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. Stephens[1], David J. McBride[1], Sean J. Humphray[2], Chris D. Greenman[1], Ignacio Varela[1], Meng-Lay Lin[1], Gonzalo R. Ordóñez[1], Graham R. Bignell[1], Kai Ye[3], Julie Alipaz[4], Markus J. Bauer[2], David Beare[1], Adam Butler[1], Richard J. Carter[2], Lina Chen[1], Anthony J. Cox[2], Sarah Edkins[1], Paula I. Kokko-Gonzales[2], Niall A. Gormley[2], Russell J. Grocock[2], Christian D. Haudenschild[5], Matthew M. Hims[2], Terena James[2], Mingming Jia[1], Zoya Kingsbury[2], Catherine Leroy[1], John Marshall[1], Andrew Menzies[1], Laura J. Mudie[1], Zemin Ning[1], Tom Royce[4], Ole B. Schulz-Trieglaff[2], Anastassia Spiridou[2], Lucy A. Stebbings[1], Lukasz Szajkowski[2], Jon Teague[1], David Williamson[5], Lynda Chin[6], Mark T. Ross[2], Peter J. Campbell[1], David R. Bentley[2], P. Andrew Futreal[1] & Michael R. Stratton[1,7]

Tuesday, February 14 lecture

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

ARTICLES

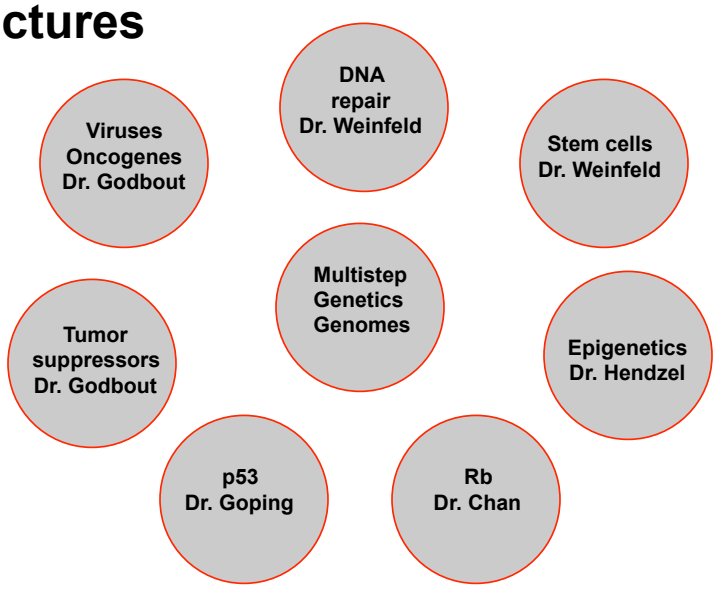
A small-cell lung cancer genome with complex signatures of tobacco exposure

Erin D. Pleasance[1], Philip J. Stephens[1], Sarah O'Meara[1,2], David J. McBride[1], Alison Meynert[3], David Jones[1], Meng-Lay Lin[1], David Beare[1], King Wai Lau[1], Chris Greenman[1], Ignacio Varela[1], Serena Nik-Zainal[1], Helen R. Davies[1], Gonzalo R. Ordoñez[1], Laura J. Mudie[1], Calli Latimer[1], Sarah Edkins[1], Lucy Stebbings[1], Lina Chen[1], Mingming Jia[1], Catherine Leroy[1], John Marshall[1], Andrew Menzies[1], Adam Butler[1], Jon W. Teague[1], Jonathon Mangion[2], Yongming A. Sun[4], Stephen F. McLaughlin[5], Heather E. Peckham[5], Eric F. Tsung[5], Gina L. Costa[5], Clarence C. Lee[5], John D. Minna[6], Adi Gazdar[6], Ewan Birney[3], Michael D. Rhodes[4], Kevin J. McKernan[5], Michael R. Stratton[1,7], P. Andrew Futreal[1] & 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

