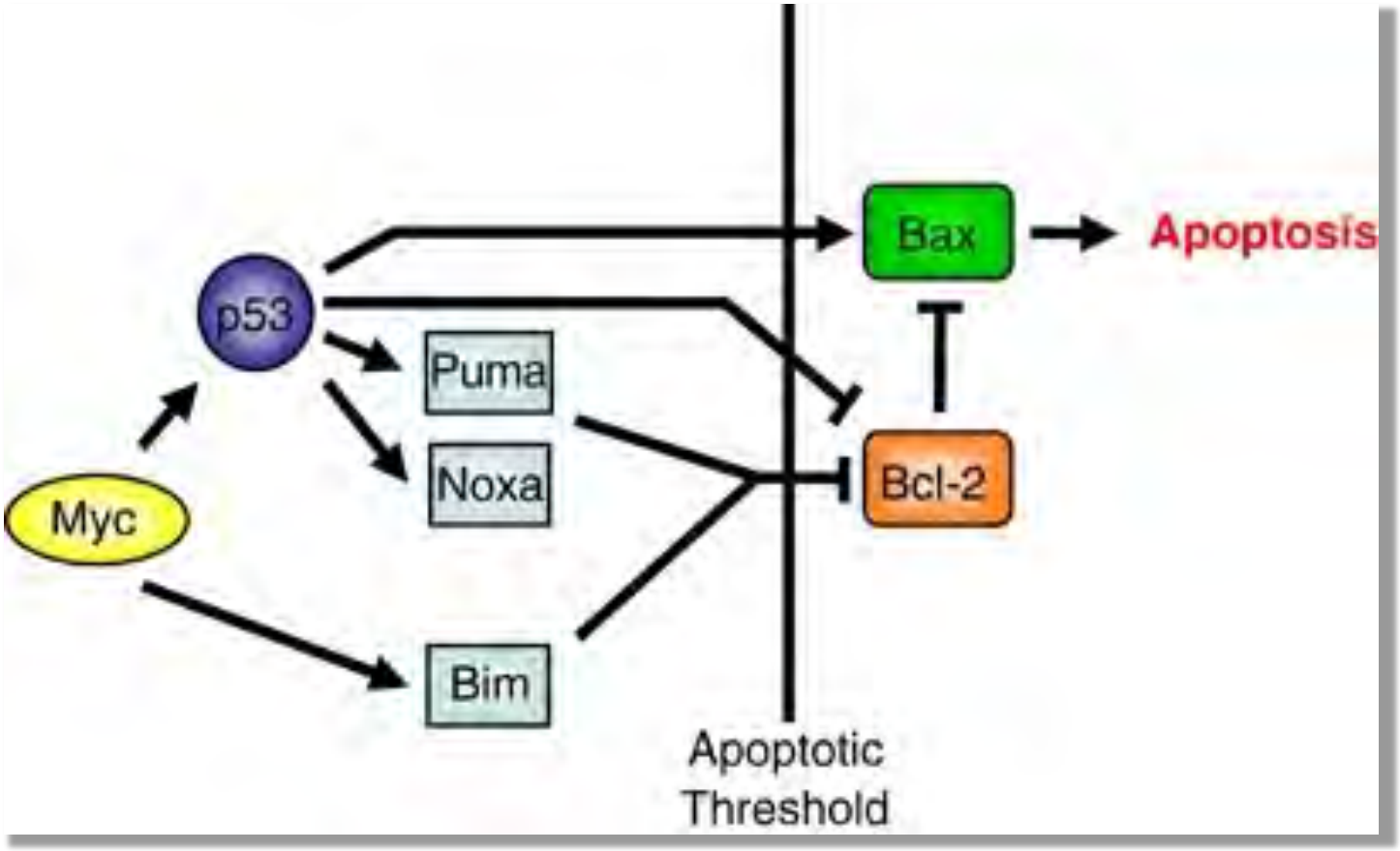
Clemens A. Schmitt, Mila E. McCurrach, Elisa de Stanchina, Rachel R. Wallace-Brod
and Scott W. Lowe[1]

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724 USA



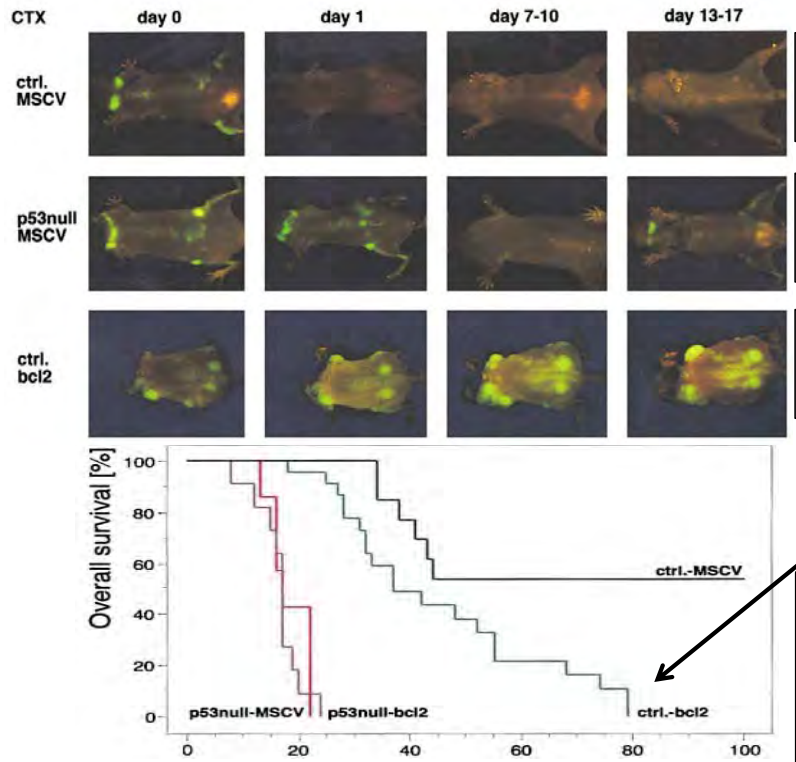
Eμ-myc lymphomagenesis




Bax
Apoptosis
p53
Puma
Noxa
Bcl-2
Myc
Bim
Apoptotic
Threshold

p53 and Bcl-2 uniquely control treatment responses in vivo

Cyclophosphamide (CTX) treatment



Control tumors completely respond to treatment

p53-null tumors undergo delayed response, and ultimately relapse

Bcl2 overexpressing tumors displayed no response to CTX

…but the mice survive very well

Figure 1. Contribution of p53 and Bcl2 to Treatment Responses (A) Mice harboring ctrl.-MSCV, *p53* null-MSCV, and ctrl.-bcl2 lymphomas were treated at comparable tumor burdens (day 0) with a single dose of cyclophosphamide (CTX) and monitored by whole-body fluorescence imaging. Representative examples are shown.

Cell, Vol. 109, 335–346, May 3, 2002, Copyright © 2002 by Cell Press

A Senescence Program Controlled by p53 and p16^{INK4a} Contributes to the Outcome of Cancer Therapy

Clemens A. Schmitt,[1,4] Jordan S. Fridman,[1] Meng Yang,[2] Soyoung Lee,[1,4] Eugene Baranov,[2] Robert M. Hoffman,[2] and Scott W. Lowe[1,3]

p53 and Bcl-2 uniquely control treatment responses in vivo

Cyclophosphamide (CTX) treatment

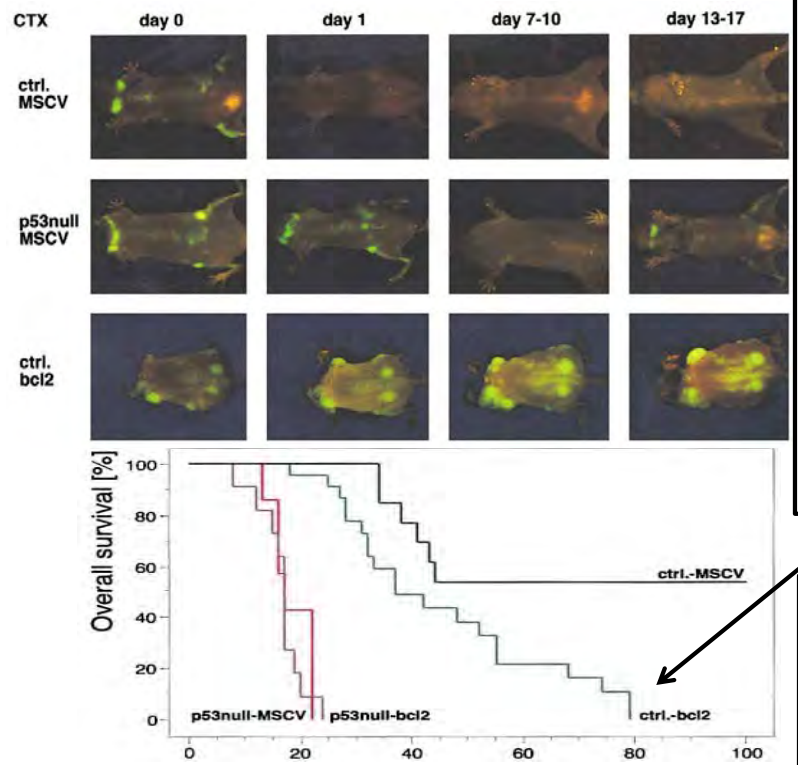
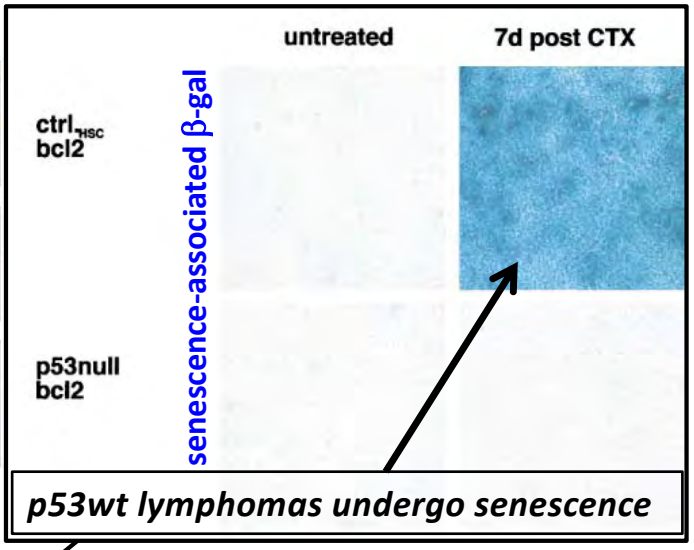


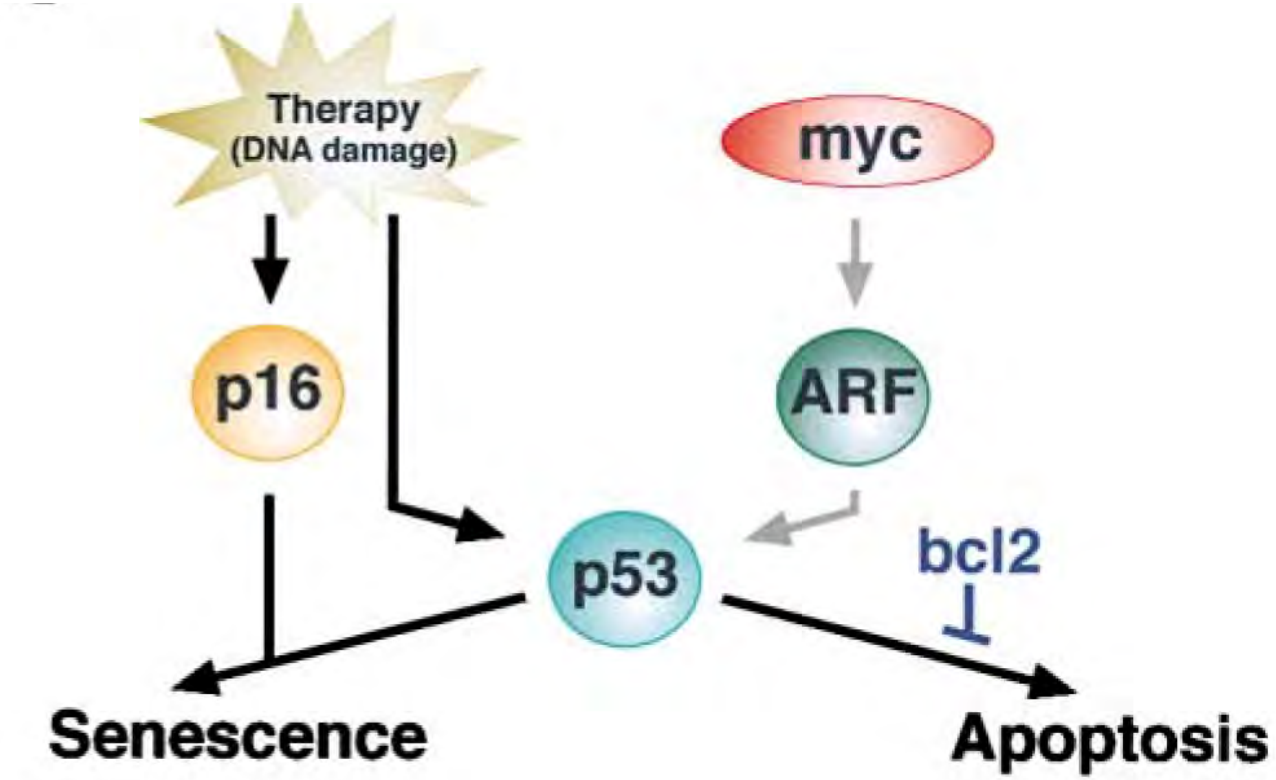
Figure 1. Contribution of p53 and Bcl2 to Treatment Responses (A) Mice harboring ctrl.-MSCV, *p53* null-MSCV, and ctrl.-bcl2 lymphomas were treated at comparable tumor burdens (day 0) with a single dose of cyclophosphamide (CTX) and monitored by whole-body fluorescence imaging. Representative examples are shown.



