Genomic Instability

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RPN-530 Oncology for Scientist-I October 18, 2016

Previous lecturers supplying slides/notes/inspiration

- **Daniel L. Stoler, Ph.D.**
- **Bill Burhans, Ph.D.**
- **Amin Mahpour, (almost Ph.D.?)**

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Questions/Outline

- What is Genomic instability?
- What factors contribute to genome integrity?
- How we identify these aberrations?

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A renewed model of pancreatic cancer evolution based on genomic rearrangement patterns

- Notta, et al, Nature October 12, 2016
- **doi:10.1038/nature19823**

Genomic instability

- Cells maintain genome integrity and promote faithful genome propagation by:
  - Coordinated DNA replication
  - DNA-damage sensing and repair
  - Cell-cycle checkpoints
    - Most checkpoints evolutionarily conserved and are tumor suppressors

Genomic instability

- Drives evolution at the molecular level and generates genetic variation/diversity
- Specialized role in generation of variability in developmentally regulated processes
  - Immunoglobulin diversification
- Associated with pathological disorders
  - Premature aging
  - Inherited disease
  - Cancer

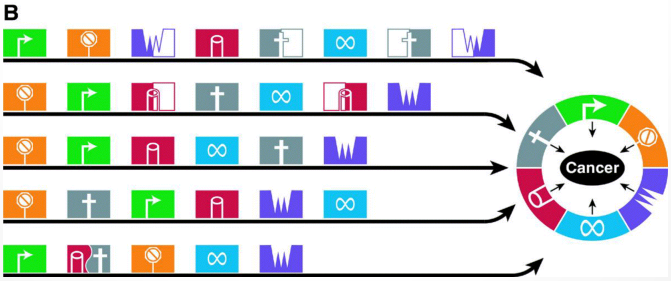
Cancer

- Evolution at a vastly accelerated rate with natural selection favoring the growing tumor mass over the organism.
- Successive gene mutations activating oncogenes and inactivating tumor suppressors.

A

Component	Acquired Capability	Example of Mechanism
	Self-sufficiency in growth signals	Activate H-Ras oncogene
	Insensitivity to anti-growth signals	Lose retinoblastoma suppressor
	Evading apoptosis	Produce IGF survival factors
	Limitless replicative potential	Turn on telomerase
	Sustained angiogenesis	Produce VEGF inducer
	Tissue invasion & metastasis	Inactivate E-cadherin

B



Cell, Vol. 100, 57–70, January 7, 2000,

Early Molecular Model of Tumor Progression - Vogelstein

- Hypothesis: Mutation in one gene associated with each step in progression.

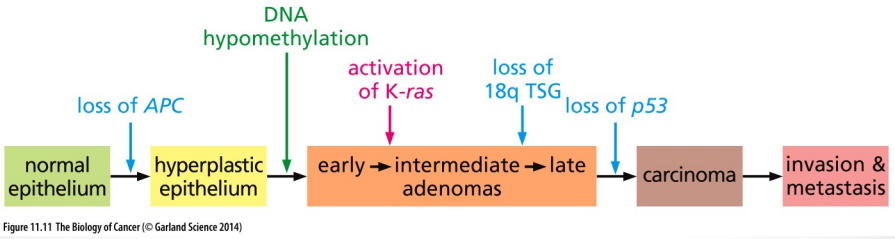
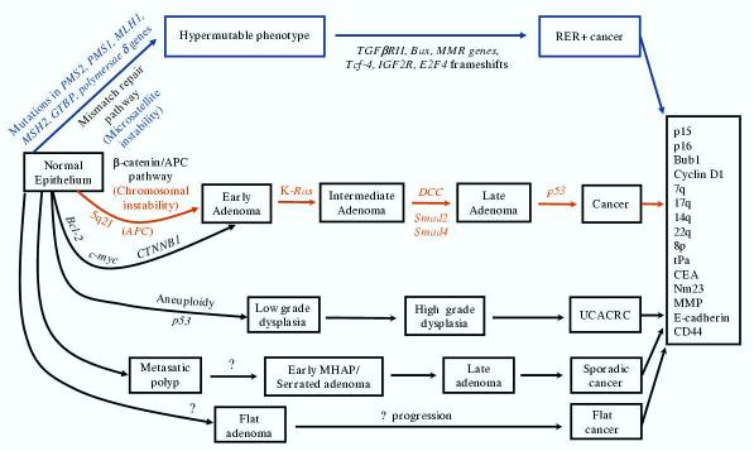


Figure 11.11 The Biology of Cancer (© Garland Science 2014)

Fearon & Vogelstein, Cell 61, 759–767 (**1990**).