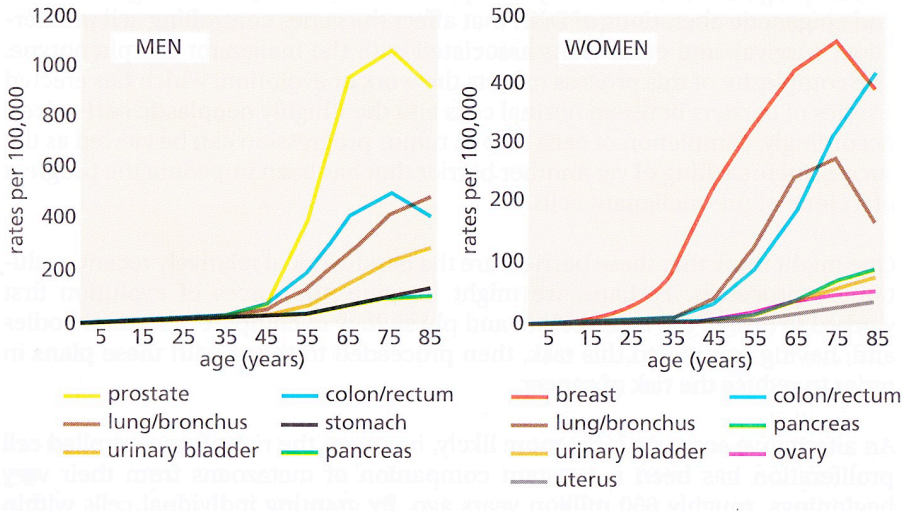


Figure 11.1 *The Biology of Cancer* (© Garland Science 2007)

Cancer: it takes time

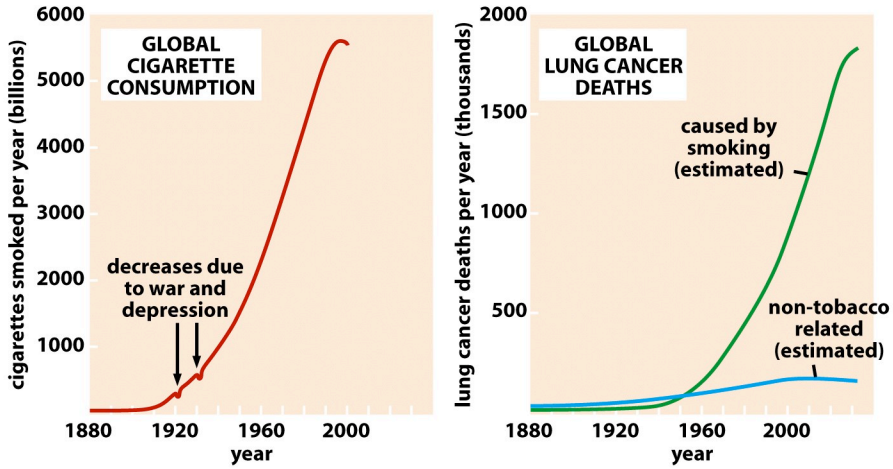


Figure 11.2 *The Biology of Cancer* (© Garland Science 2007)

Multistep change in tissue architecture

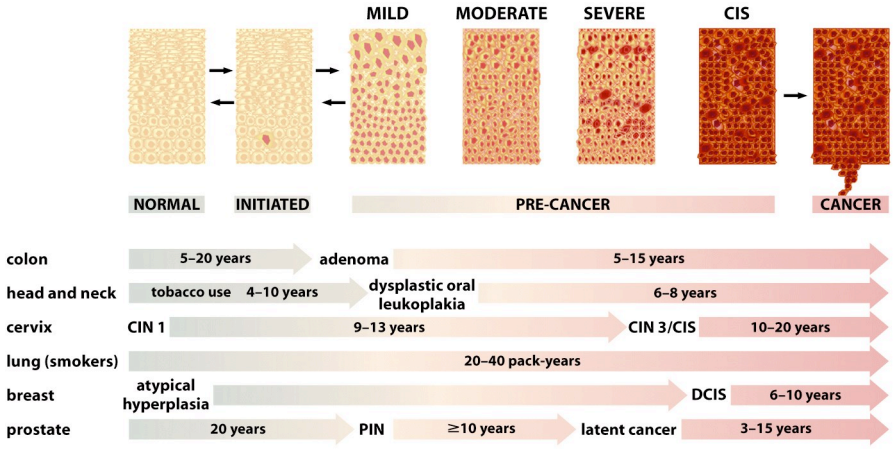
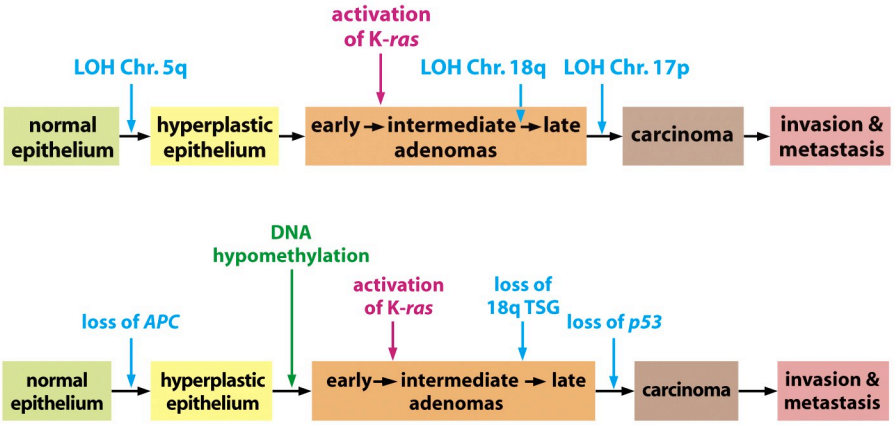