Cell, Vol. 109, 335–346, May 3, 2002, Copyright © 2002 by Cell Press

A Senescence Program Controlled by p53 and p16^{INK4a} Contributes to the Outcome of Cancer Therapy

Clemens A. Schmitt,[1,4] Jordan S. Fridman,[1]
Meng Yang,[2] Soyoung Lee,[1,4] Eugene Baranov,[2]
Robert M. Hoffman,[2] and Scott W. Lowe[1,3]


Therapy
(DNA damage)
myc
p16
ARF
p53
bcl2
Senescence
Apoptosis

Some cell types may be easier to transform than others?

B cell: barriers to tumorigenic transformation may primarily involve proliferation and apoptosis

Myc + Bcl-2 (or *p53* loss) drives lymphomagenesis

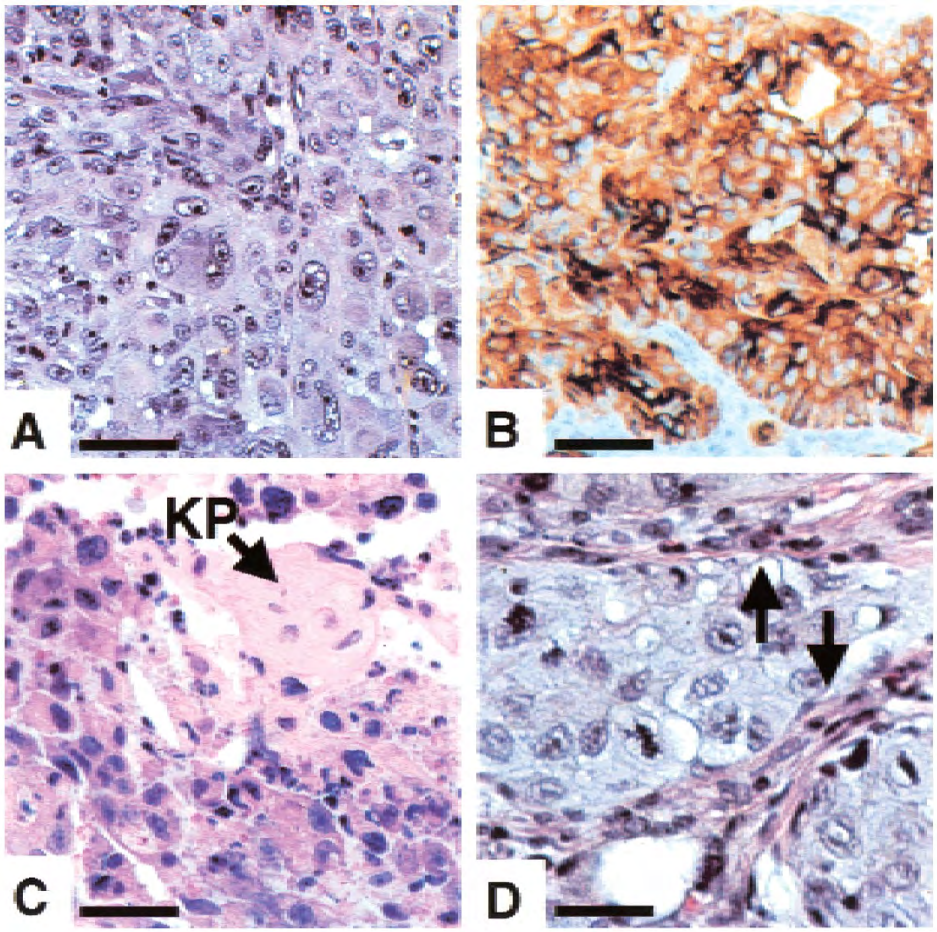
Mammary epithelial cell:

p53 loss
RB loss
Ras
Myc
PI-3-kinase
Matrix?

Primary human mammary epithelial cells (HMEC) could be rendered tumorigenic by the introduction of Ras and LT-Ag:

Table 1. *Formation of subcutaneous tumors in nude mice*

Cells	Genotype	No. tumors/ injection	Ras over-expression
HMEC	hTERT, V	0/3	—
	hTERT, Ras-puro	0/6	12.0
	LT, V, V	0/3	—
	LT, hTERT, V	0/6	—
	LT, Ras-puro	0/3	12.0
	LT, hTERT, Ras-hygro	0/24	3.5
	LT, hTERT, Ras-zeo	1/15	7.2
	LT, hTERT, Ras-puro	14/27	12.0
PHMEC	LT, hTERT, V	0/9	0
	LT, hTERT, Ras-puro	6/9	14.0
HEK	LT, hTERT, Ras-hygro	1/7	9.5
	LT, hTERT, Ras-puro	15/15	60



Human breast cancer cells generated by oncogenic transformation of primary mammary epithelial cells

Brian Elenbaas,[1] Lisa Spirio,[1] Frederick Koerner,[2] Mark D. Fleming,[3] Drazen B. Zimonjic,[4] Joana Liu Donaher,[1] Nicholas C. Popescu,[4] William C. Hahn,[1,5] and Robert A. Weinberg[1,6]

GENES & DEVELOPMENT 15:50–65 © 2001

Human breast cancer cells generated by oncogenic transformation of primary mammary epithelial cells

GENES & DEVELOPMENT 15:50–65 © 2001

Brian Elenbaas,[1] Lisa Spirio,[1] Frederick Koerner,[2] Mark D. Fleming,[3] Drazen B. Zimonjic,[4] Joana Liu Donaher,[1] Nicholas C. Popescu,[4] William C. Hahn,[1,5] and Robert A. Weinberg[1,6]

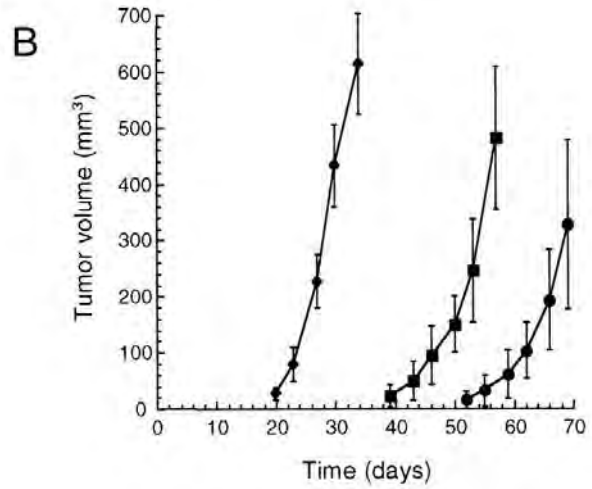


Figure 6. The latency of subcutaneous tumor formation is enhanced by mixing HMLER cells with Matrigel or early-passage human RMFs. (*A*) Comparison of the latency and rate of tumor formation of HEK cells (♦), BJ fibroblasts (■), and HMECs (●) each expressing LT, *hTERT*, and H-*ras*V12. (*B*) The latency of tumor formation of HMLER cells (●) was decreased by addition of Matrigel (■) or primary RMFs (RMF.1, ♦). Results are expressed as the mean of six tumors +/– S.D. at the indicated time points after injection.

"Mixing the epithelial tumor cells with Matrigel or primary human mammary fibroblasts substantially increased the efficiency of tumor formation and decreased the latency of tumor formation, demonstrating a significant influence of the stromal microenvironment on tumorigenicity."

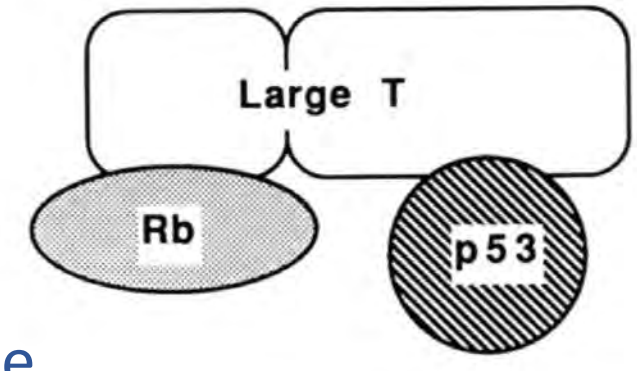
Some cell types may be easier to transform than others?

B cell: barriers to tumorigenic transformation may primarily involve proliferation and apoptosis

Myc + Bcl-2 (or *p53* loss) drives lymphomagenesis

Mammary epithelial cell:

p53 loss
RB loss
Ras
Myc
PI-3-kinase
Matrix?



Cell

Leading Edge
Review

Hallmarks of Cancer: The Next Generation

Douglas Hanahan[1,2,*] and Robert A. Weinberg[3,*]

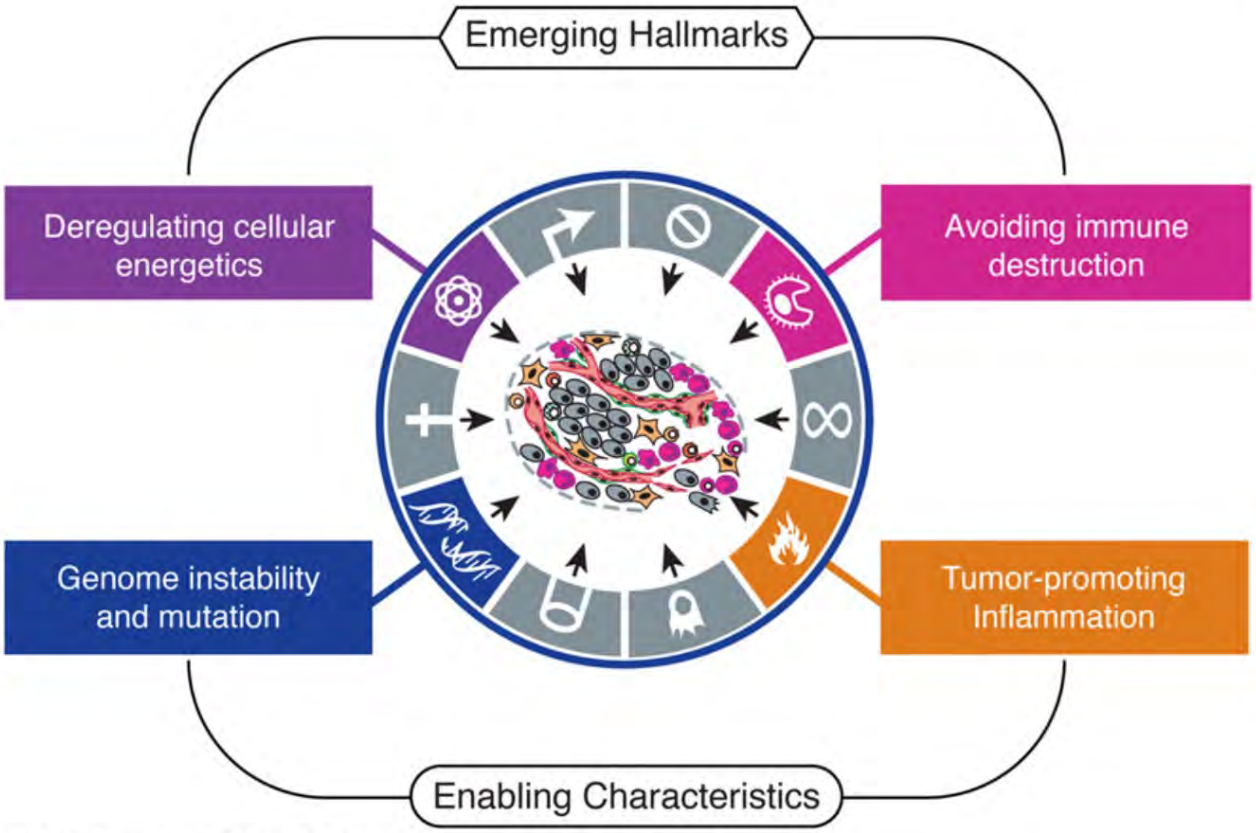
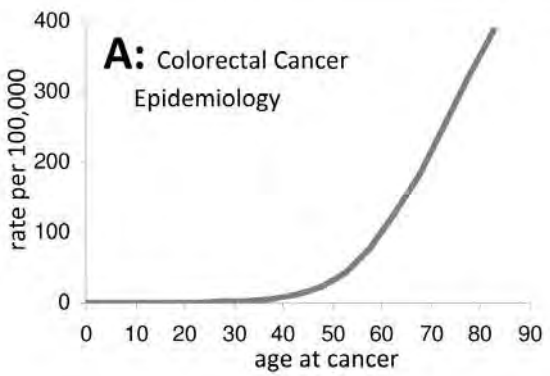


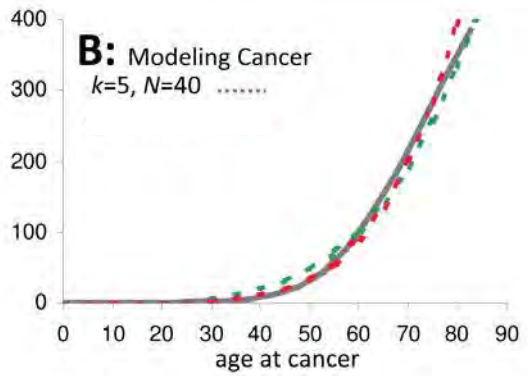
Figure 3. Emerging Hallmarks and Enabling Characteristics

An increasing body of research suggests that two additional hallmarks of cancer are involved in the pathogenesis of some and perhaps all cancers. One involves the capability to modify, or reprogram, cellular metabolism in order to most effectively support neoplastic proliferation. The second allows cancer cells to evade immunological destruction, in particular by T and B lymphocytes, macrophages, and natural killer cells. Because neither capability is yet generalized and fully validated, they are labeled as emerging hallmarks. Additionally, two consequential characteristics of neoplasia facilitate acquisition of both core and emerging hallmarks. Genomic instability and thus mutability endow cancer cells with genetic alterations that drive tumor progression. Inflammation by innate immune cells designed to fight infections and heal wounds can instead result in their inadvertent support of multiple hallmark capabilities, thereby manifesting the now widely appreciated tumor-promoting consequences of inflammatory responses.