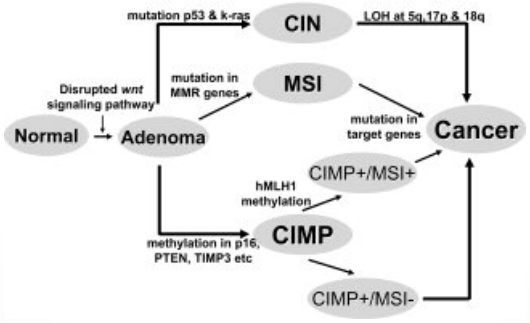
Early Molecular Model of Tumor Progression- Vogelstein

- Problem 1: Vogelstein pathway is no more than a rough outline…

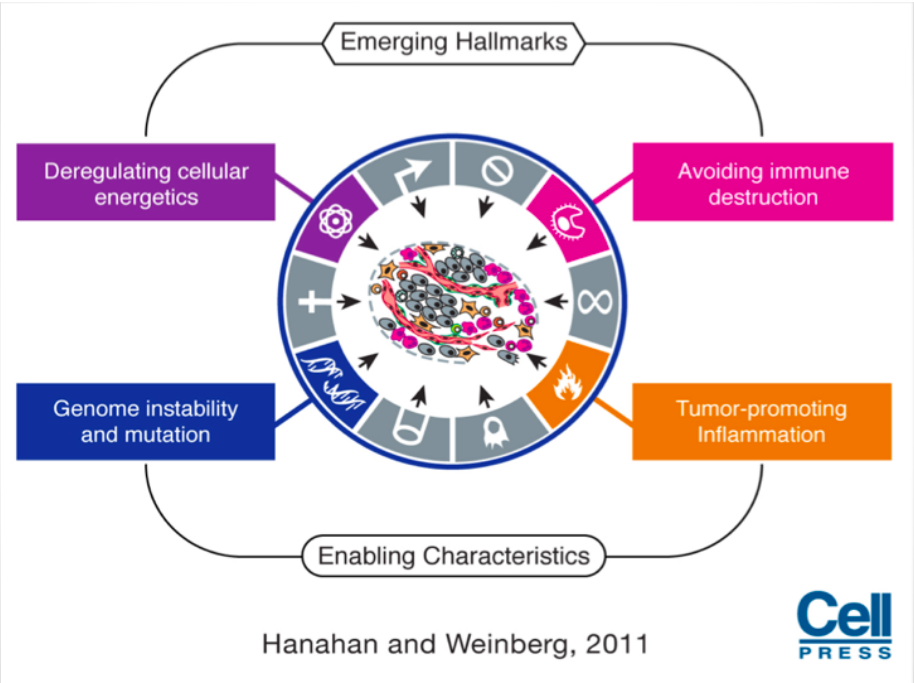


Early Molecular Model of Tumor Progression

- Problem 2: How do you accumulate all of these mutations in one cell?



- Molecular pathogenesis of colorectal cancer **Cancer** Volume 104, Issue 10, pages 2035-2047, 4 OCT 2005 DOI: 10.1002/cncr.21462



Cancer is a genetic disease

- Genetic selection at the level of single cells.
- Aneuploidy is a hallmark of cancer cells.
- Somatic mutations occur in most cancers.
- Inherited germline mutations occur in rare familial cancer syndromes.
- Increases in mutation rate or genomic instability increase frequency of cancer.

Genomic Instability in cancer

- Genomic instability is a fundamentally important feature of (all) cancer cells.
  - Chromosomal instability
  - Intrachromosomal instability
  - Microsatellite instability → MSI
  - Epigenetic instability

  } CIN

- (Is instability a cause or consequence of cancer?)

Genomic Instability in cancer

- **1990**: TIsty. Tumor cells growing in culture are genomically unstable; elevated gene amplification rates. ***Genomic instability is a continuous process since it was inherited by daughter tumor cells.***
- **1991**: Loeb. Normal rate of mutation (1.4 x 10-10 nucleotides/ cell division) was insufficient to produce the estimated necessary mutations to achieve cancer. ***Need for Mutator Phenotype***.
- **1992**: Kallioniemi and Pinkel. Comparative genomic hybridization showed **amplifications and deletions** in cancer, more than existing dogma.
- **1993**: Fishel and Kolodner. Hereditary nonpolyposis colorectal cancer arises from defects in DNA mismatch repair. **Microsatellite instability**.

Familial Cancer Syndromes

- Hereditary Nonpolyposis Colorectal Cancer –
  - Mismatch Repair Genes
- Familial Breast/Ovarian Cancer
  - BRCA1/2
- Ataxia Telangiectasia
  - ATM
- Li- Fraumeni Syndrome
  - p53
- Werner's and Bloom's Syndromes
  - DNA Helicases

Mutator Hypothesis

Forms of instability

- Chromosomal Instability(CIN)
  - Microscopic changes in the Karyotype
    - Chromosomal gain or loss (Aneuploidy)
    - Chromosomal translocation
  - Failures in either mitotic chromosome transmission or the spindle mitotic checkpoint
  - Can be studied by Cytogenetics techniques
- Microsatellite Instability(MSI or MIN)
  - Repetitive DNA expansions and contractions.
    - Replication slippage
    - Mismatch repair (MMR) impairment
    - Homologous recombination
  - Require molecular techniques(i.e. PCR) to identify them
- Mutations, small deletions, insertions, inversions, etc.
  - Identify by sequencing
- Epigenetic instability
  - Identify by ChIP-seq, WGBS, etc