Figure 11.7 *The Biology of Cancer* (© Garland Science 2007)

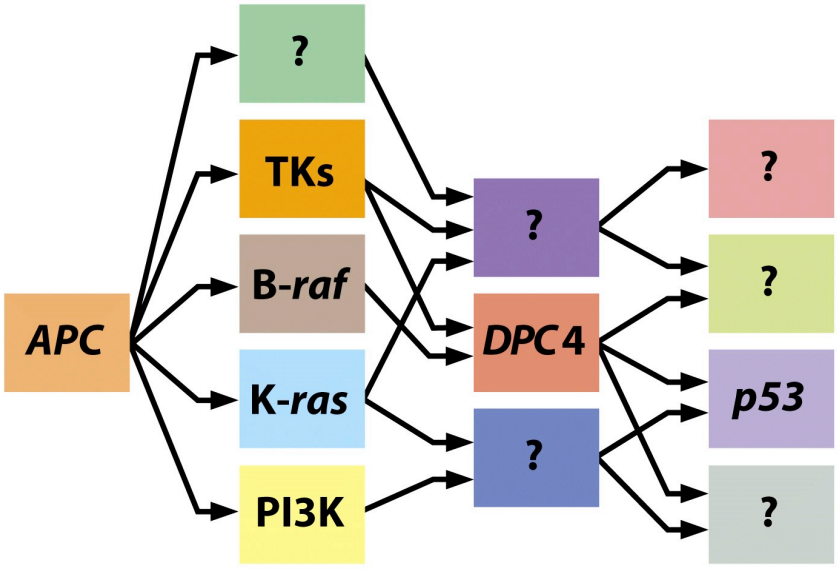
Multistep change in genomic alterations



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)