How many driver mutations does it take?

- *Estimations involving epidemiology*





Calabrese and Shibata *BMC Cancer* 2010, **10**:3
http://www.biomedcentral.com/1471-2407/10/3

BMC Cancer

CORRESPONDENCE Open Access

A simple algebraic cancer equation: calculating how cancers may arise with normal mutation rates

Peter Calabrese[1], Darryl Shibata[2*]

Table 1 Model Parameters

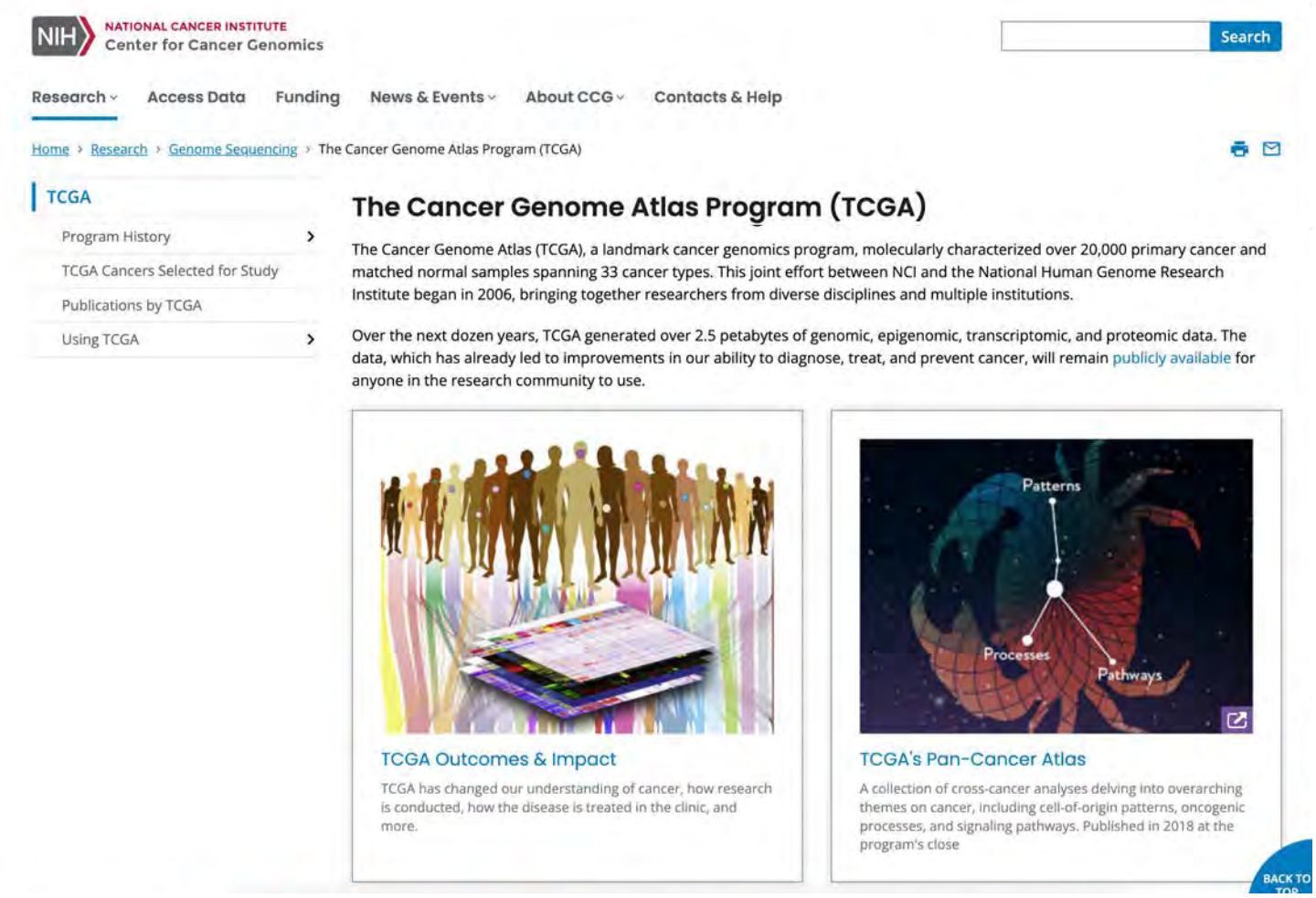
Parameter	Description	Colorectal Cancer With Specific Gene Targets*
k	rate-limiting stages	5 driver gene mutations
m	number of crypts	15,000,000
n	stem cells per crypt	40
u	target mutation rate	1×10^{-6} per gene per division
d	divisions since birth	once every four days
p	probability of cancer	-

*In this model, five specific driver genes must be mutated for transformation

How many driver mutations does it take?

- *Estimations from epidemiology*
- *Estimations from sequencing efforts*

How many driver mutations does it take?

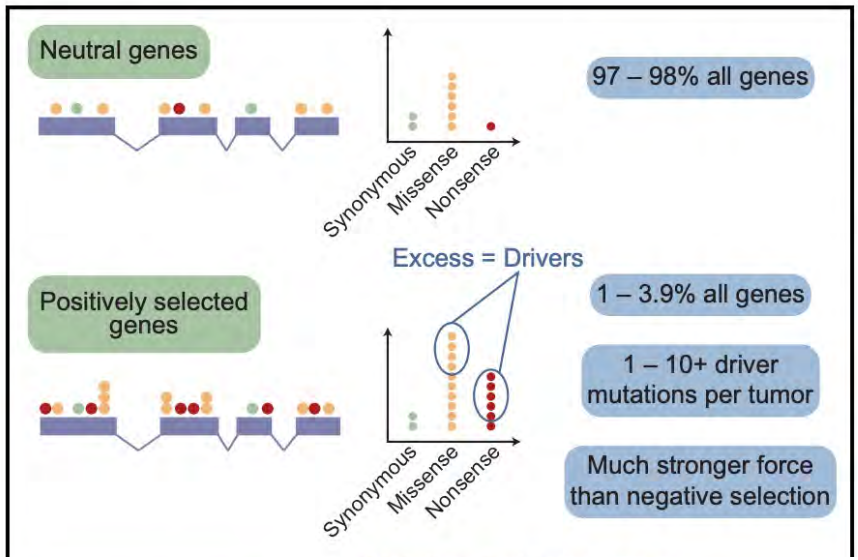


How many driver mutations does it take?

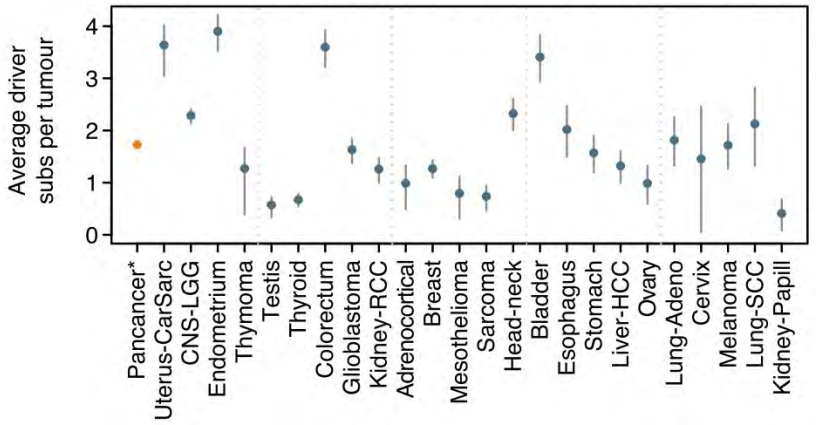
Universal Patterns of Selection in Cancer and Somatic Tissues

Iñigo Martincorena,[1,6,*] Keiran M. Raine,[1] Moritz Gerstung,[2] Kevin J. Dawson,[1] Kerstin Haase,[3] Peter Van Loo,[3,4] Helen Davies,[1] Michael R. Stratton,[1] and Peter J. Campbell[1,5,*]

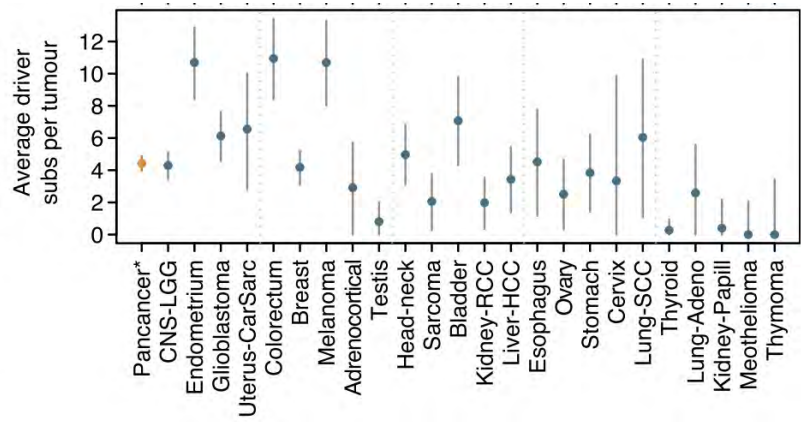
Graphical Abstract



Only considering mutations in 369 known cancer genes

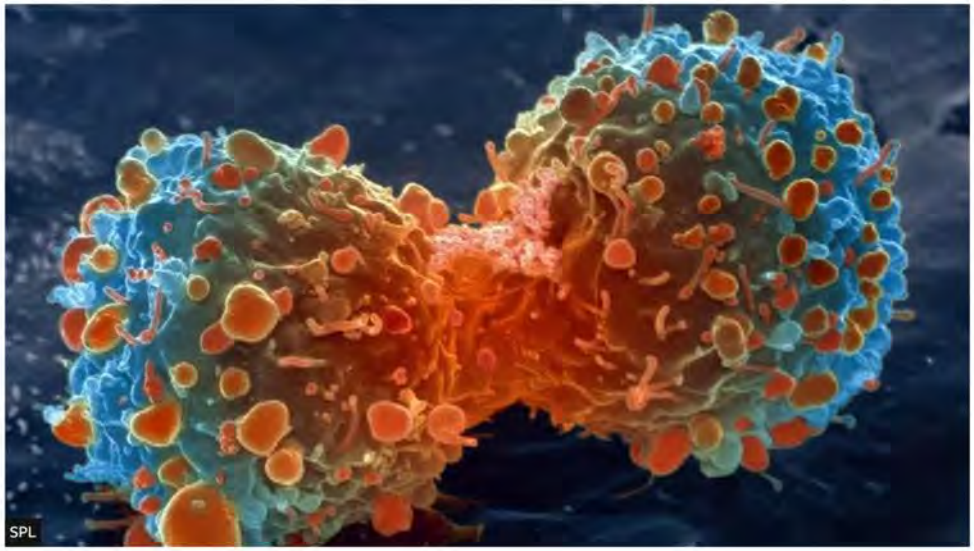


Considering all mutations in protein-coding genes



'Handful of changes' make cancer

19 October 2017



By James Gallagher
Health and science reporter, BBC News website

Top Stories

Credit Suisse to borrow $54bn to shore up finances
31 minutes ago

N Korea fires long range missile ahead of Japan talks
1 hour ago

S Korea and Japan: A 'milestone' meeting of frenemies
2 hours ago

Cell

Leading Edge

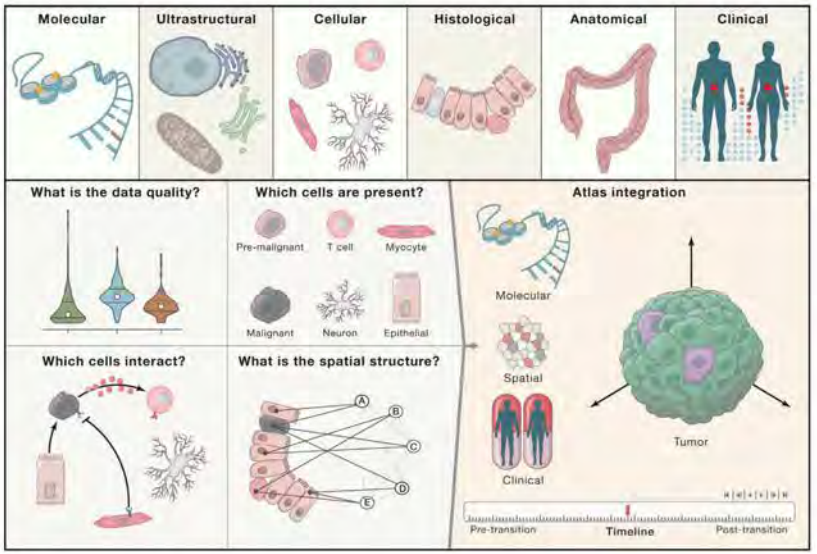
Perspective

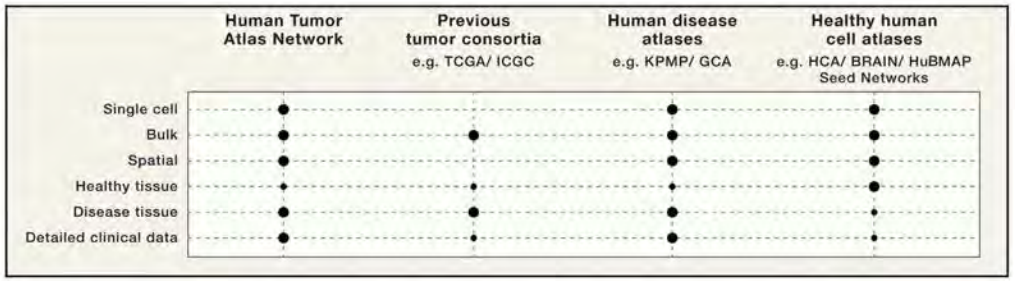
The Human Tumor Atlas Network: Charting Tumor Transitions across Space and Time at Single-Cell Resolution

