

Genetics-multistep tumorigenesis genomic integrity & cancer

Sections 11.1-11.8 from
Weinberg's 'the biology of
Cancer'

Cancer genetics and genomics
Selected publications (more of a
journal club format)

Tuesday, February 14 lecture

Vol 463 | 14 January 2010 | doi:10.1038/nature08658

nature

ARTICLES

A comprehensive catalogue of somatic mutations from a human cancer genome

Erin D. Pleasance^{1*}, R. Keira Cheetham^{2*}, Philip J. Stephens¹, David J. McBride¹, Sean J. Humphray², Chris D. Greenman¹, Ignacio Varela¹, Meng-Lay Lin¹, Gonzalo R. Ordóñez¹, Graham R. Bignell¹, Kai Ye³, Julie Alipaz⁴, Markus J. Bauer², David Beare¹, Adam Butler¹, Richard J. Carter², Lina Chen¹, Anthony J. Cox², Sarah Edkins¹, Paula I. Kokko-Gonzales², Niall A. Gormley², Russell J. Grocock², Christian D. Haudenschild², Matthew M. Hims², Terena James², Mingming Jia¹, Zoya Kingsbury², Catherine Leroy¹, John Marshall¹, Andrew Menzies¹, Laura J. Mudie¹, Zemin Ning¹, Tom Royce⁴, Ole B. Schulz-Trieglaff², Anastassia Spiridou², Lucy A. Stebbings¹, Lukasz Szajkowski², Jon Teague¹, David Williamson², Lynda Chin⁶, Mark T. Ross², Peter J. Campbell¹, David R. Bentley², P. Andrew Futreal¹ & Michael R. Stratton^{1,7}

Tuesday, February 14 lecture

nature

Vol 463 | 14 January 2010 | doi:10.1038/nature08629

ARTICLES

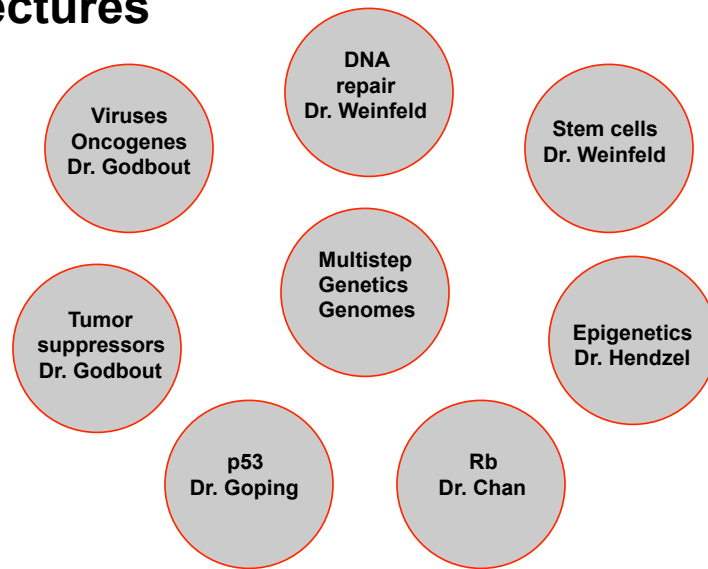
A small-cell lung cancer genome with complex signatures of tobacco exposure

Erin D. Pleasance¹, Philip J. Stephens¹, Sarah O'Meara^{1,2}, David J. McBride¹, Alison Meynert³, David Jones¹, Meng-Lay Lin¹, David Beare¹, King Wai Lau¹, Chris Greenman¹, Ignacio Varela¹, Serena Nik-Zainal¹, Helen R. Davies¹, Gonzalo R. Ordoñez¹, Laura J. Mudie¹, Calli Latimer¹, Sarah Edkins¹, Lucy Stebbings¹, Lina Chen¹, Mingming Jia¹, Catherine Leroy¹, John Marshall¹, Andrew Menzies¹, Adam Butler¹, Jon W. Teague¹, Jonathon Mangion², Yongming A. Sun⁴, Stephen F. McLaughlin⁵, Heather E. Peckham⁵, Eric F. Tsung⁵, Gina L. Costa⁵, Clarence C. Lee⁵, John D. Minna⁶, Adi Gazdar⁶, Ewan Birney³, Michael D. Rhodes⁴, Kevin J. McKernan⁵, Michael R. Stratton^{1,7}, P. Andrew Futreal¹ & Peter J. Campbell^{1,8}

Exam Question
(Tuesday, February 28)

10 marks for 10 points

Integration with other ONCOL520 lectures



“All cancers arise as a result of changes that have occurred in the DNA sequence of the genomes of cancer cells”

Stratton, MR (2009) *Nature* 458:719

Key concepts

- ❑ Sequential acquisition of genomic (epigenomic) alterations that favor tumorigenesis
- ❑ Clonal evolution and tumor heterogeneity
- ❑ Cancer genetics and cancer genomes