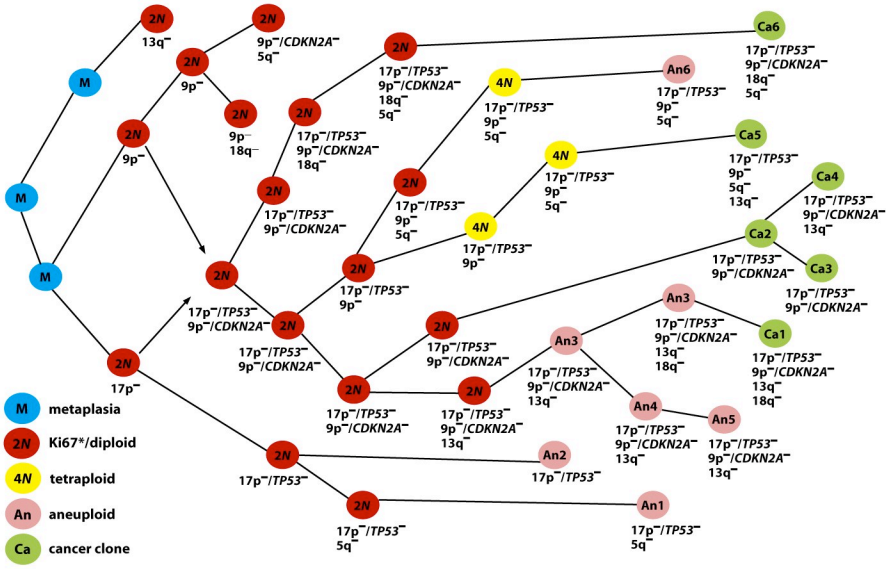
Multiple roads to the same destination



Think heterogeneity in tumorigenesis

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

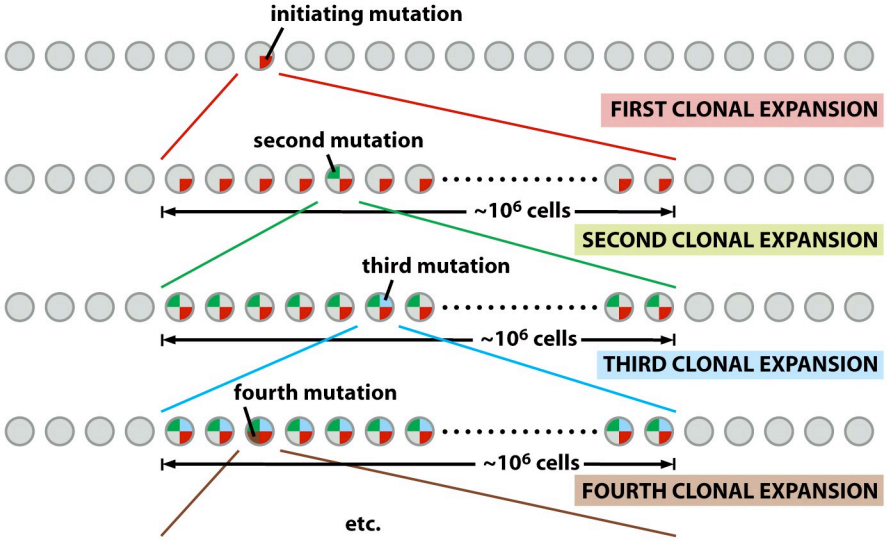
Multiple roads to the same destination



Think heterogeneity in tumorigenesis

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

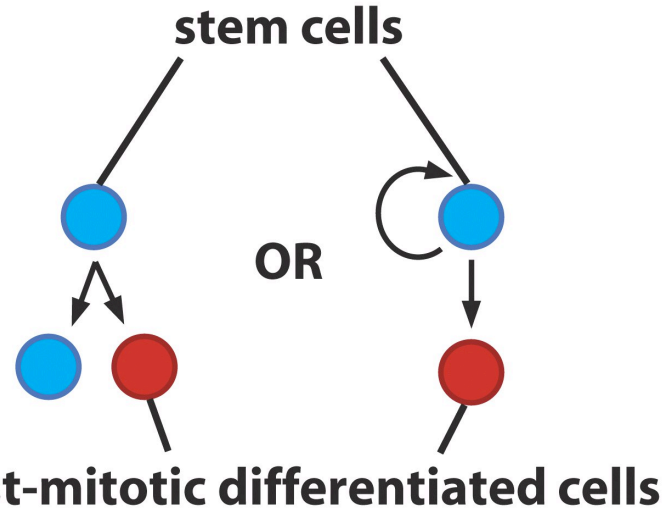
Clonal evolution & expansion



Don't be fooled by the seemingly homogenous expanded pools

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

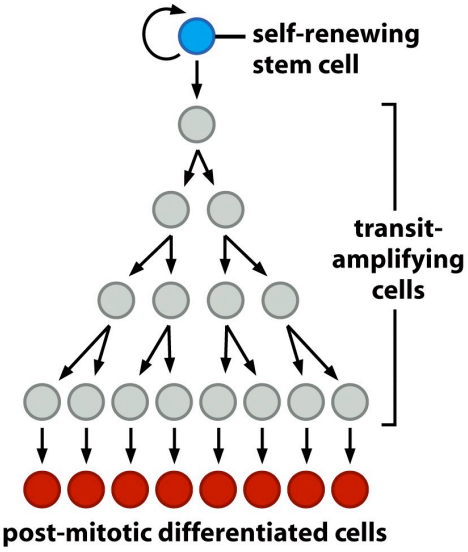
Stem cells & clonal evolution



Not all tumor cells are created equal

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

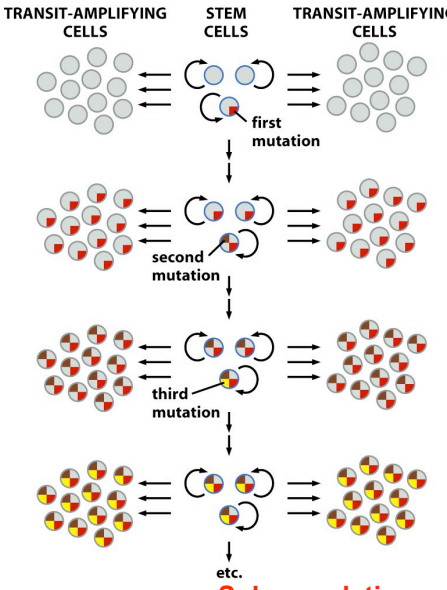