Orit Rozenblatt-Rosen,[1,35] Aviv Regev,[1,2,3,35,36,*] Philipp Oberdoerffer,[4,35] Tal Nawy,[5,35] Anna Hupalowska,[1] Jennifer E. Rood,[1] Orr Ashenberg,[1] Ethan Cerami,[6] Robert J. Coffey,[7] Emek Demir,[8] Li Ding,[9] Edward D. Esplin,[10] James M. Ford,[10,11] Jeremy Goecks,[12] Sharmistha Ghosh,[13] Joe W. Gray,[14] Justin Guinney,[15,16] Sean E. Hanlon,[17] Shannon K. Hughes,[4] E. Shelley Hwang,[18,19] Christine A. Iacobuzio-Donahue,[20] Judit Jané-Valbuena,[1]

Bruce E. Johnson,[21] Ken S. Lau,[7] Tracy Lively,[22] Sarah A. Mazzilli,[23] Dana Pe'er,[5] Sandro Santagata,[24,25] Alex K. Shalek,[1,26,27,28,29] Denis Schapiro,[1,24] Michael P. Snyder,[10] Peter K. Sorger,[24] Avrum E. Spira,[23,30] Sudhir Srivastava,[13] Kai Tan,[31,32] Robert B. West,[33] Elizabeth H. Williams[6,34] and the Human Tumor Atlas Network

Cell *181*, April 16, 2020





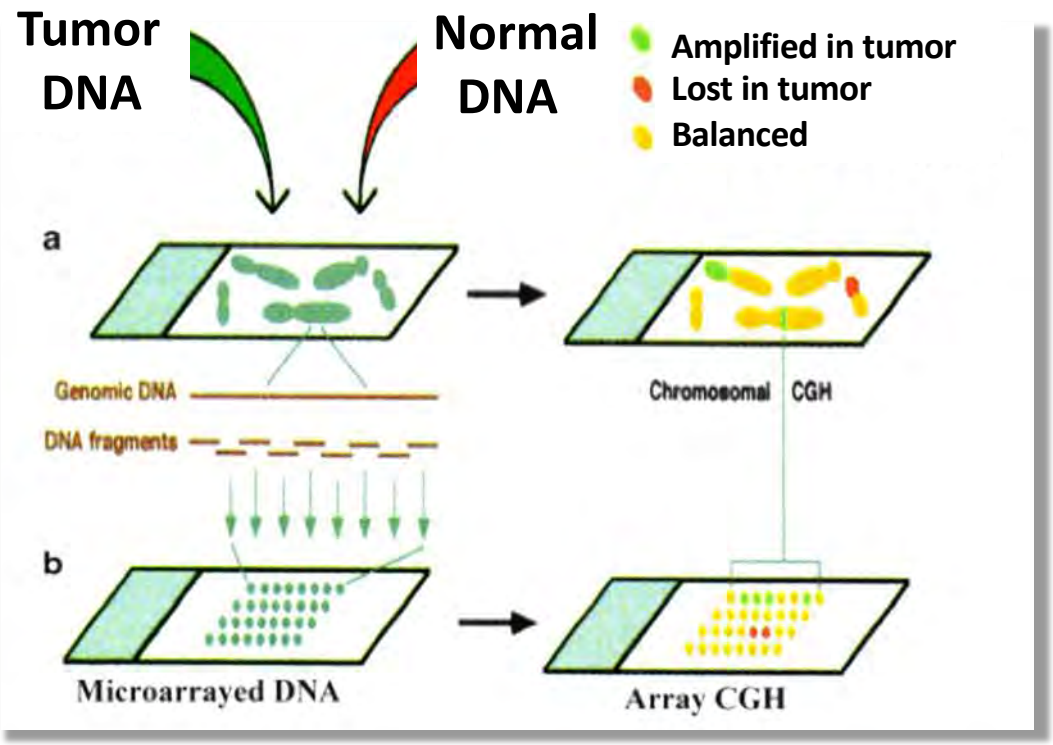
Box 1. Toolbox Used for Building 3D Tumor Atlases across Scales

Molecular		Spatiomolecular	Histological	Anatomical
sc/snRNA-seq	RNA-seq	EM	H&E	MRI
sc/snEpigenomics	Epigenomics	Sequencing-based		CT
CITE-seq	WES	Fluorescence-based		PET
	Metabolomics	Antibody-based		
	Proteomics			
	Microbiome			

Single cell · Bulk · Multiplex transcriptomics · Multiplex proteomics

The HTAN toolbox includes molecular, spatiomolecular, histological, and anatomical profiling.

My PhD Project: chromosomal instability in osteosarcoma



The presence of p53 mutations in human osteosarcomas correlates with high levels of genomic instability

Michael Overholtzer*†, Pulivarthi H. Rao‡, Reyna Favis§, Xin-Yan Lu‡, Michael B. Elowitz*, Francis Barany§, Marc Ladanyi¶, Richard Gorlick‖, and Arnold J. Levine*,**

PNAS | September 30, 2003 | vol. 100 | no. 20 | 11547–11552

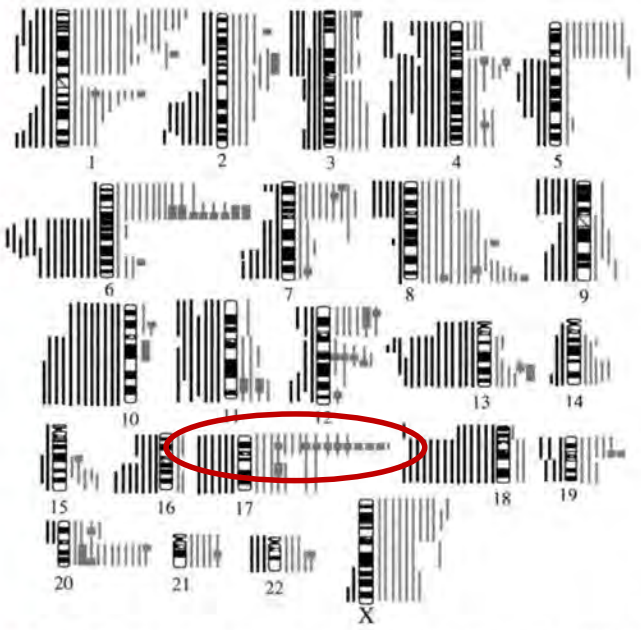
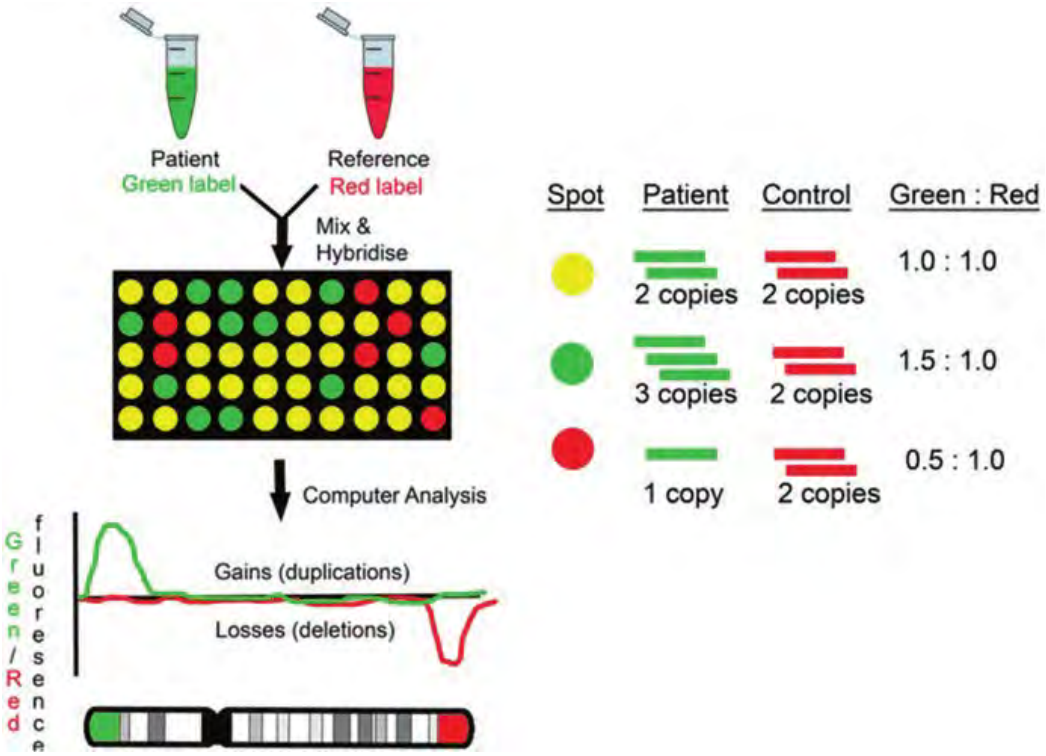
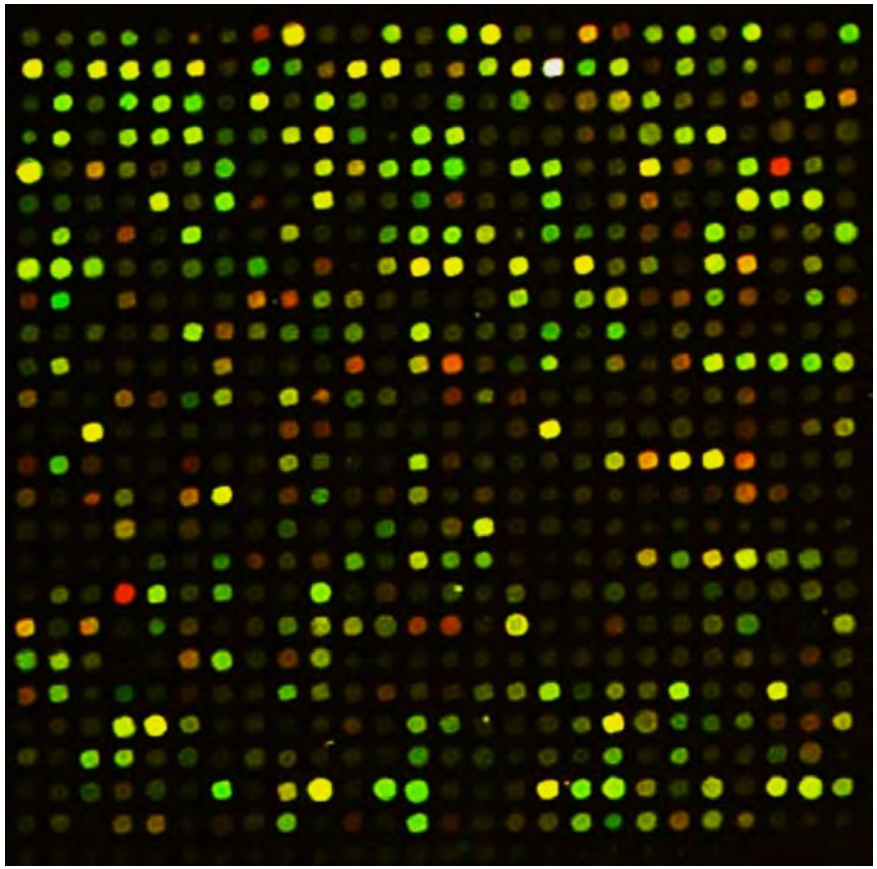


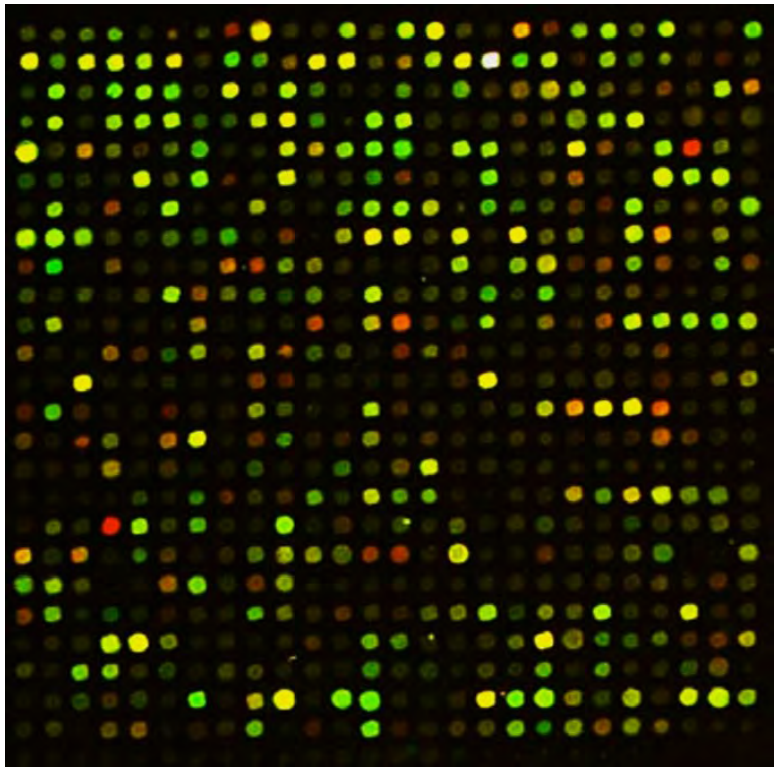
Fig. 1. Ideogram of CGH-detectable copy-number changes in 34 osteosarcoma tumors. Copy-number gains are depicted by thin gray lines to the right of each chromosome, high-level amplifications (approximately >5-fold) are depicted by thick gray bars, and losses are depicted by thin black lines to the left of each chromosome.