Cancer: a disease of ageing

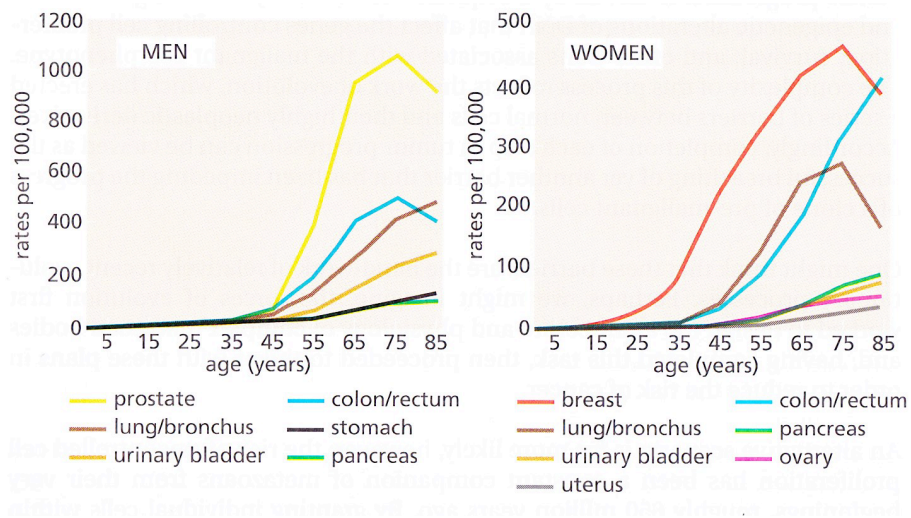


Figure 11.1 *The Biology of Cancer* (© Garland Science 2007)

Cancer: it takes time

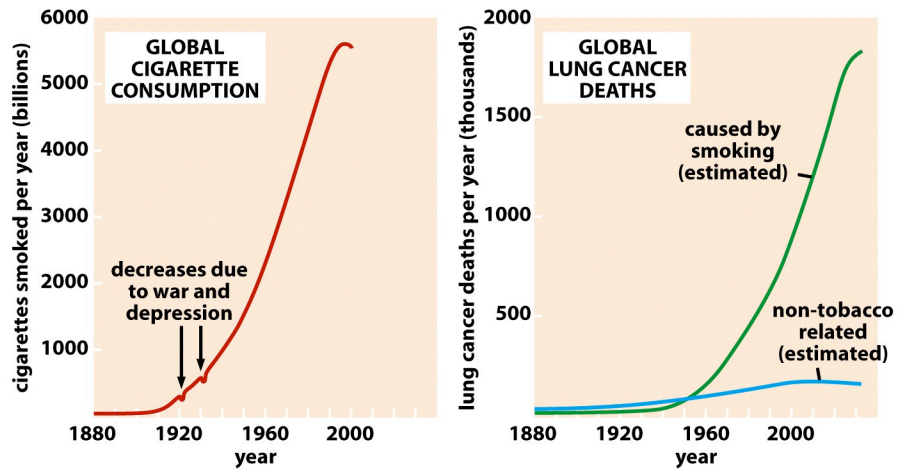


Figure 11.2 *The Biology of Cancer* (© Garland Science 2007)

Multistep change in tissue architecture

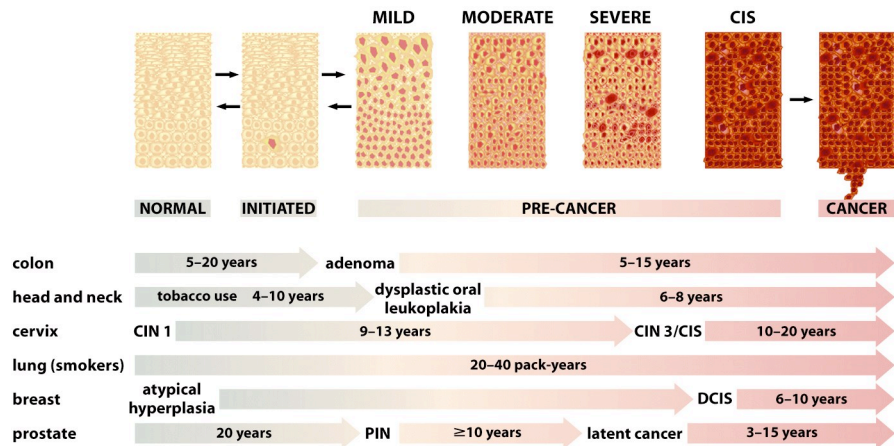
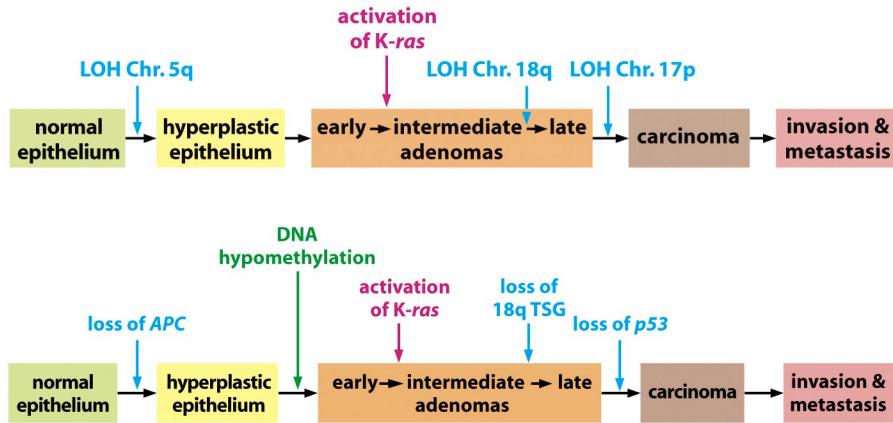


Figure 11.7 *The Biology of Cancer* (© Garland Science 2007)