Stem cells & clonal evolution



Not all cells created equal: cell hierarchies

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

Stem cells & clonal evolution

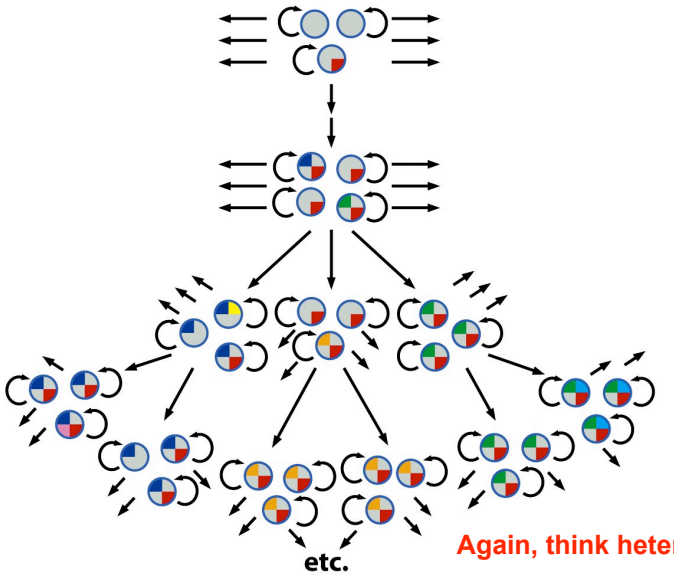


Multiple rounds of mutation and selection

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

Sub-populations of tumor stem cells

Stem cells & clonal evolution

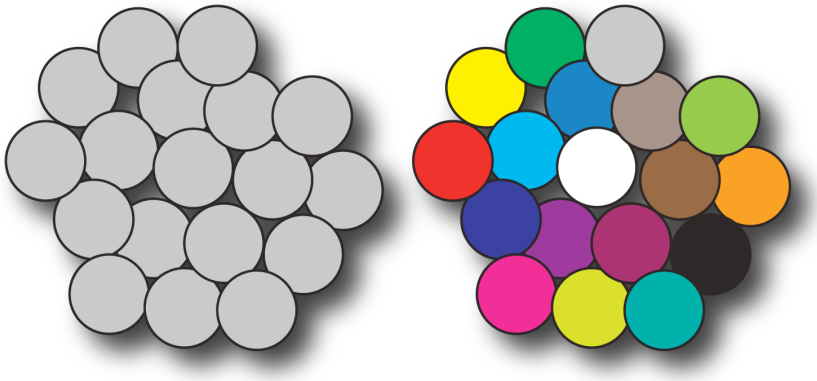


Again, think heterogeneity

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

Co-existence of multiple stem cell pools

…in other words, visualize



Not this

This

The concept of genetic heterogeneity

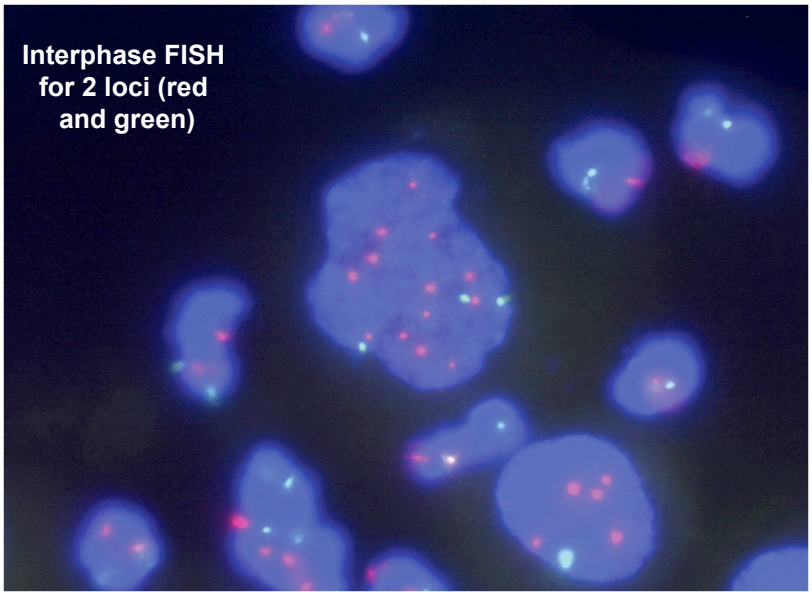


Figure 11.19 *The Biology of Cancer* (© Garland Science 2007)

Why are all these things different?