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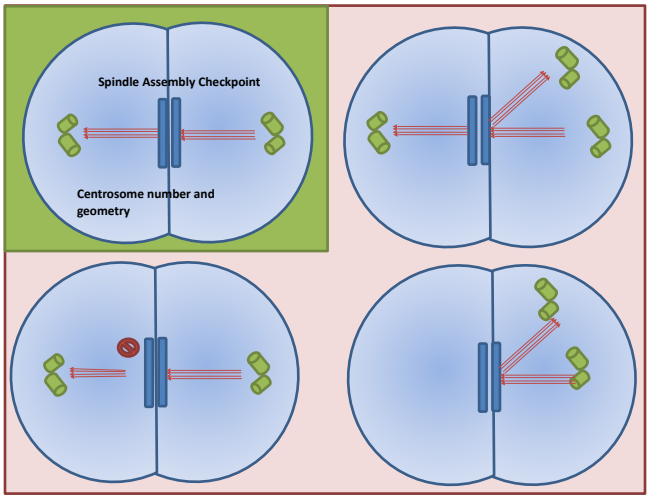
Chromosomal Instability



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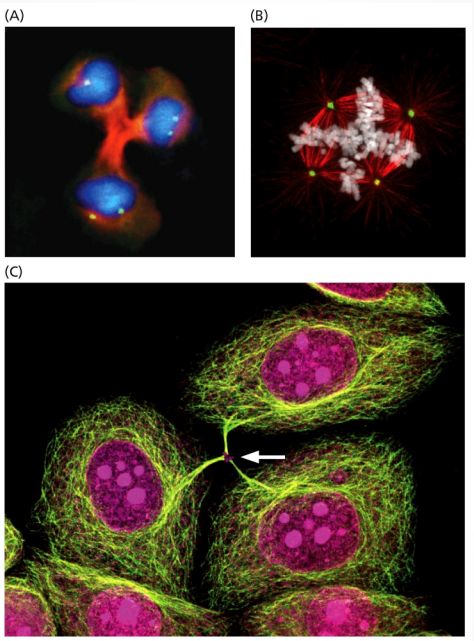


Figure 12.39 The Biology of Cancer (© Garland Science 2014)

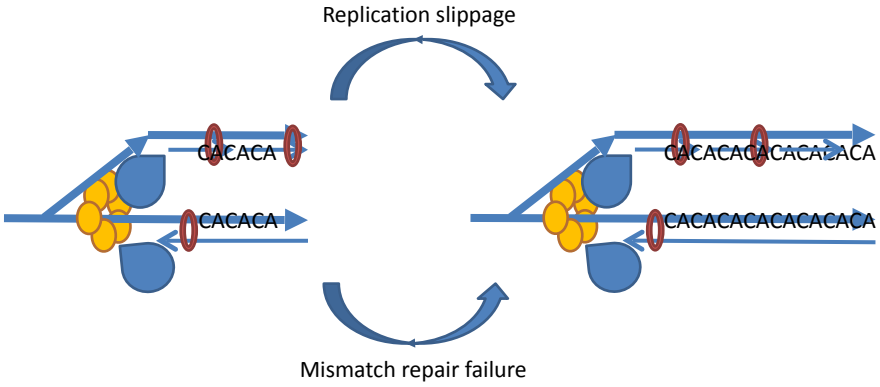
Table 12.3 Mutated, methylated, and overexpressed genes in cancer cells that perturb chromosomal stability

Gene	Function of gene product	Consequence of alteration in cancer cells
BUBR1/ BUB1[a]	spindle assembly checkpoint	progress through mitosis, even in the presence of microtubule inhibitors[a]
MAD1[b]	spindle assembly checkpoint	large-scale aneuploidy
MAD2[b,c]	spindle assembly checkpoint	premature entrance into anaphase,[d] aneuploidy
Securin	attachment of sister chromatids	nondisjunction of chromosomes[e]
cohesin complex	attachment of sister chromatids	aneuploidy
Aurora-A, -B	separation of chromatids at anaphase	premature entrance into anaphase[d]
CHFR[e]	spindle assembly checkpoint	nondisjunction[f], chromosome loss
14-3-3σ	DNA damage checkpoint	segregation of unrepaired chromosomes
RB	cell-cycle regulator	aneuploidy
APC[g]	regulation of proliferation	mitotic defects, cytokinesis failure

[a]Humans with heritable compromised BubR1 function suffer the cancer predisposition syndrome termed mosaic variegated aneuploidy. Mice with inherited Bub1 and BubR1 insufficiency are also cancer prone under certain conditions.
[b]Mad1 and Mad2 form complexes at the kinetochore that prevent chromatid separation until complexes with spindle fibers have been properly formed. *Mad1*$^{+/-}$ mouse heterogyzotes develop a variety of tumors.
[c]The *Mad2* gene is transcriptionally repressed in a number of solid tumors and is frequently mutated in gastric carcinomas. Mice that are heterozygous at the *Mad2* locus (i.e., are *Mad2*$^{+/-}$) develop lung cancers as adults, while those that overexpress wild-type Mad2 protein develop a variety of malignancies.
[d]Premature entrance into anaphase can lead to loss of entire chromosomes.
[e]*Chfr*$^{+/-}$ mice develop lymphomas early in life and carcinomas of the liver, lung, and gastrointestinal tract later in life.
[f]Nondisjunction is the failure of sister chromatids to separate at anaphase.
[g]Anaphase-promoting complex.