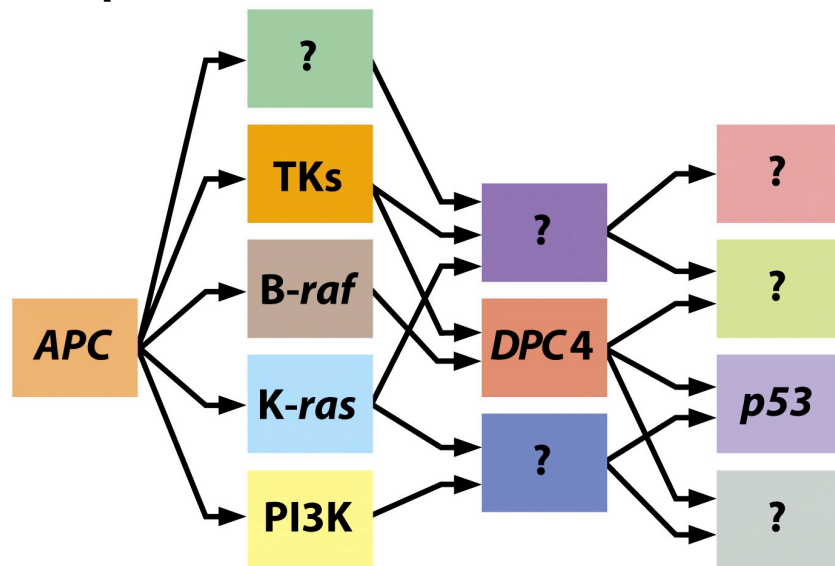
Multistep change in genomic alterations



Ability to identify requires distinguishing alleles
Also, note the limited number of 'driver' events during progression

Figure 11.9 *The Biology of Cancer* (© Garland Science 2007)

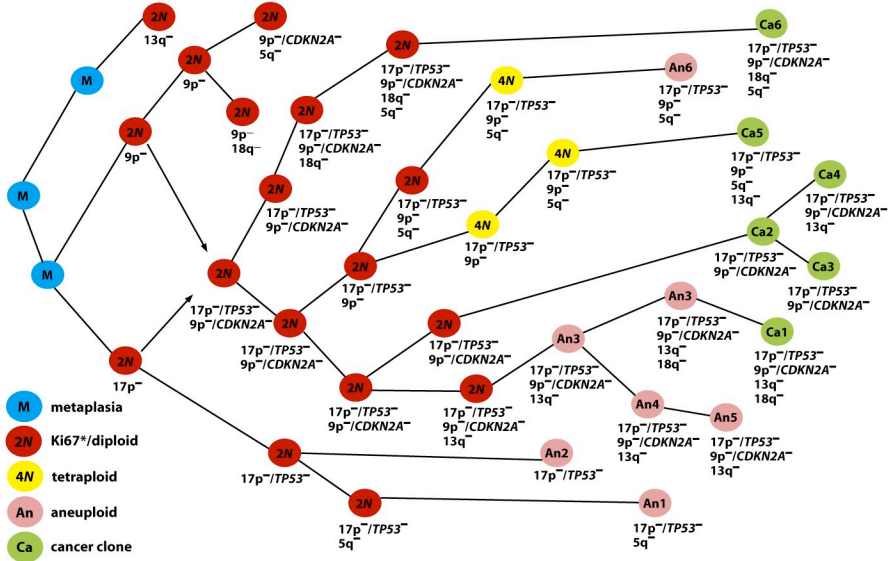
Multiple roads to the same destination



Think heterogeneity in tumorigenesis

Figure 11.11a *The Biology of Cancer* (© Garland Science 2007)

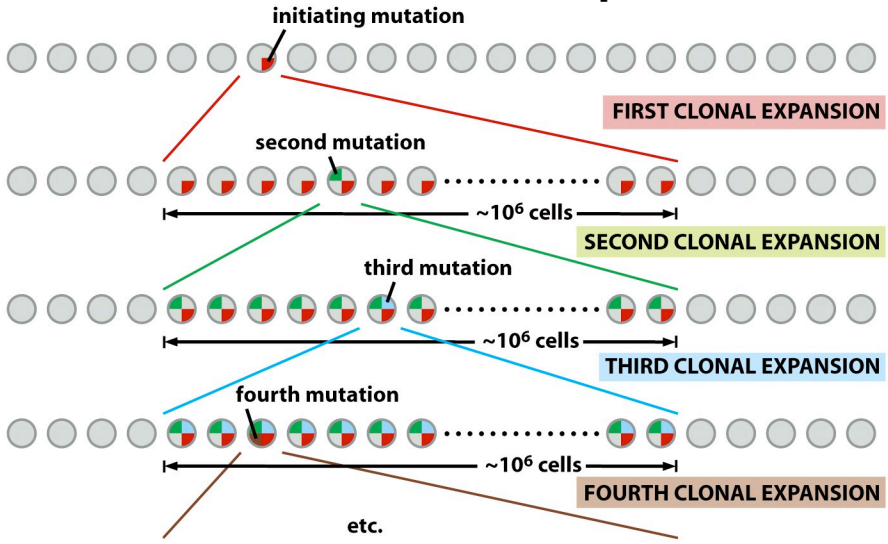
Multiple roads to the same destination



Think heterogeneity in tumorigenesis

Figure 11.11b *The Biology of Cancer* (© Garland Science 2007)