- These are just two genomic loci
- What about whole genomes (the post-textbook era)?

Today's reality

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

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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)

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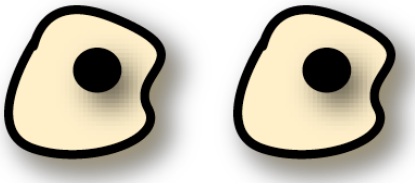
- The need to understand cancer genomes (& personal genomes)
  - Your genome vs. the cancer genome
  - Germline vs. sporadic
- Evolution of technologies to enable this goal (fallout from the human genome project)



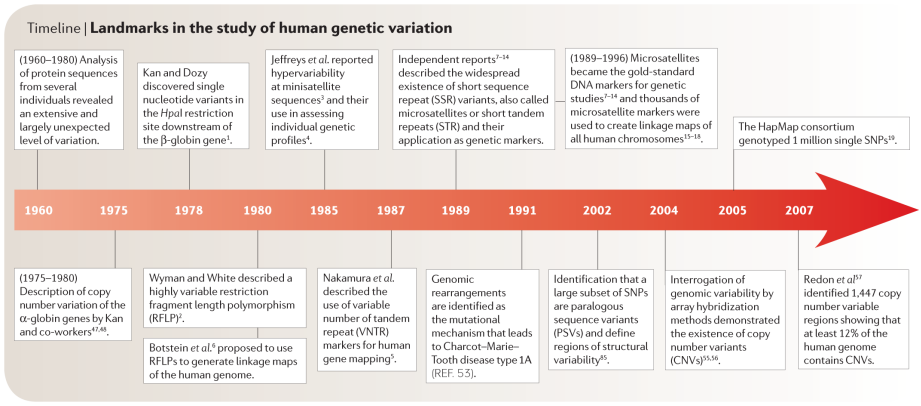
Normal cell Cancer cell

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



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 affect on levels of expression of the oncogene. Instead, it affects the structure of the oncogene-encoded protein.