Table 12.3 The Biology of Cancer (© Garland Science 2014)

Microsatellite instability



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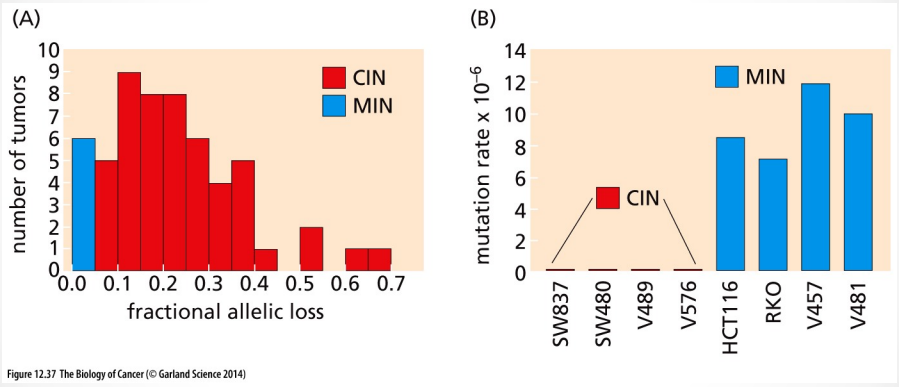
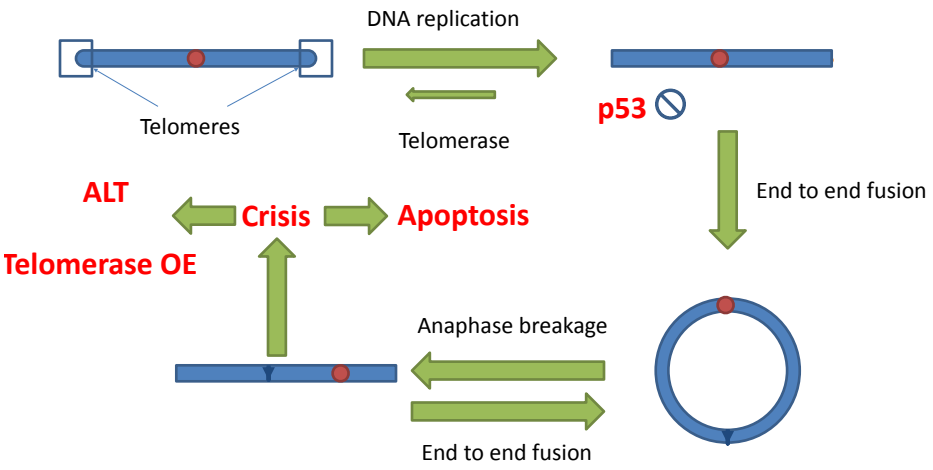


Figure 12.37 The Biology of Cancer (© Garland Science 2014)

Telomere attrition contribute to genomic instability



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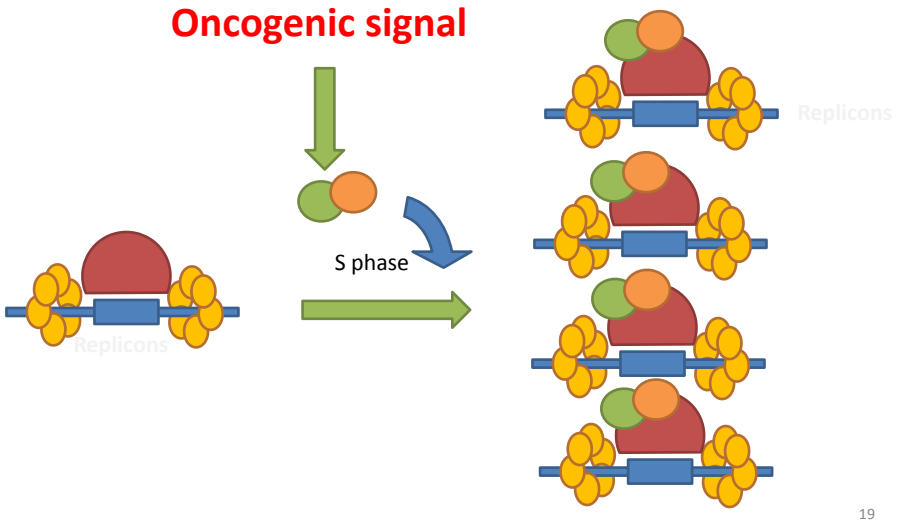
Oncogenes promote DNA replication



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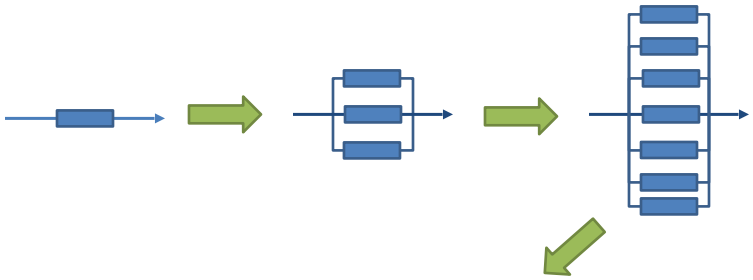
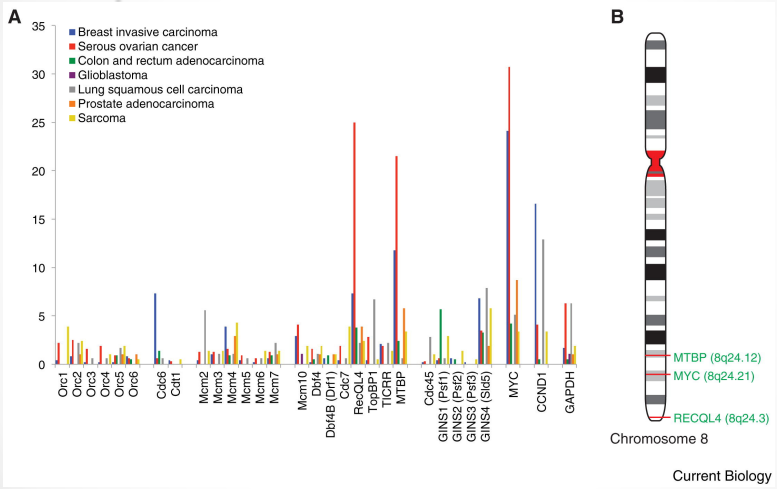
Oncogenes and local replication/ Amplifications