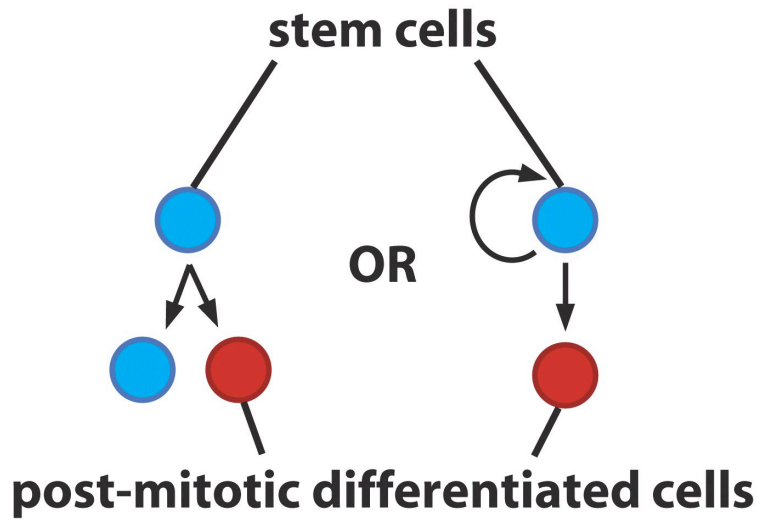
Clonal evolution & expansion



Don't be fooled by the seemingly homogenous expanded pools

Figure 11.12 *The Biology of Cancer* (© Garland Science 2007)

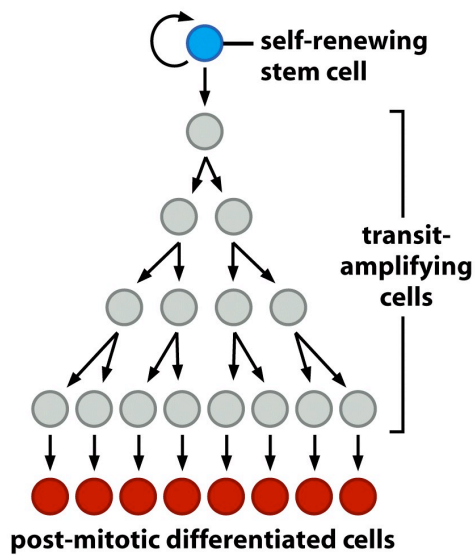
Stem cells & clonal evolution



Not all tumor cells are created equal

Figure 11.16a *The Biology of Cancer* (© Garland Science 2007)

Stem cells & clonal evolution



Not all cells created equal: cell hierarchies

Figure 11.16b *The Biology of Cancer* (© Garland Science 2007)

Stem cells & clonal evolution

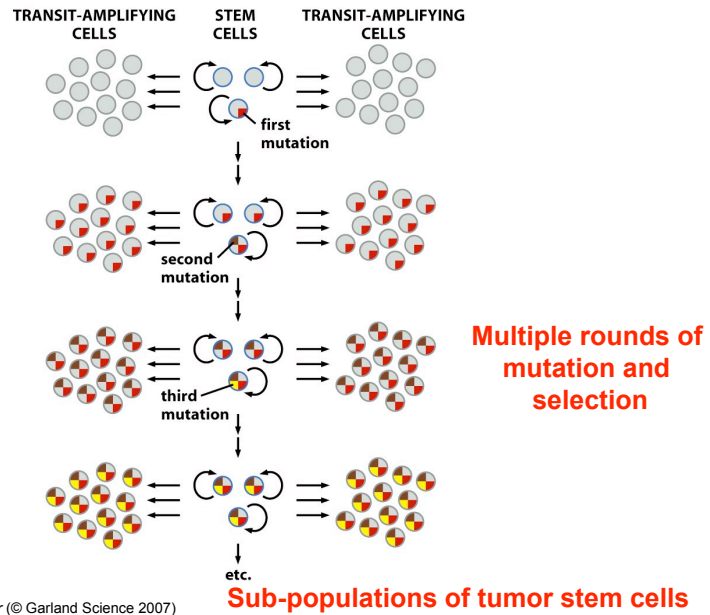


Figure 11.17 *The Biology of Cancer* (© Garland Science 2007)

Stem cells & clonal evolution

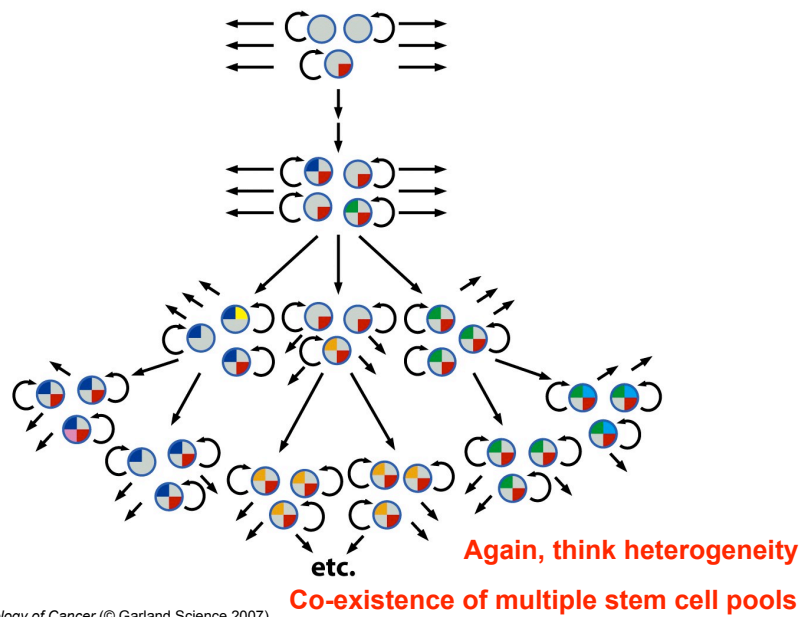
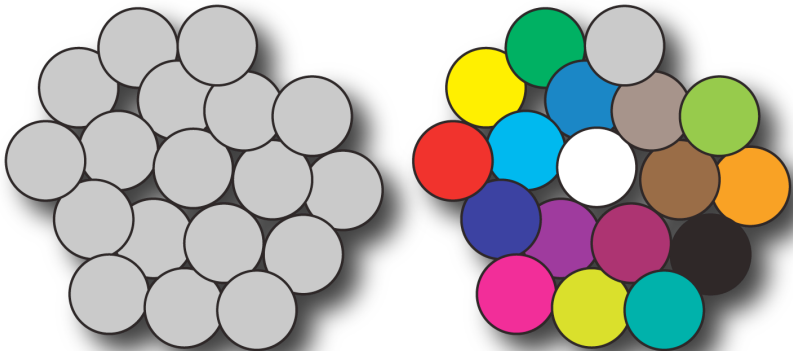