0028-0836/82/450143—04$01.00

circa 1982

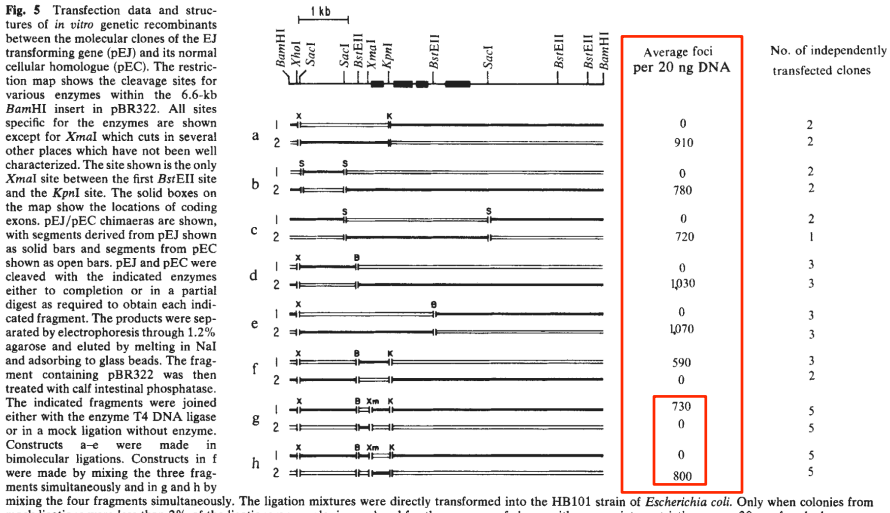


Fig. 5 Transfection data and structures of *in vitro* genetic recombinants between the molecular clones of the EJ transforming gene (pEJ) and its normal cellular homologue (pEC). The restriction map shows the cleavage sites for various enzymes within the 6.6-kb *Bam*HI insert in pBR322. All sites specific 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
RB1	familial retinoblastoma; *dominant*	13q14	transcriptional/ cell cycle regulator	retinoblastoma
P16^{INK4a}	familial melanoma; *dominant*	9p21	CDK inhibitor	melanoma
CDK4	familial melanoma; *dominant*	12q13	CDK	melanoma
P53	Li-Fraumeni; *dominant*	17p13.1	transcription factor	sarcomas, breast cancer
APC	familial adenomatous polyposis; *dominant*	5q21	growth factor signalling	colorectal cancer
CDH1	hereditary diffuse gastric cancer; *dominant*	16q22.1	cell-to-cell adhesion	diffuse gastric cancer
LKB1	Peutz-Jeghers; *dominant*	19p13.3	serine threonine kinase	gastrointestinal cancer
PTEN	Cowden syndrome; juvenile polyposis coli; *dominant*	10q23.3	phosphatase, cytoskeletal protein?	breast cancer, gastrointestinal cancer
SMAD4	juvenile polyposis coli; *dominant*	18q21.2	growth factor signalling	gastrointestinal cancer
MEN1	multiple endocrine neoplasia type 1; *dominant*	11q13	transcription co-factor	endocrine
RET	multiple endocrine neoplasia type 2; *dominant*	10q11.2	receptor tyrosine kinase	endocrine
MET	Hereditary papillary renal cancer; *dominant*	7q31	receptor tyrosine kinase	papillary renal cancer
KIT	familial gastrointestinal stromal tumours; *dominant*	4q12	receptor tyrosine kinase	gastrointestinal cancer (stromal)
PTCH	basal cell nevus syndrome; *dominant*	9q22.3	membrane receptor	basal cell (skin)
NF1	neurofibromatosis type 1; *dominant*	17q11.2	GTPase-activating protein	neurofibrosarcomas
NF2	neurofibromatosis type 2; *dominant*	22q12.2	cytoskeletal protein?	central nervous system tumours
VHL	von Hippel-Lindau *dominant*	3p25	protein maturation? RNA elongation?	renal clear cell carcinomas, pheochromocytomas
WT1	Wilms tumour; *dominant*	11p13	transcription factor	nephroblastoma
BLM	Bloom syndrome; *recessive*	15q26.1	dsDNA repair?	leukaemia, lymphoma
FANCA; *FANCC*; others	Fanconi anaemia; *recessive*	16q24.3; 9q22.3; ?	dsDNA repair?	leukaemia
XPB; *XPD* others	xeroderma pigmentosum; *recessive*	2q21; 19q13; ?	helicases, nucleotide excision repair	basal cell and squamous cell carcinomas
ATM	ataxia telangiectasia; *recessive*	11q22.3	serine-threonine protein kinase	lymphoma, leukaemia
NBS1	Nijmegen breakage syndrome; *recessive*	8q21	transcription factor? dsDNA repair?	lymphoma
BRCA1	familial breast/ovarian cancer; *dominant*	17q21	transcription factor? dsDNA repair	breast, ovarian cancer
BRCA2	familial breast/ovarian cancer; *dominant*	13q12	transcription factor? dsDNA repair	breast, ovarian cancer
MLH1; *MSH2* *PMS1*; *PMS2*; *MSH6*	hereditary non-polyposis colorectal cancer; *dominant*	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

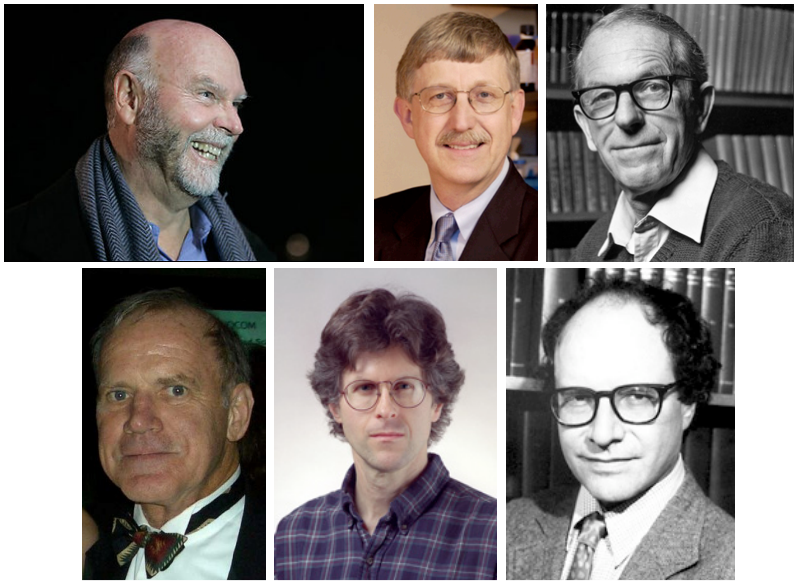
Keep in mind the mechanistic heterogeneity; multiple ways to gain or loose protein function

Status thus far?