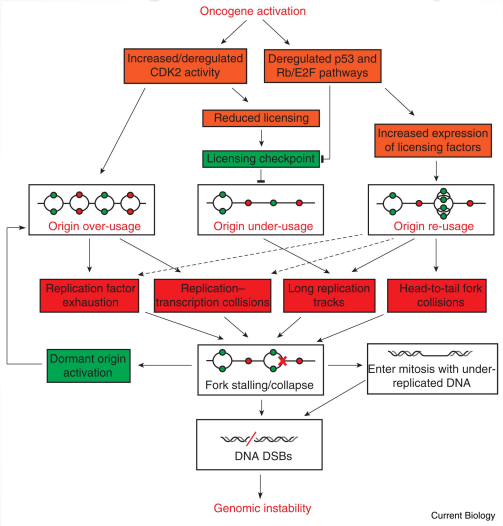
Growth and survival advantage (e.g. Myc)

Copy number increase of replication factors in cancer



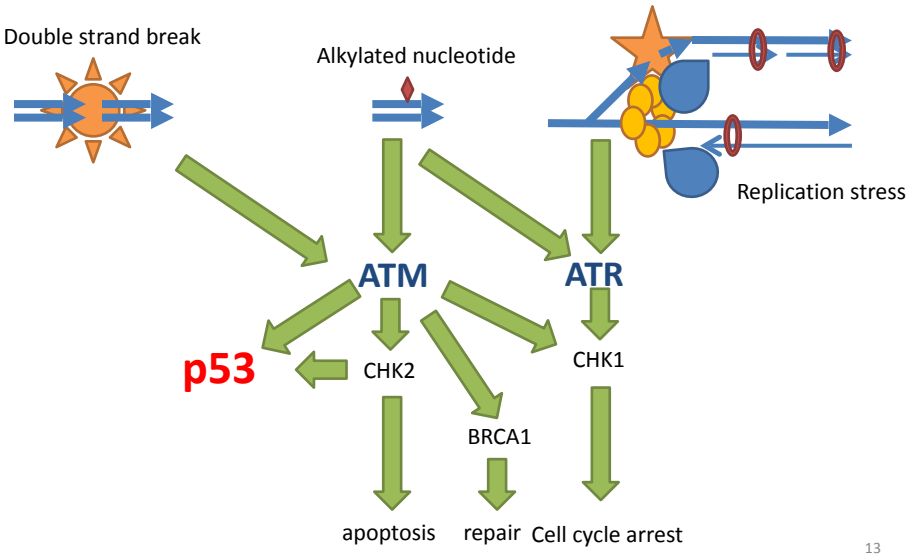
Current Biology 2014 24, R435-R444DOI: (10.1016/j.cub.2014.04.012)

Oncogene activation causes replicative stress and genomic instability.



Current Biology 2014 24, R435-R444DOI: (10.1016/j.cub.2014.04.012)

DNA damage checkpoint



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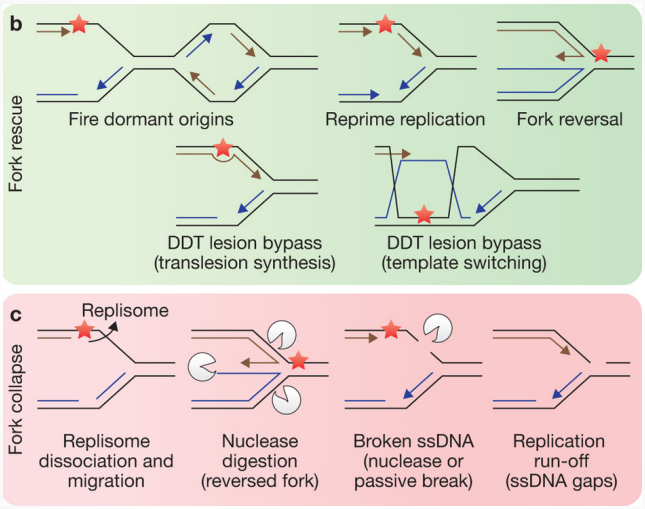
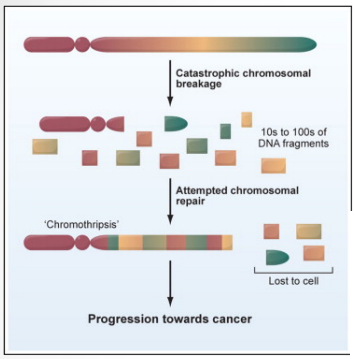
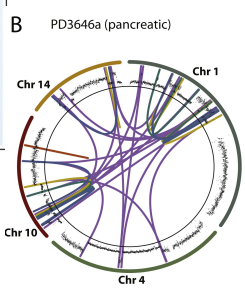
Replication stress induced DNA damage