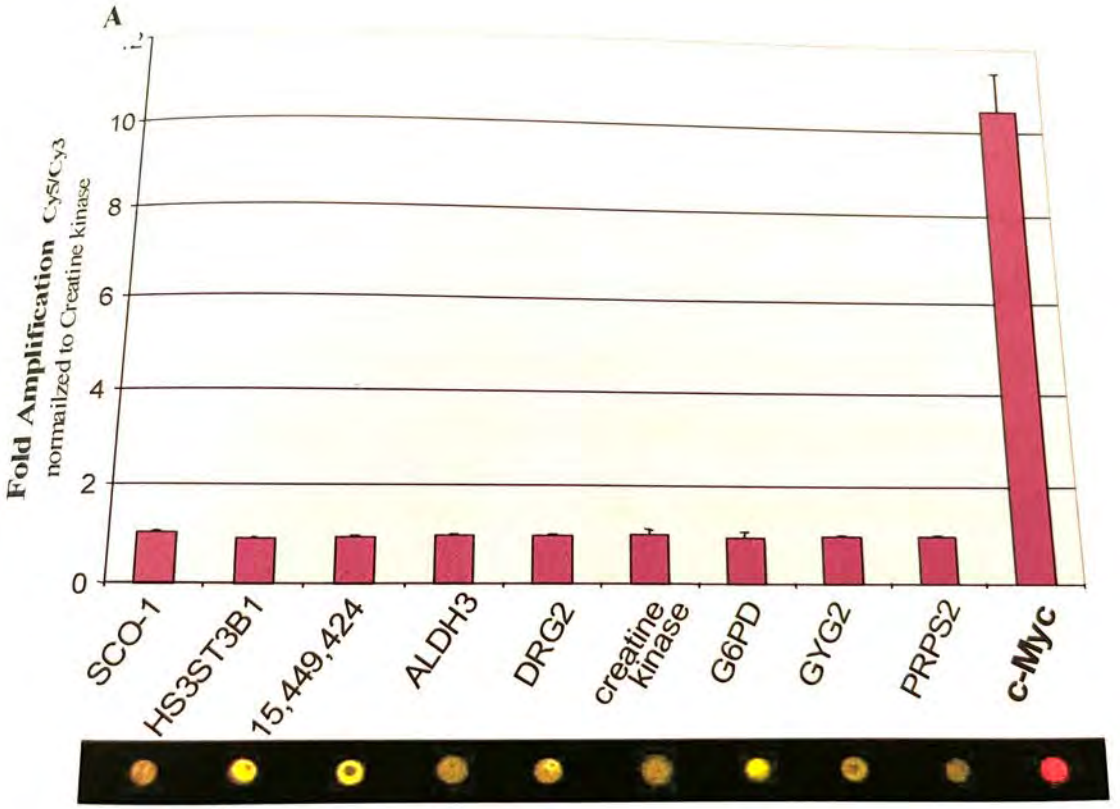
My PhD Project in Levine lab: use (homemade) array CGH to identify novel oncogenes or tumor suppressors in osteosarcoma



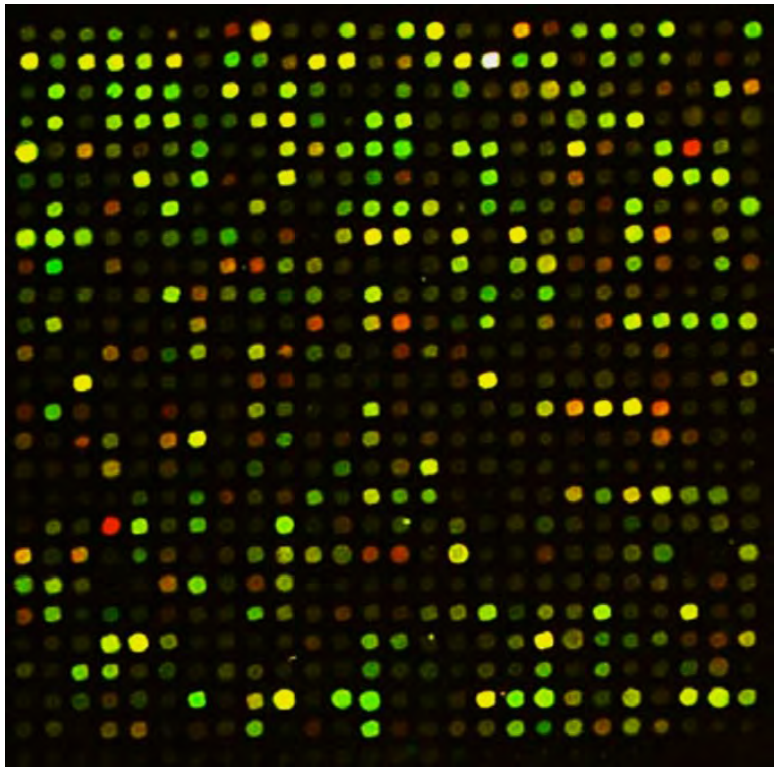


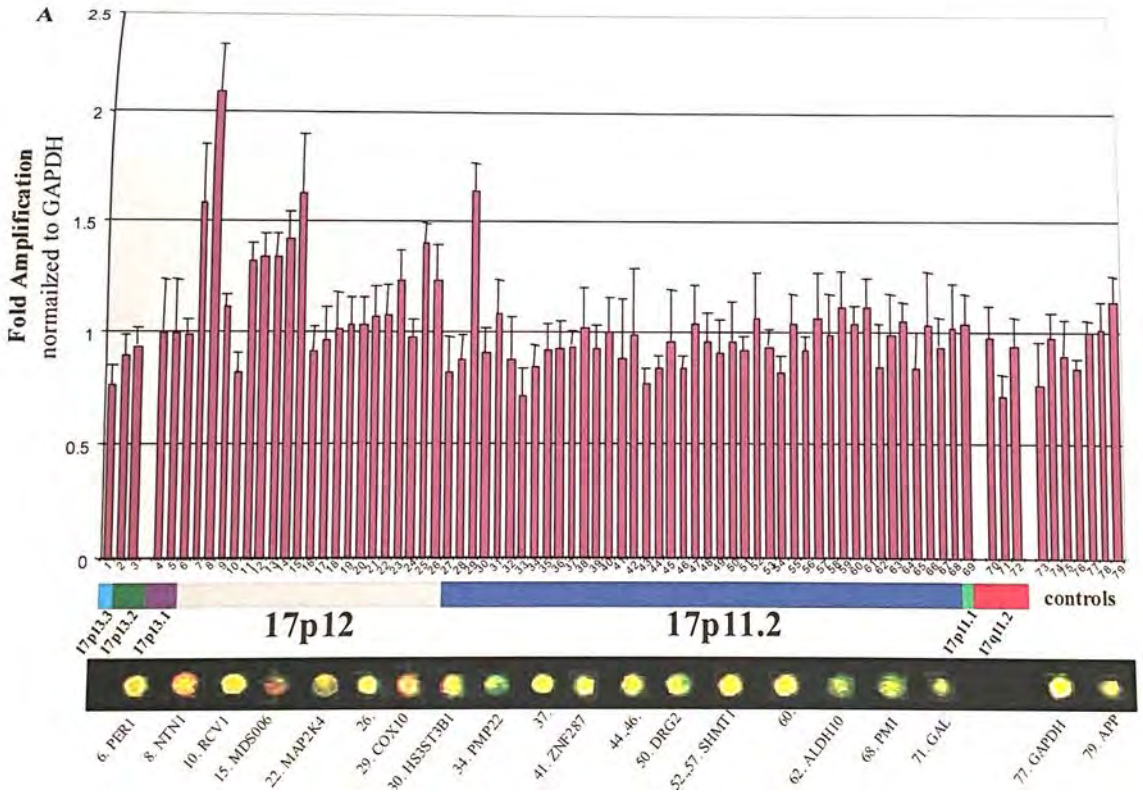
Homemade array CGH to identify novel oncogenes or tumor suppressors in osteosarcoma



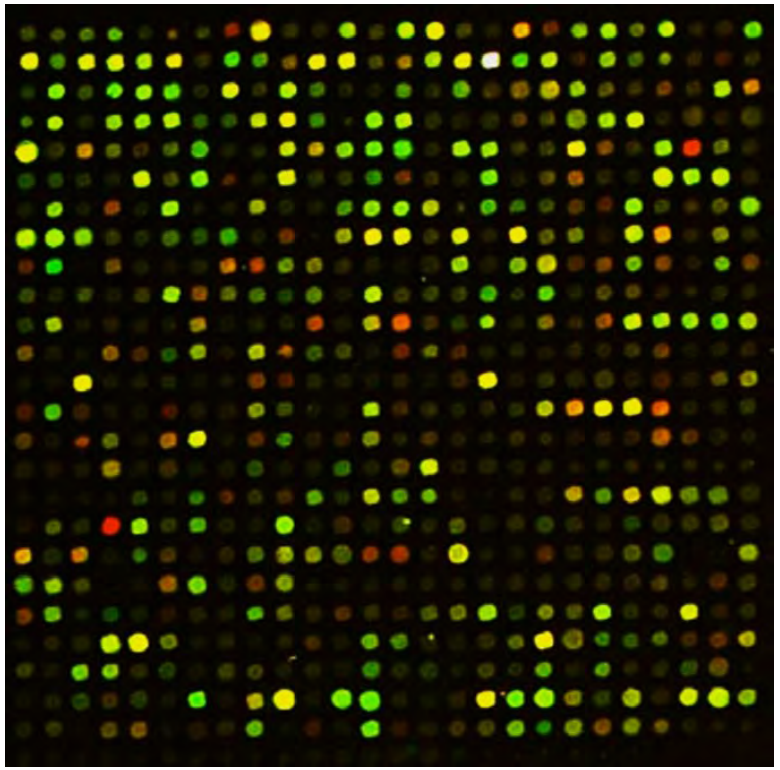


My PhD Project in Levine lab: use (homemade) array CGH to identify novel oncogenes or tumor suppressors in osteosarcoma