Figure 11.18 *The Biology of Cancer* (© Garland Science 2007)

...in other words, visualize



Not this

This

The concept of genetic heterogeneity

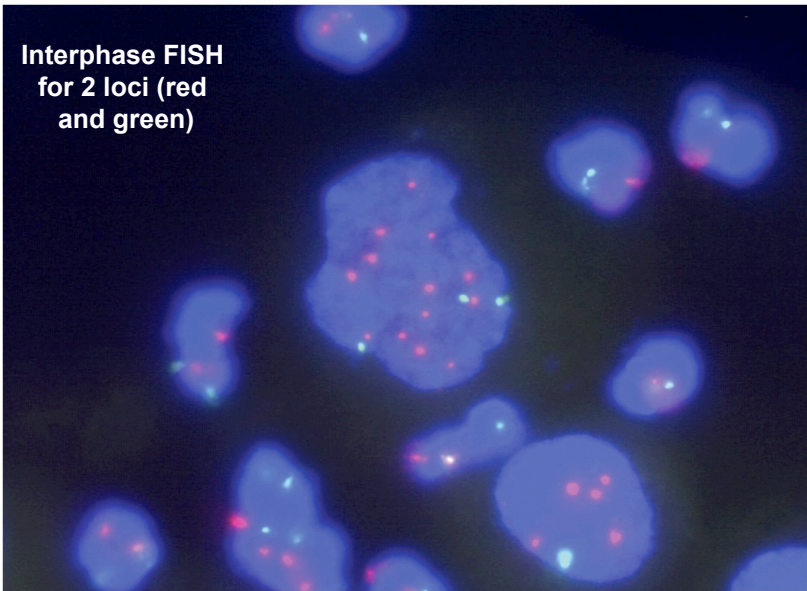


Figure 11.19 *The Biology of Cancer* (© Garland Science 2007)

Why are all these things different?

❑ These are just two genomic loci

❑ What about whole genomes (the post-textbook era)?

Today's reality

CBCnews | Technology & Science

IN THE NEWS

- Canada's oil pipelines
- U.S. campaign blog
- CES: Gadget list
- Haiti two years later

[Home](#) [World](#) [Canada](#) [Politics](#) [Business](#) [Health](#) [Arts & Entertainment](#) [Technology & Science](#) [Community](#) [Weather](#) [Video](#)

[Technology & Science](#) [Quirks & Quarks Blog](#) [Photo Galleries](#)

Human DNA decoded for \$1K in 1 day

Machine decodes human genome's 3 billion chemical building blocks

The Associated Press | Posted: Jan 11, 2012 11:02 AM ET | Last Updated: Jan 11, 2012 10:58 AM ET 2



A scientist works with a machine that reads DNA at the Life Technologies laboratory in Carlsbad, Calif. The company says its machine can decode a human genome in a day for \$1,000 US. (Gregory Bull/Associated Press)

Stay Connected with CBC News



YOUR IDEAL CAR:
FIND IT HERE IN TWO CLICKS



kijiji
AUTOS

SELL YOUR CAR
BROWSE CARS

AP/UP BY CCM/PA/T

❑ The need to understand cancer genomes (& personal genomes)

❑ Your genome vs. the cancer genome

❑ Germline vs. sporadic

❑ Evolution of technologies to enable this goal (fallout from the human genome project)



Normal cell



Cancer cell

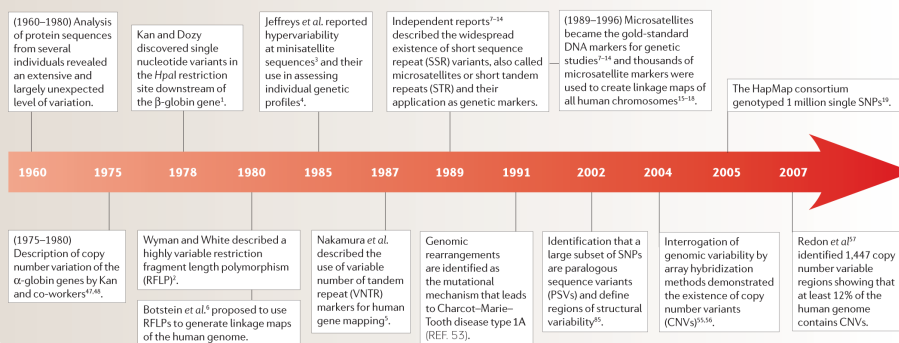
One of these men will succumb to cancer

❑ **Somatic: acquisition of mutations that convert a normal cell to a cancer cell**

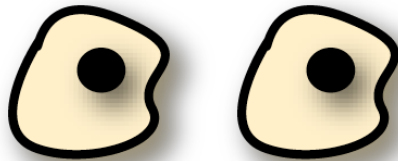
❑ **Germline: inheritance of an alteration or mutation that will cause or predispose to cancer**