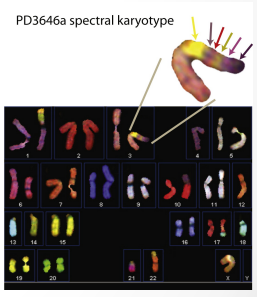


Causes and consequences of replication stress **Michelle K. Zeman & Karlene A. Cimprich** *Nature Cell Biology* **16**, 2–9 (2014) doi:10.1038/ncb2897

Chromothripsis







Massive Genomic Rearrangement Acquired in a Single Catastrophic Event during Cancer Development, Stephens et al, Cell 2011

Chromothripsis

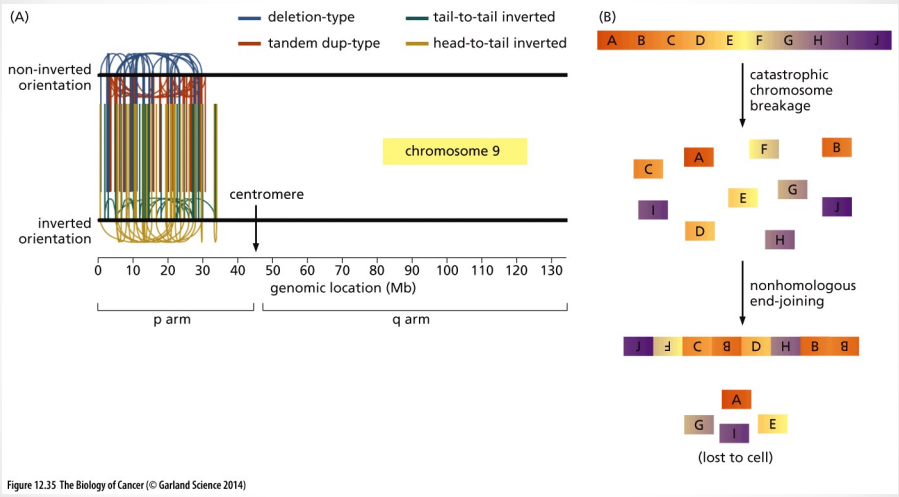


Figure 12.35 The Biology of Cancer (© Garland Science 2014)

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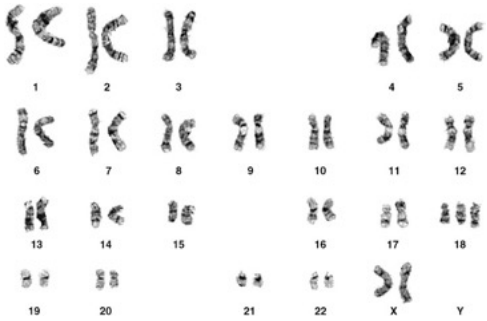
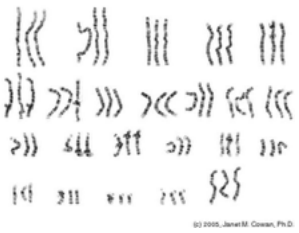
Part III: Measurements of Genomic Instabilities

- Inter-Sample Sequence, Repeat PCR
- Allelotyping (SNP arrays)
- Comparative Genomic Hybridization
  - BAC-Array
- Spectral Karyotyping
- Karyotyping

Size of DNA damage detected increases.

Cytogenetic - Karyotyping





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Spectral Karyotyping (SKY)

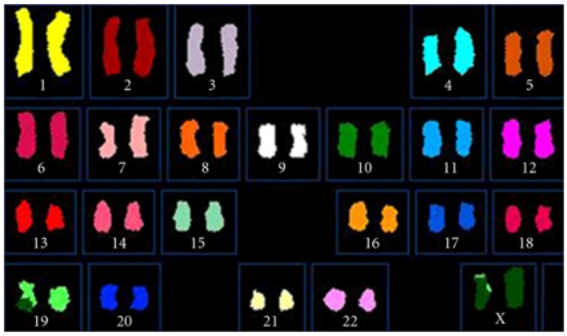

Photo: Autism Spectrum Disorder in a Girl with a De Novo X;19 Balanced Translocation. Hindawi 2012