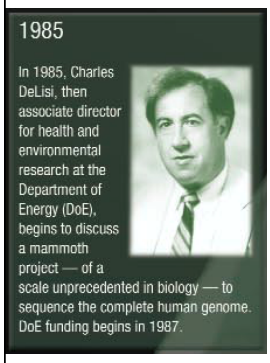
- ~350 of 22,000 protein encoding genes show recurrent somatic mutations
- Most by genetic and physical mapping (again, painstaking)
- Identifying genes with transforming activity (i.e., H-RAS)
- Animal models
- Guess work?

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

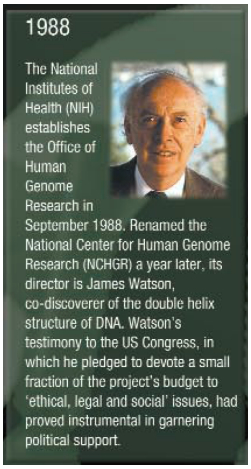
Understanding the cancer genome requires that we understand our own genomes

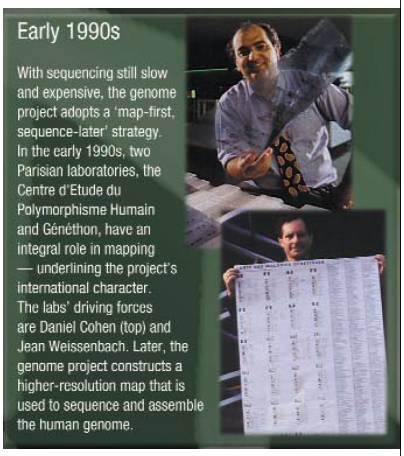


Human genome timeline



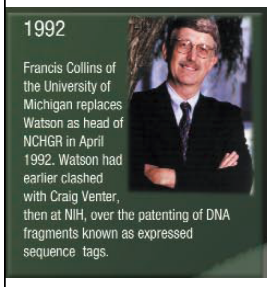
Interested in affect of radiation on mutation (Department of Energy)



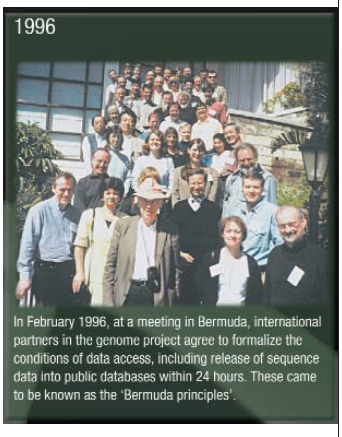


Officially launched

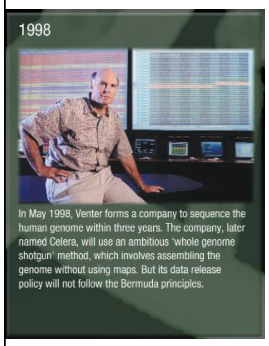
Human genome timeline



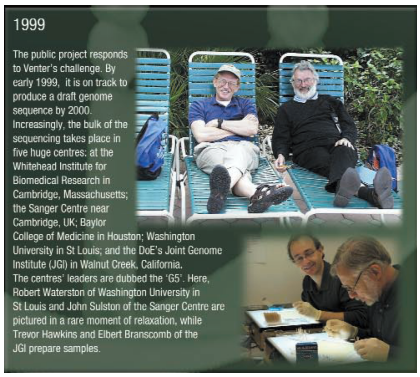
Collins takes over

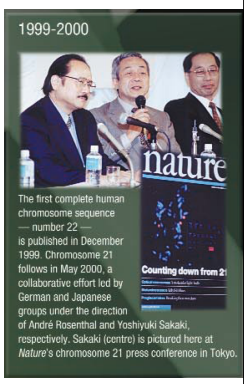


Human genome timeline



Venter enters the picture ($$$$$)





Completion of draft announced in 2000

Draft version published in 2001 (complete in 2003)

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"