A brief history of genetic variation

Timeline | Landmarks in the study of human genetic variation



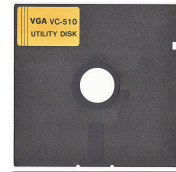
Circa 1980 (the way it was)



Normal cell

Cancer cell

DNA-mediated gene transfer



...and no internet

Activating oncogenic mutations

A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene

E. Premkumar Reddy, Roberta K. Reynolds, Eugenio Santos & Mariano Barbacid
Laboratory of Cellular and Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, USA

The genetic change that leads to the activation of the oncogene in T24 human bladder carcinoma cells is shown to be a single point mutation of guanosine into thymidine. This substitution results in the incorporation of valine instead of glycine as the twelfth amino acid residue of the T24 oncogene-encoded p21 protein. Thus, a single amino acid substitution appears to be sufficient to confer transforming properties on the gene product of the T24 human bladder carcinoma oncogene.

Mechanism of activation of a human oncogene

Clifford J. Tabin, Scott M. Bradley, Cornelia I. Bargmann & Robert A. Weinberg
Whitehead Institute for Biomedical Research, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Alex G. Papageorge & Edward M. Scolnick
Merck Laboratories, West Point, Pennsylvania 19486, USA

Ravi Dhar, Douglas R. Lowy & Esther H. Chang*

Laboratories of Molecular Virology and Dermatology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, USA
* Present address: Department of Pathology, Uniformed Services University for the Health Sciences, Bethesda, Maryland 20014, USA

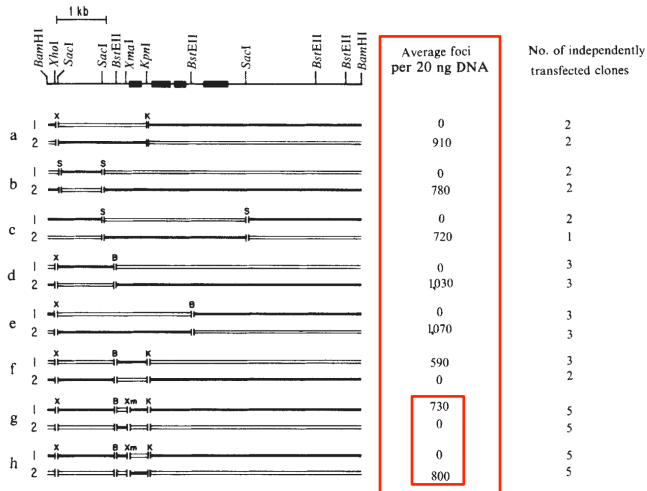
The oncogene of the human EJ bladder carcinoma cell lines arose via alteration of a cellular proto-oncogene. Experiments are presented that localize the genetic lesion that led to activation of the oncogene. The lesion has no effect on levels of expression of the oncogene. Instead, it affects the structure of the oncogene-encoded protein.

0028-0836/82/450143-04\$01.00

© 1982 Macmillan Journals Ltd

circa 1982

Fig. 5 Transfection data and structures of *in vitro* genetic recombinants between the molecular clones of the EJ transforming gene (pEJ) and its normal cellular homologue (pEC). The restriction map shows the cleavage sites for various enzymes within the 6.6-kb BamHI insert in pBR322. All sites specific for the enzymes are shown except for *Xma*I which cuts in several other places which have not been well characterized. The site shown is the only *Xma*I site between the first *Bst*EII site and the *Kpn*I site. The solid boxes on the map show the locations of coding exons. pEJ/pEC chimaeras are shown, with segments derived from pEJ shown as solid bars and segments from pEC shown as open bars. pEJ and pEC were cleaved with the indicated enzymes either to completion or in a partial digest as required to obtain each indicated fragment. The products were separated by electrophoresis through 1.2% agarose and eluted by melting in NaI and adsorbing to glass beads. The fragment containing pBR322 was then treated with calf intestinal phosphatase. The indicated fragments were joined either with the enzyme T4 DNA ligase or in a mock ligation without enzyme. Constructs a-e were made in bimolecular ligations. Constructs in f were made by mixing the three fragments simultaneously and in g and h by mixing the four fragments simultaneously. The ligation mixtures were directly transformed into the HB101 strain of *Escherichia coli*. Only when colonies from mock ligations were less than 2% of the ligations were colonies analysed for the presence of clones with appropriate restriction maps; 20 ng of each clone were transfected to NIH 3T3 cells as described in Fig. 2 legend and then carried without selection until foci were visualized in 10-14 days. Results of the transfections are shown in the first column. The second column shows the number of independent bacterial colonies screened and then transfected into NIH 3T3 cells.



Swapped tumor and normal DNA to find critical region

Activity could be mapped to a 350bp fragment, which was sequenced; single mutation changes Gly to Val

FYI: current day sequencing projects involve 100,000,000,000bp (and increasing rapidly)

❑ Catalyzed the discovery of many more cancer genes, primarily through genetic linkage (positional cloning).

❑ ...but painstakingly slow.

Table 1. Summary of genes responsible for inherited cancer predisposition, their chromosomal location, the syndromes they cause and the functions of the gene products (mode of inheritance is shown in italics).