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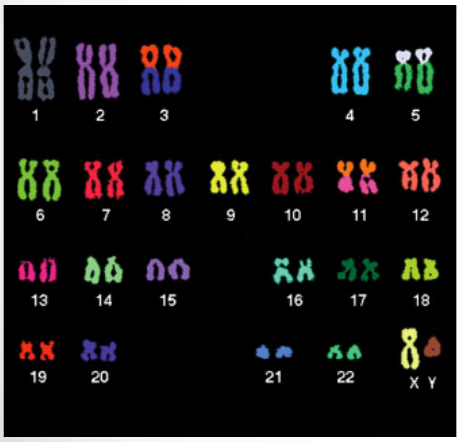
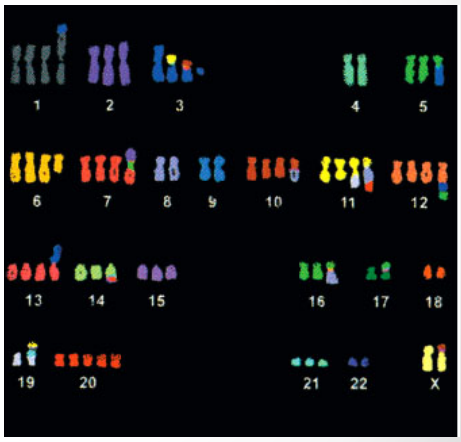
Changes produce genomic and karyotypic instability and often show gross rearrangements

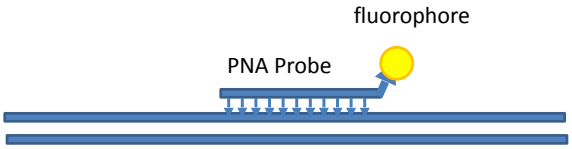
Normal cells



Cancerous cells



Fluorescent In Situ Hybridization(FISH)



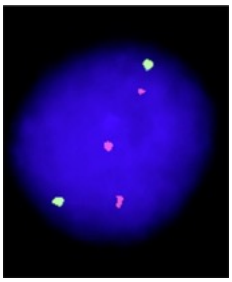


Photo: Swiss perinatal institute

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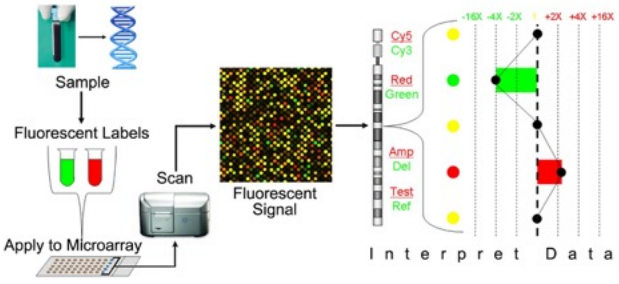
Microarray Comparative Genomic Hybridization(M-CGH)



The basic technique for using microarrays to detect genomic microdeletions and microduplications associated with autism and other disorders. Microarrays can be used to examine hundreds of thousands of segments of DNA or RNA simultaneously.

Photo: Agilent

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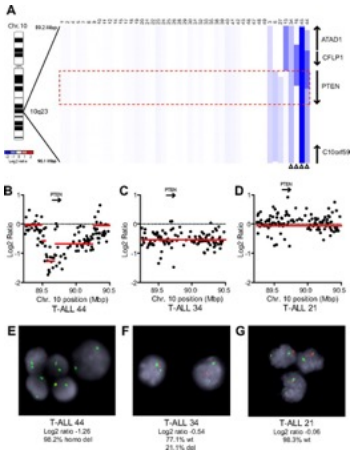
Utilization of CGH in cancer



High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia. Blood. 2009

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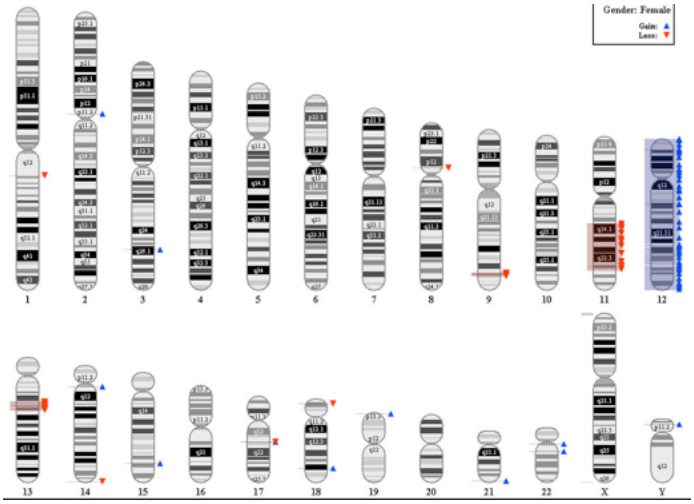
Virtual Karyotype

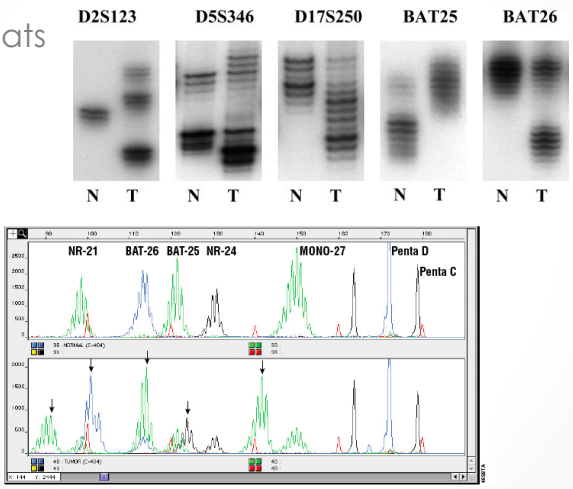


Next-generation sequencing(NGS)