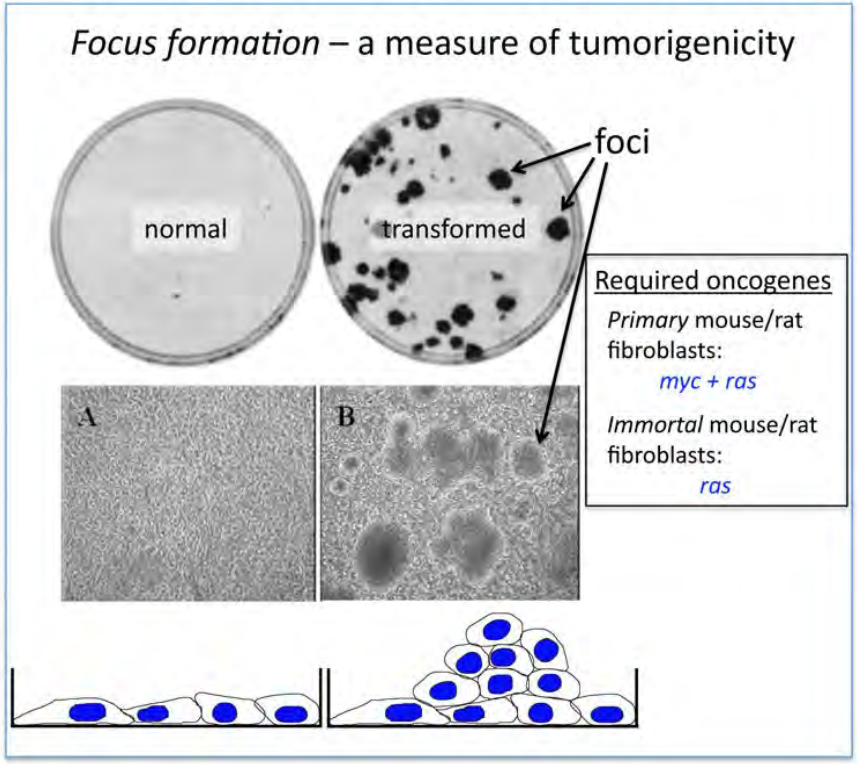




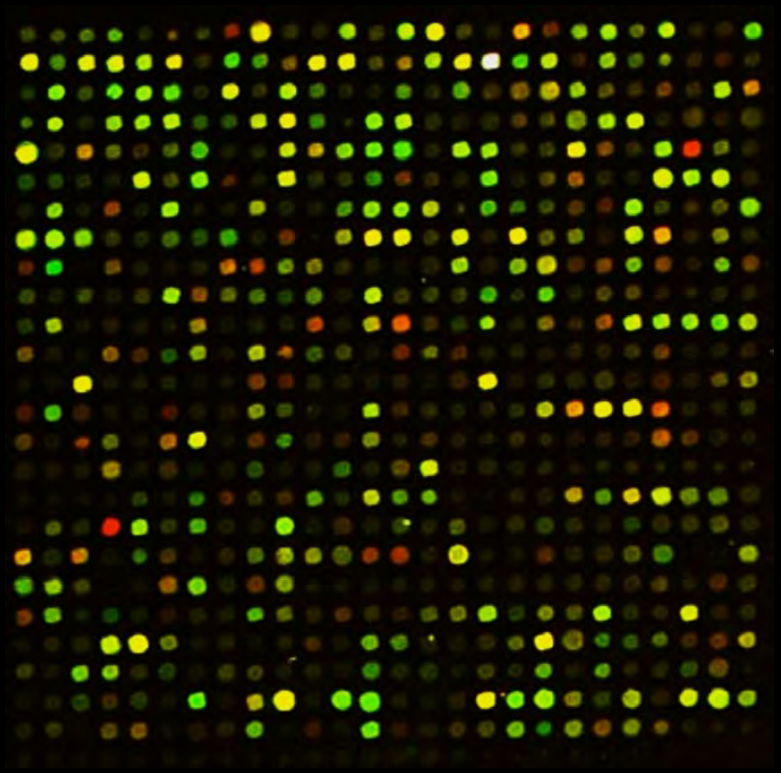
Candidate gene ID



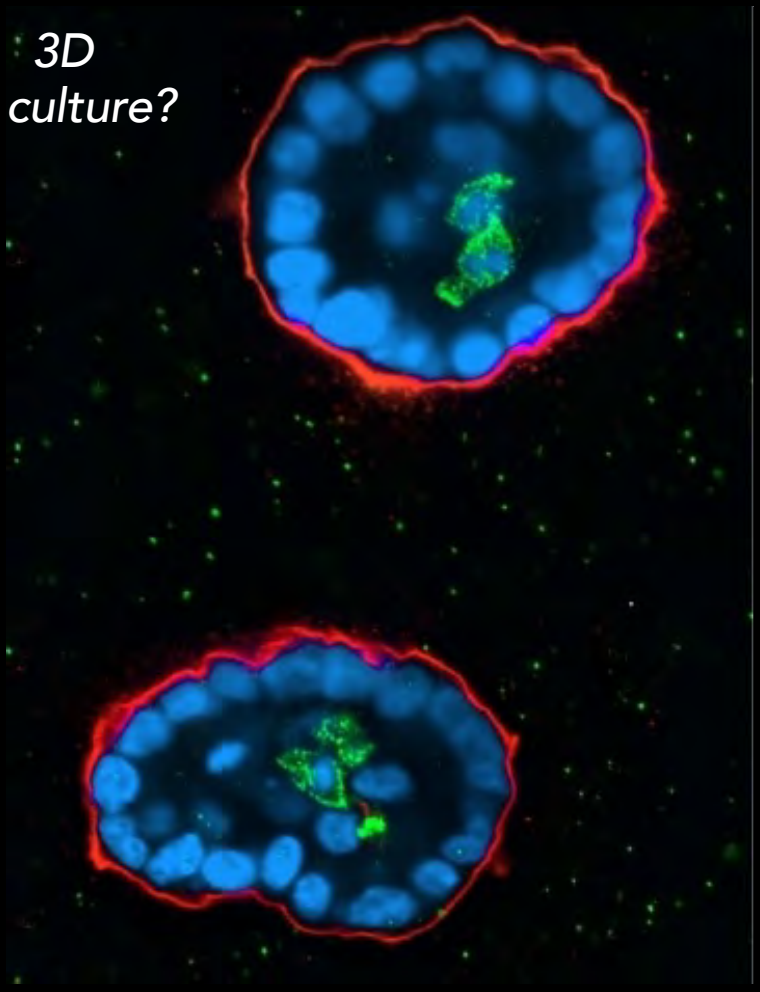
Functional Assay?

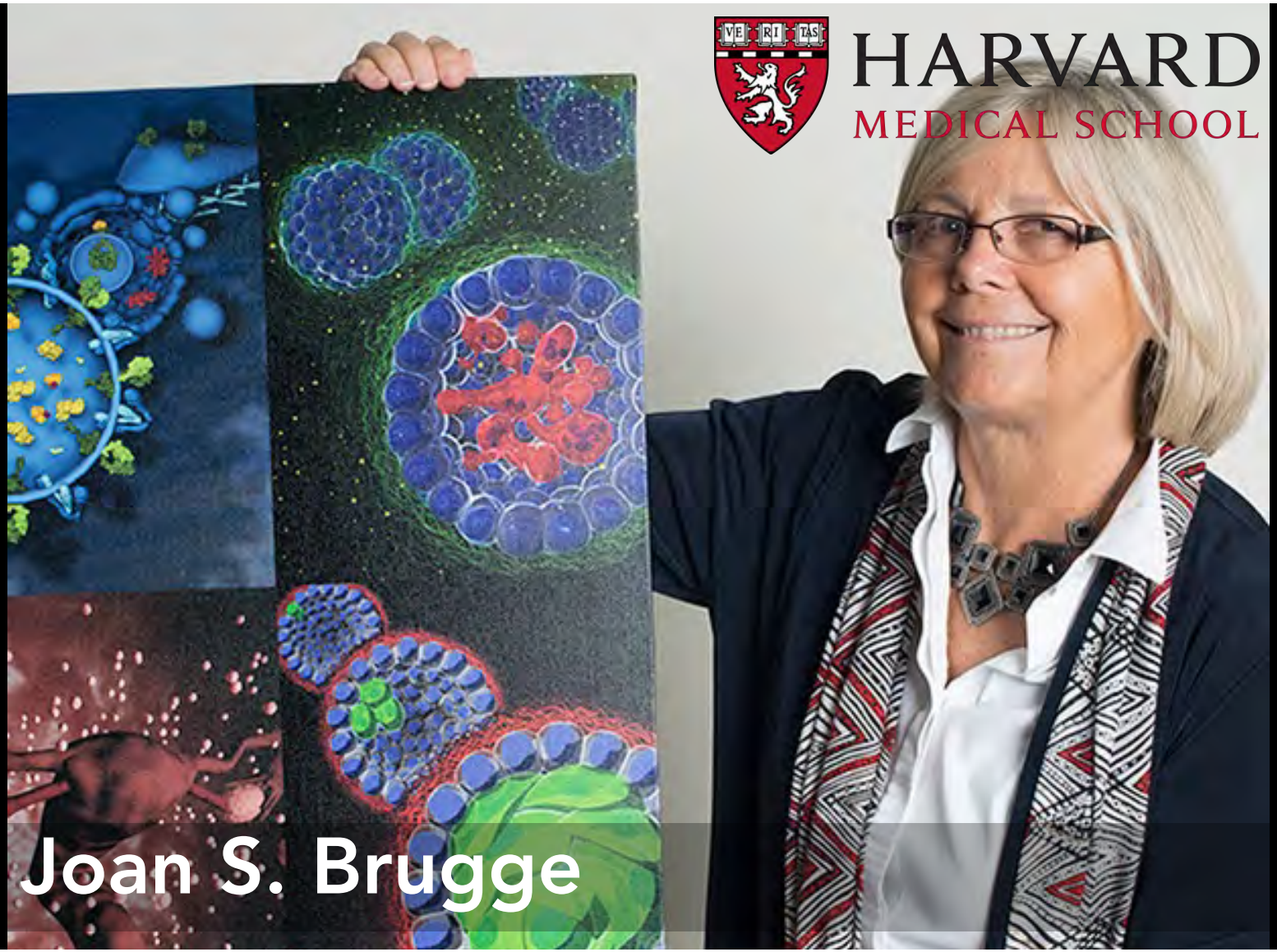


Mapped new candidate oncogenes, but how to assay for function?

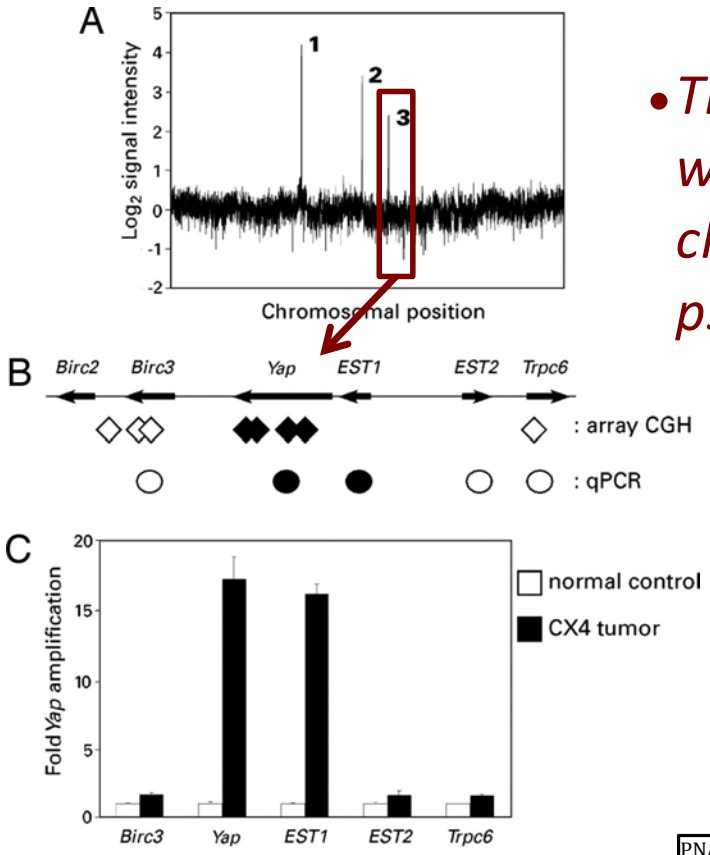


3D culture?




VE RI TAS
HARVARD
MEDICAL SCHOOL
Joan S. Brugge

A gene called Yap was found to be amplified in a genomic screen of mouse breast tumors



- *The Yap gene was amplified, without co-amplification of cIAP1 and cIAP2, in a mouse p53+/-, Brca1 -/- breast tumor*

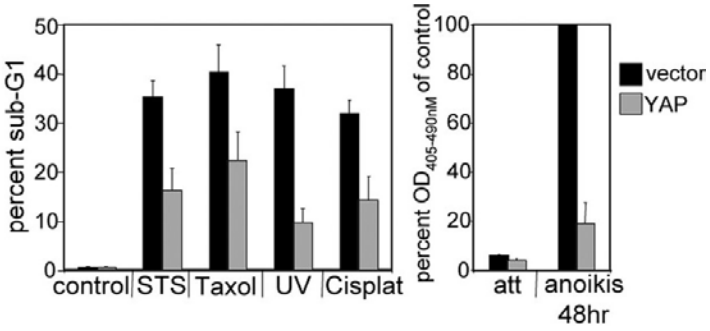
PNAS _ **August 15, 2006** _ vol. 103 _ no. 33 _ **12405–12410**

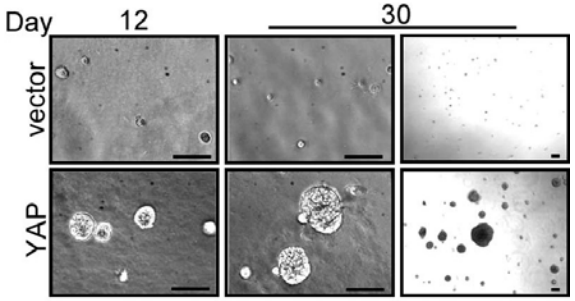
Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon

Michael Overholtzer, Jianmin Zhang, Gromoslaw A. Smolen, Beth Muir, Wenmei Li, Dennis C. Sgroi, Chu-Xia Deng, Joan S. Brugge, and Daniel A. Haber.

Yap overexpression transforms mammary epithelial cells

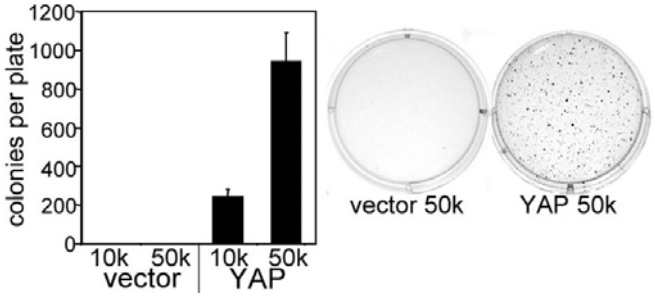
Yap overexpression inhibited cell death in mammary epithelial cells





Yap overexpression promoted growth factor-independent proliferation

The combined upregulation of proliferation and inhbition of apoptosis transformed mammary epithelial cells

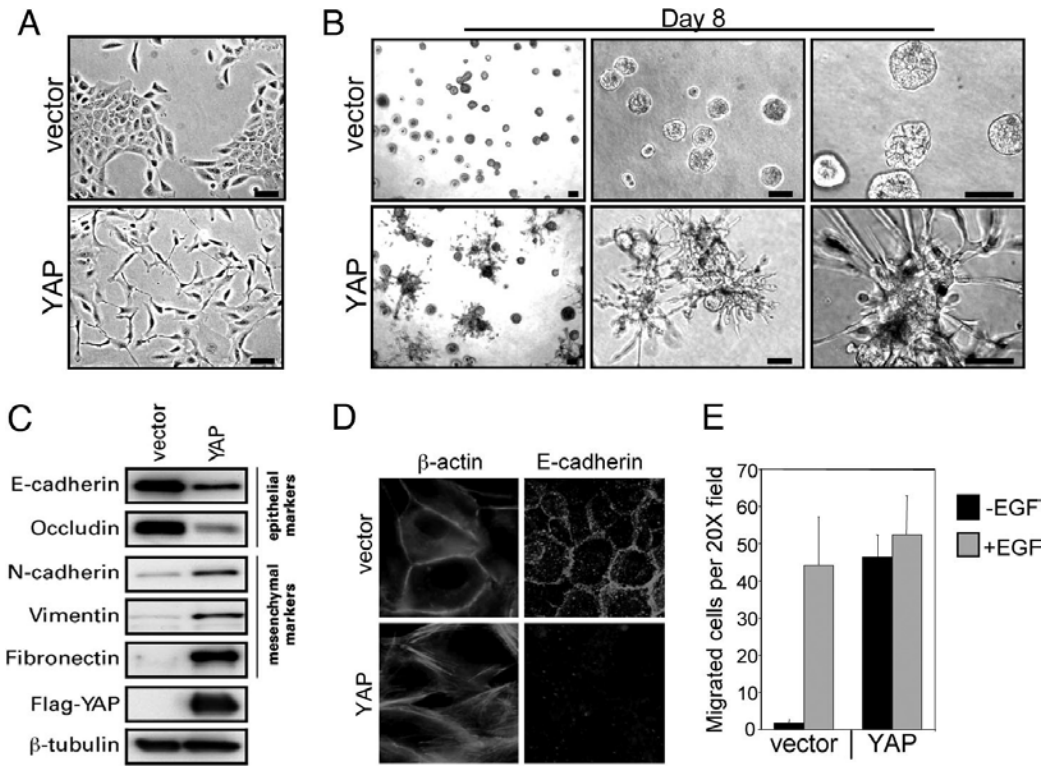


PNAS _ **August 15, 2006** _ vol. 103 _ no. 33 _ **12405–12410**

Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon

Michael Overholtzer, Jianmin Zhang, Gromoslaw A. Smolen, Beth Muir, Wenmei Li, Dennis C. Sgroi, Chu-Xia Deng, Joan S. Brugge, and Daniel A. Haber.