Gene	Syndrome	Location	Principal function	Principal malignancies
<i>RB1</i>	familial retinoblastoma; <i>dominant</i>	13q14	transcriptional/ cell cycle regulator	retinoblastoma
<i>P16^{INK4a}</i>	familial melanoma; <i>dominant</i>	9p21	CDK inhibitor	melanoma
<i>CDK4</i>	familial melanoma; <i>dominant</i>	12q13	CDK	melanoma
<i>PS3</i>	Li-Fraumeni; <i>dominant</i>	17p13.1	transcription factor	sarcomas, breast cancer
<i>APC</i>	familial adenomatous polyposis; <i>dominant</i>	5q21	growth factor	colorectal cancer
<i>CDH1</i>	hereditary diffuse gastric cancer; <i>dominant</i>	16q22.1	signalling cell-to-cell adhesion	diffuse gastric cancer
<i>LKB1</i>	Peutz-Jeghers; <i>dominant</i>	19p13.3	serine threonine kinase	gastrointestinal cancer
<i>PTEN</i>	Cowden syndrome; juvenile polyposis coli; <i>dominant</i>	10q23.3	phosphatase, cytoskeletal protein?	breast cancer, gastrointestinal cancer
<i>SMAD4</i>	juvenile polyposis coli; <i>dominant</i>	18q21.2	growth factor signalling	gastrointestinal cancer
<i>MEN1</i>	multiple endocrine neoplasia type 1; <i>dominant</i>	11q13	transcription co-factor	endocrine
<i>RET</i>	multiple endocrine neoplasia type 2; <i>dominant</i>	10q11.2	receptor tyrosine kinase	endocrine
<i>MET</i>	Hereditary papillary renal cancer; <i>dominant</i>	7q31	receptor tyrosine kinase	papillary renal cancer
<i>KIT</i>	familial gastrointestinal stromal tumours; <i>dominant</i>	4q12	receptor tyrosine kinase	gastrointestinal cancer (stromal)
<i>PTCH</i>	basal cell nevus syndrome; <i>dominant</i>	9q22.3	membrane receptor	basal cell (skin)
<i>NF1</i>	neurofibromatosis type 1; <i>dominant</i>	17q11.2	GTPase-activating protein	neurofibrosarcomas
<i>NF2</i>	neurofibromatosis type 2; <i>dominant</i>	22q12.2	cytoskeletal protein?	central nervous system tumours
<i>VHL</i>	von Hippel-Lindau <i>dominant</i>	3p25	protein maturation? RNA elongation?	renal clear cell carcinomas, pheochromocytomas
<i>WT1</i>	Wilms tumour; <i>dominant</i>	11p13	transcription factor	nephroblastoma
<i>BLM</i>	Bloom syndrome; <i>recessive</i>	15q26.1	dsDNA repair?	leukaemia, lymphoma
<i>FANCA; FANCC; others</i>	Fanconi anaemia; <i>recessive</i>	16q24.3; 9q22.3; ?	dsDNA repair?	leukaemia
<i>XPD; NFD</i> others	xeroderma pigmentosum; <i>recessive</i>	2q21; 19q13; ?	helicases, nucleotide excision repair	basal cell and squamous cell carcinomas
<i>ATM</i>	ataxia telangiectasia; <i>recessive</i>	11q22.3	serine-threonine protein kinase	lymphoma, leukaemia
<i>NBS1</i>	Nijmegen breakage syndrome; <i>recessive</i>	8q21	transcription factor? dsDNA repair?	lymphoma
<i>BRC41</i>	familial breast/ovarian cancer; <i>dominant</i>	17q21	transcription factor? dsDNA repair	breast, ovarian cancer
<i>BRC42</i>	familial breast/ovarian cancer; <i>dominant</i>	13q12	transcription factor? dsDNA repair	breast, ovarian cancer
<i>MLH1; MSH2</i>	hereditary non-polyposis colorectal cancer; <i>dominant</i>	3p21; 2p16; 2q32; 7p22; 2p16	DNA mismatch repair	colorectal, endometrial cancer

Guilford (2000) *Cell. Mol. Life Sci* 57:589

All of these are associated with some obvious change to the gene and usually the encoded protein

...what can these include?

- **Substitutions**
- **Insertions (varying)**
- **Deletions (varying)**
- **Rearrangements (inter and intrachromosomal)**
- **Copy number increase**
- **Copy number decrease**
- **Viral DNA**
- **Mitochondria**
- **Epigenetic**

All of these are associated with some obvious change to the gene and usually the encoded protein

...but not always. What are some other possibilities?

- Amino acid change
- Splicing
- Regulatory (promoter or enhancer)
- UTR
- Non-coding RNA

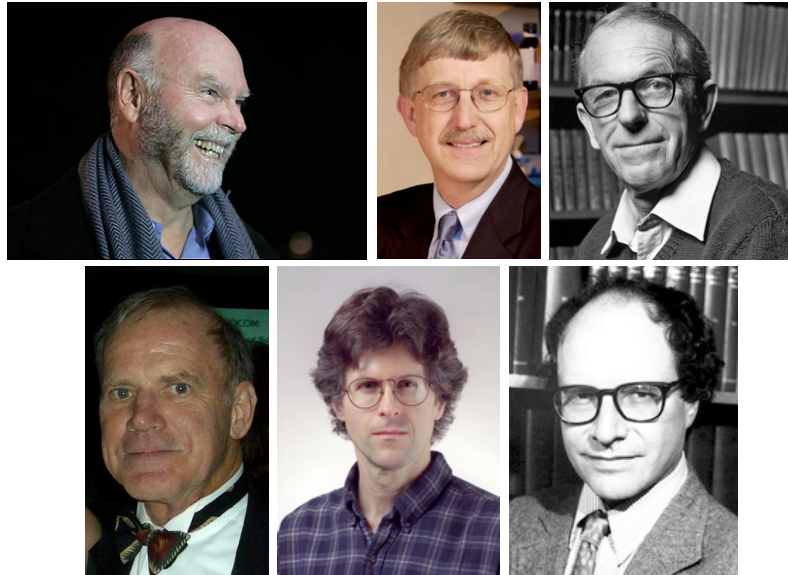
Keep in mind the mechanistic heterogeneity; multiple ways to gain or lose protein function

Status thus far?