Yap induces EMT in mammary epithelial cells



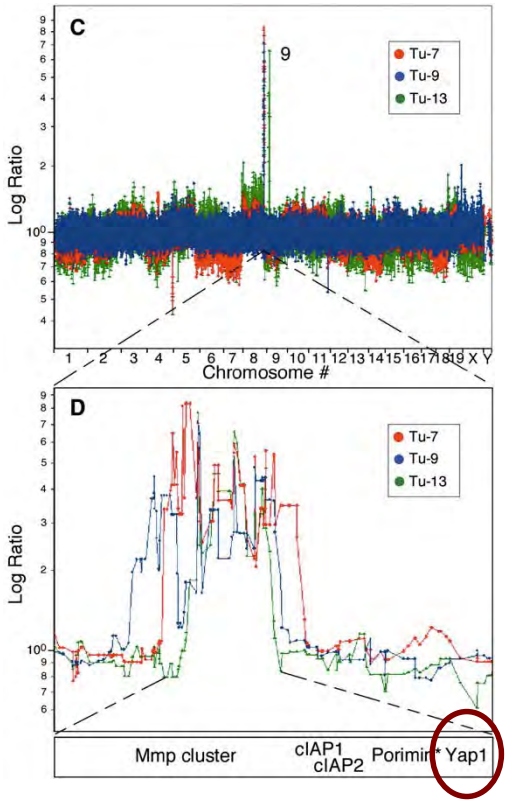
Yap overexpression downregulates E-cadherin, induces mesenchymal gene expression, and promotes migration and invasion

PNAS _ **August 15, 2006** _ vol. 103 _ no. 33 _ **12405–12410**

Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon

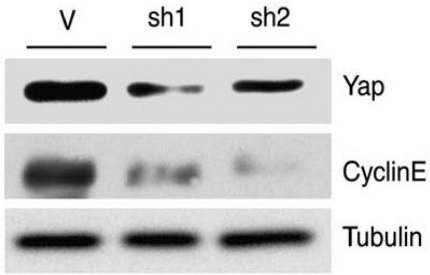
Michael Overholtzer, Jianmin Zhang, Gromoslaw A. Smolen, Beth Muir, Wenmei Li, Dennis C. Sgroi, Chu-Xia Deng, Joan S. Brugge, and Daniel A. Haber.

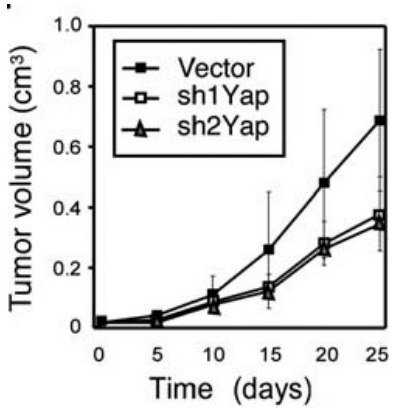
Transforming functions of Yap were also identified by Scott Lowe's lab



Yap is amplified in liver tumors from a p53-/- c-myc overexpressed mouse model

Yap overexpression promotes liver tumorigenesis in vivo



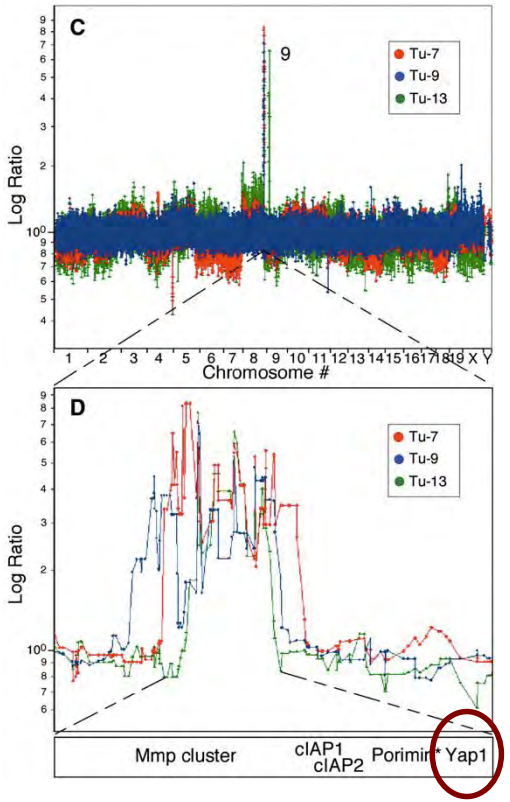


Cell 125, 1253–1267, June 30, 2006

Identification and Validation of Oncogenes in Liver Cancer Using an Integrative Oncogenomic Approach

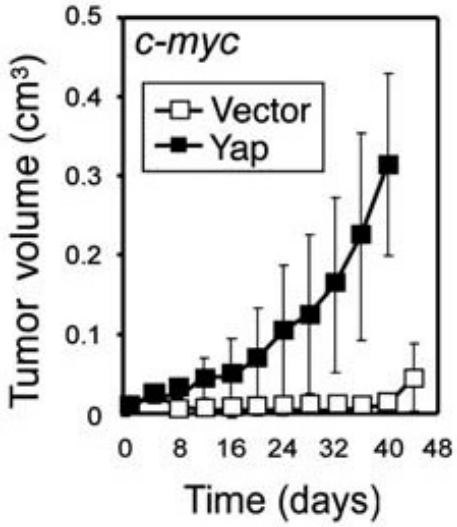
Lars Zender, Mona S. Spector, Wen Xue, Peer Flemming, Carlos Cordon-Cardo, John Silke, Sheung-Tat Fan, John M. Luk, Michael Wigler, Gregory J. Hannon, David Mu, Robert Lucito, Scott Powers, and Scott W. Lowe.

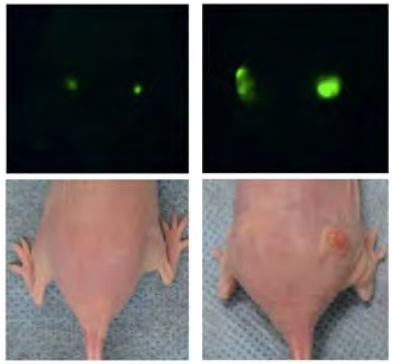
Transforming functions of Yap were also identified by Scott Lowe's lab



Yap is amplified in liver tumors from a p53-/- c-myc overexpressed mouse model

Yap overexpression promotes liver tumorigenesis in vivo





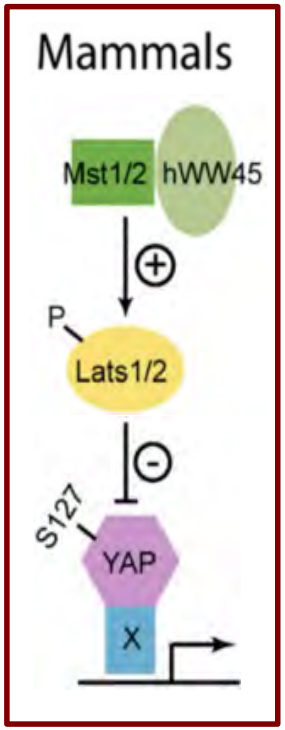
Cell 125, 1253–1267, June 30, 2006

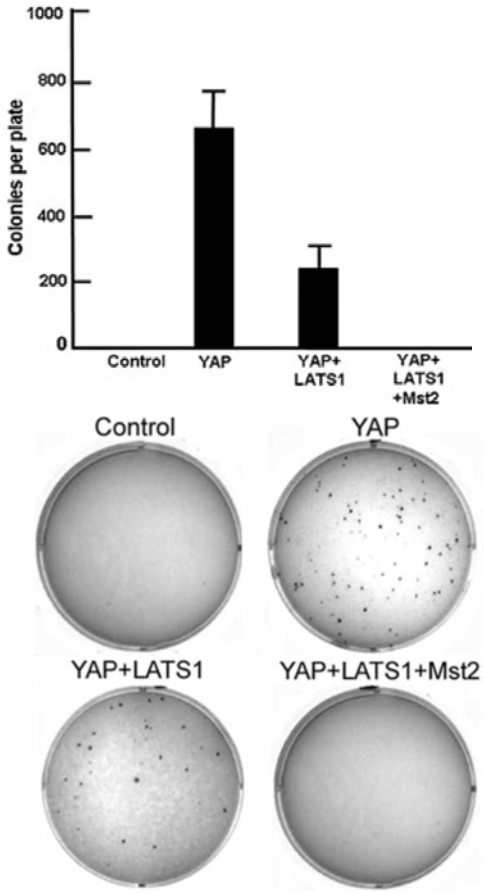
Identification and Validation of Oncogenes in Liver Cancer Using an Integrative Oncogenomic Approach

Lars Zender, Mona S. Spector, Wen Xue, Peer Flemming, Carlos Cordon-Cardo, John Silke, Sheung-Tat Fan, John M. Luk, Michael Wigler, Gregory J. Hannon, David Mu, Robert Lucito, Scott Powers, and Scott W. Lowe.

The cellular assays in mammary epithelial cells provided a platform to dissect the mammalian "Hippo" pathway

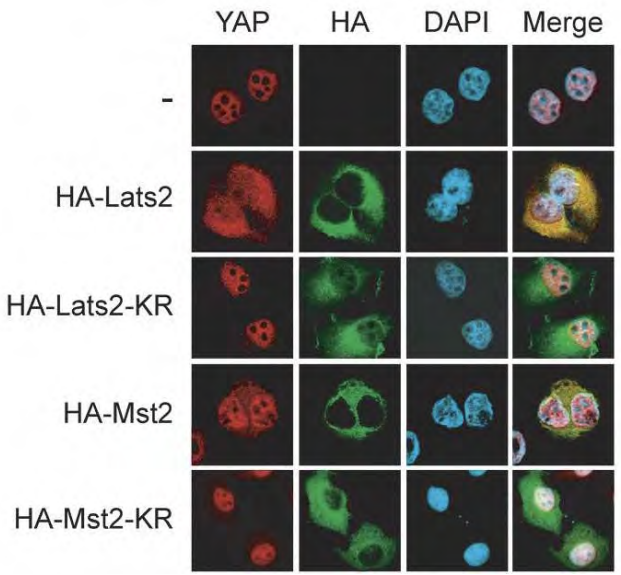
Yap-driven transformation is inhibited by Lats and Mst





(human mammary epithelial cells)

Phosphorylated Yap is excluded from the nucleus

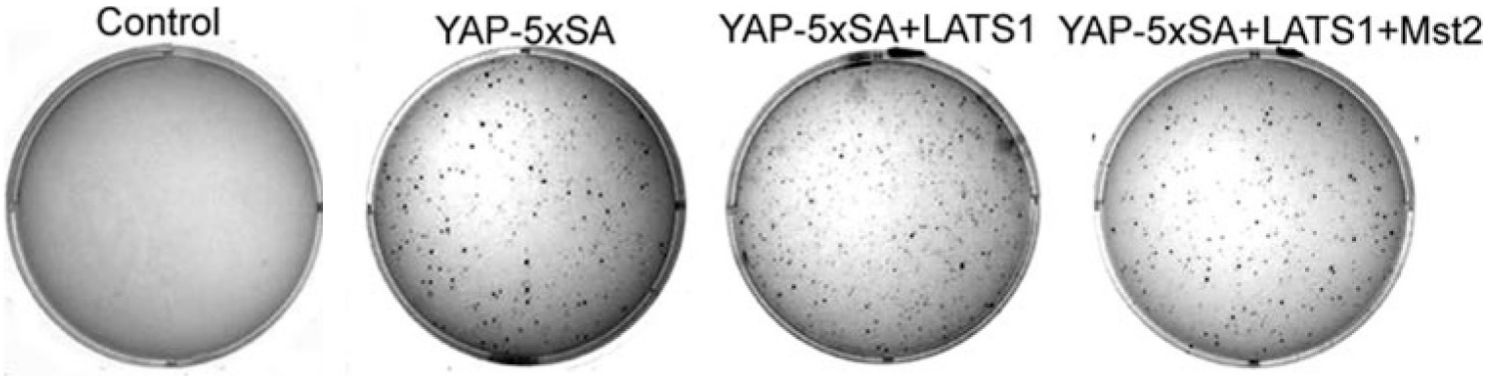


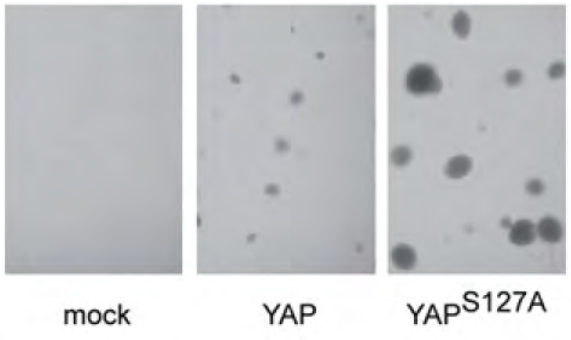
THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 283, NO. 9, pp. 5496–5509, February 29, 2008

Tumor Suppressor LATS1 Is a Negative Regulator of Oncogene *YAP**

Yawei Hao, Alex Chun, Kevin Cheung, Babak Rashidi, and Xiaolong Yang.

Non-phosphorylatable Yap is not inhibited by expression of Lats and Mst





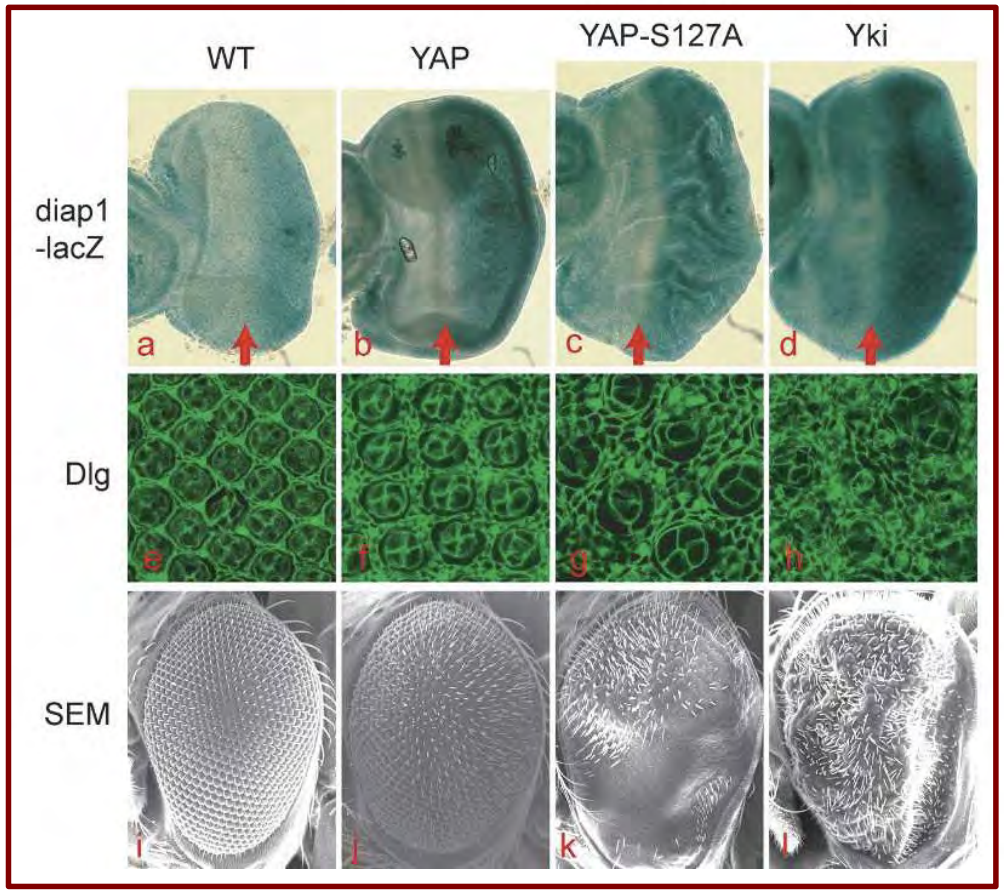
Yap S127A is generally a more potent transformer than wt Yap

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 283, NO. 9, pp. 5496–5509, February 29, 2008

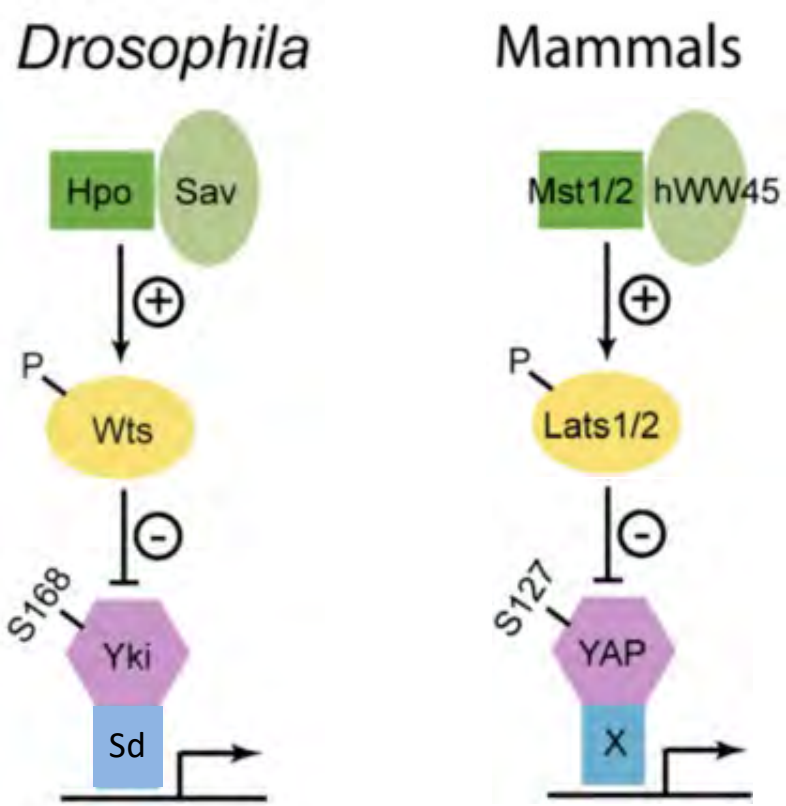
Tumor Suppressor LATS1 Is a Negative Regulator of Oncogene *YAP**

Yawei Hao, Alex Chun, Kevin Cheung, Babak Rashidi, and Xiaolong Yang.

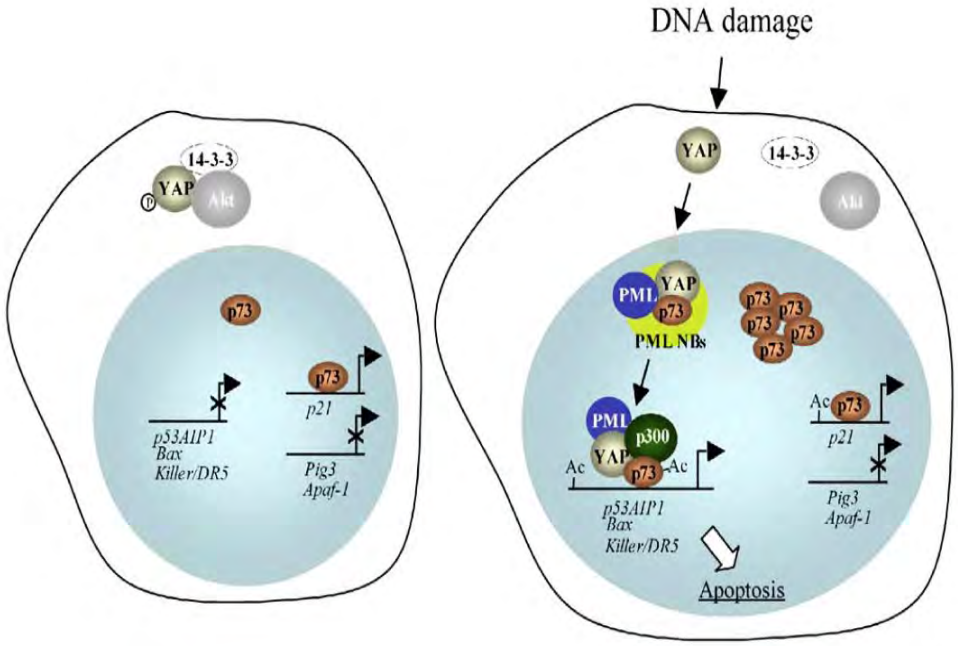
Non-phosphorylatable Yap drives more tissue overgrowth in Drosophila



Genes Dev. 2007 21: 2747-2761
Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control.
Bin Zhao, Xiaomu Wei, Weiquan Li, Ryan S. Udan, Qian Yang, Joungmok Kim, Joe Xie, Tsuneo Ikenoue, Jindan Yu, Li Li, Pan Zheng, Keqiang Ye, Arul Chinnaiyan, Georg Halder, Zhi-Chun Lai, and Kun-Liang Guan.

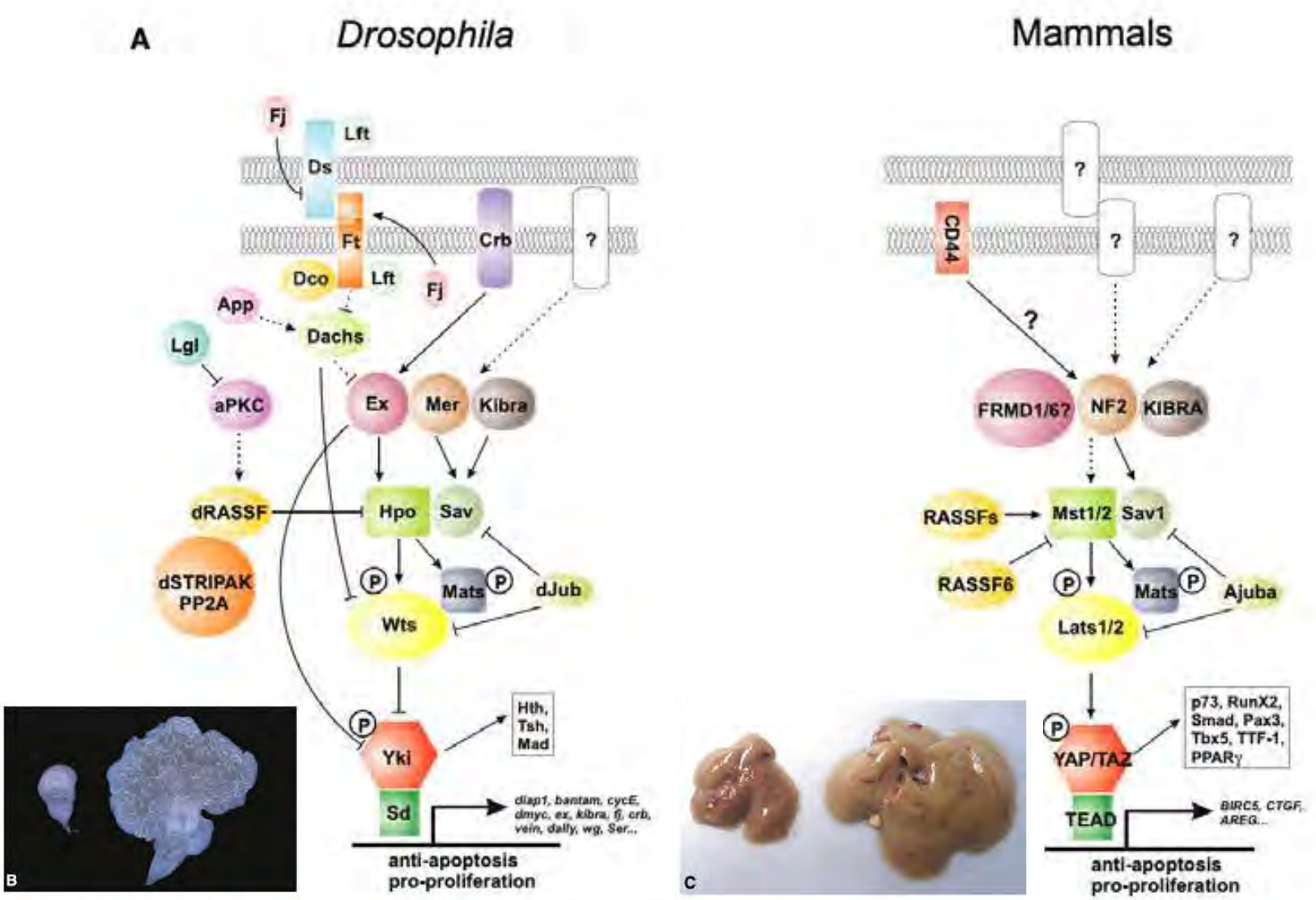

Drosophila
Hpo
Sav
P
Wts
S168
Yki
Sd
Mammals
Mst1/2
hWW45
P
Lats1/2
S127
YAP
X

Yap was originally reported to activate apoptosis:



- *Yap was shown to bind to p73 to enhance apoptosis in response to DNA damage*
- *Akt phosphorylated Yap on S127 to inhibit apoptosis by 14-3-3-dependent retention of Yap in the cytoplasm*

The significance of this pro-apoptotic pathway is unclear.


A
Drosophila
Mammals
Fj
Lft
Ds
Ft
Crb
?
Dco
Lft
Fj
App
Dachs
Lgl
aPKC
Ex
Mer
Kibra
dRASSF
Hpo
Sav
dSTRIPAK
PP2A
P
Mats
P
dJub
Wts
P
Yki
Hth,
Tsh,
Mad
Sd
dIap1, bantam, cycE,
dmyc, ex, kibra, fj, crb,
vein, dally, wg, Ser...
anti-apoptosis
pro-proliferation
B
CD44
?
?
?
?
FRMD1/6?
NF2
KIBRA
RASSFs
Mst1/2
Sav1
RASSF6
P
Mats
P
Ajuba
Lats1/2
P
YAP/TAZ
p73, RunX2,
Smad, Pax3,
Tbx5, TTF-1,
PPARγ
TEAD
BIRC5, CTGF,
AREG...
anti-apoptosis
pro-proliferation
C