- **~350 of 22,000 protein encoding genes show recurrent somatic mutations**
- **Most by genetic and physical mapping** (again, painstaking)
- **Identifying genes with transforming activity** (i.e., H-RAS)
- **Animal models**
- **Guess work?**

Can we fully define the range of somatic changes that accompany the conversion of a normal cell to a cancer cell?

Understanding the cancer genome requires that we understand our own genomes



Human genome timeline

1985

In 1985, Charles DeLisi, then associate director for health and environmental research at the Department of Energy (DoE), begins to discuss a mammoth project — of a scale unprecedented in biology — to sequence the complete human genome. DoE funding begins in 1987.



Interested in affect of radiation on mutation (Department of Energy)

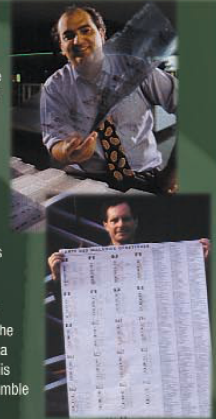
1988

The National Institutes of Health (NIH) establishes the Office of Human Genome Research in September 1988. Renamed the National Center for Human Genome Research (NCHGR) a year later, its director is James Watson, co-discoverer of the double helix structure of DNA. Watson's testimony to the US Congress, in which he pledged to devote a small fraction of the project's budget to 'ethical, legal and social' issues, had proved instrumental in garnering political support.



Early 1990s

With sequencing still slow and expensive, the genome project adopts a 'map-first, sequence-later' strategy. In the early 1990s, two Parisian laboratories, the Centre d'Etude du Polymorphisme Humain and Génethon, have an integral role in mapping — underlining the project's international character. The labs' driving forces are Daniel Cohen (top) and Jean Weissenbach. Later, the genome project constructs a higher-resolution map that is used to sequence and assemble the human genome.

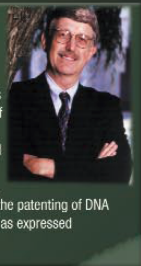


Officially launched

Human genome timeline

1992

Francis Collins of the University of Michigan replaces Watson as head of NCHGR in April 1992. Watson had earlier clashed with Craig Venter, then at NIH, over the patenting of DNA fragments known as expressed sequence tags.



Collins takes over

1992

Later that year, Venter sets up The Institute for Genomic Research (TIGR) in Rockville, Maryland. TIGR later sequences a host of bacterial genomes, starting with *Haemophilus influenzae*.



1996



In February 1996, at a meeting in Bermuda, international partners in the genome project agree to formalize the conditions of data access, including release of sequence data into public databases within 24 hours. These came to be known as the 'Bermuda principles'.

Human genome timeline

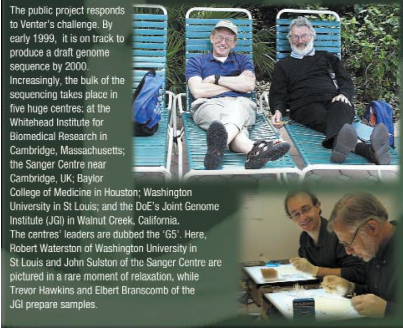
1998



In May 1998, Venter forms a company to sequence the human genome within three years. The company, later named Celera, will use an ambitious "whole genome shotgun" method, which involves assembling the genome without using maps. But its data release policy will not follow the Bermuda principles.

Venter enters the picture (\$\$\$\$\$)

1999



The public project responds to Venter's challenge. By early 1999, it is on track to produce a draft genome sequence by 2000. Increasingly, the bulk of the sequencing takes place in five huge centres: at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts; the Sanger Centre near Cambridge, UK; Baylor College of Medicine in Houston; Washington University in St Louis; and the DoE's Joint Genome Institute (JGI) in Walnut Creek, California. The centres' leaders are dubbed the 'G5'. Here, Robert Waterston of Washington University in St Louis and John Sulston of the Sanger Centre are pictured in a rare moment of relaxation. While Trevor Hawkins and Ebert Branscomb of the JGI prepare samples.

1999-2000



The first complete human chromosome sequence — number 22 — is published in December 1999. Chromosome 21 follows in May 2000, a collaborative effort led by German and Japanese groups under the direction of Andre Rosenthal and Yoshiyuki Sakaki, respectively. Sakaki (centre) is pictured here at Nature's chromosome 21 press conference in Tokyo.

2000



On 26 June 2000, leaders of the public project and Celera announce completion of a working draft of the human genome sequence. Collins and Venter are seen here on television with Ari Patrinos of the DoE, who cut through the animosity between the rival projects to broker the joint announcement at the White House in Washington.

Outside, celebrations continue with Eric Lander of the Whitehead Institute, Baylor's Richard Gibbs, and Waterston and Richard Wilson from Washington University.



Completion of draft announced in 2000

Draft version published in 2001 (complete in 2003)

This week



Finally, this week sees the publication of the draft genome, the public sequence in *Nature*, Celera's in *Science*.

13 years and \$437 million later, we have a 'complete' genome sequence

\$3 billion allocated to various genome projects over this time

“Exploiting this variation has huge implications for cancer research, treatment, and prevention”