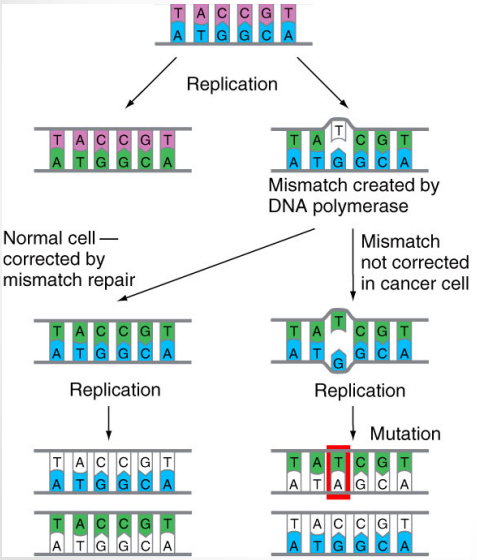
- Best way to analyze genomic instability is to sequence the cancer genome.
- Exome sequencing has been used to analyzed Lung, glioblastoma and many other cancers.
- The data is publicly available through TCGA and other sequencing consortium.
- Whole genome sequencing is expensive, but provides more details about cancer genome(e.g. Noncoding and promoter sequence).

Characterizing MSI

- CA repeats



Changes that produce genomic and karyotypic instability



- Defects in DNA replication machinery – lost capability to reproduce genome faithfully
- Increase rate of chromosomal aberrations – fidelity of chromosome reproduction greatly diminished

Quantifying Genomic Alterations by Direct DNA Sequencing - 2006

- **Sequenced 13,023** genes in each of 11 **breast** carcinomas and in each of 11 **colon** carcinomas.
  - **189 genes had above average mutation frequencies.**
  - **The average tumor had 93 genes mutated.**
  - **This set varied from tumor to tumor.**
  - In addition to point mutations, many "indels" of 1-100 bases.
- Sjoblom et al, Science 314:268-274, **2006.**
- Kaiser, Science 313:1370, **2006**

Quantifying Genomic Alterations by Direct DNA Sequencing

- *This pattern of diversity applies to most solid tumors*
  - • Breast, • Gastric, • Lung • Ovarian • Renal • Colorectal
  - • Head and Neck • Mesothelioma • Pancreatic
  - Nature 446: 153, 2007
  - PNAS 105:3521, 2008
- Driver Mutations
  - confer growth advantages on the cancer cell -> positive selection
- Passenger Mutations
  - don't confer growth advantages -> no positive selection
- **"drivers appear to be distributed across a large number of genes, each of which is mutated infrequently"**
  - Stratton et al. Nature 458:719, 2009.

When does genomic instability occurduring tumor progression?

- Early -> driver of progression
- Late -> result of progression

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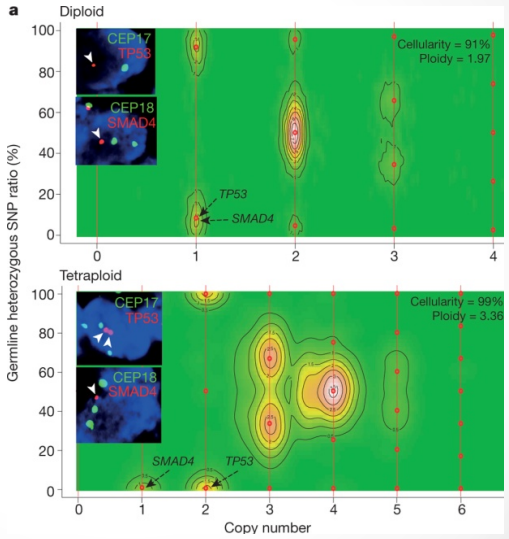
- High genomic instability in PancCa
- Sequence 100 whole genomes
- Purified primary and metastatic pancreatic ductal Ca

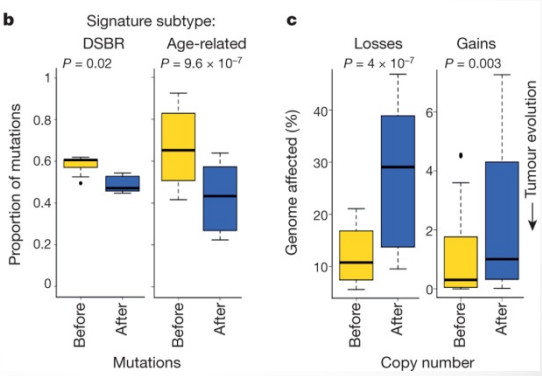
A renewed model of pancreatic cancer evolution based on genomic rearrangement patterns

- Polyploidization in pancreatic cancer
- 45% of tumors



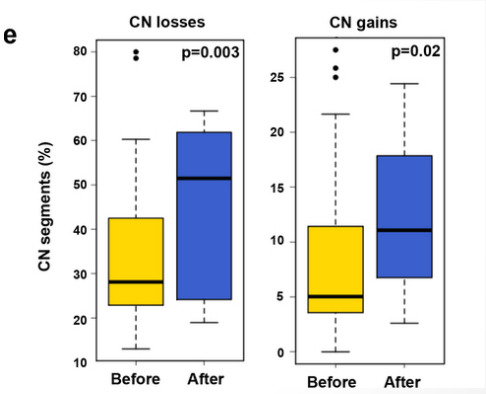
A renewed model of pancreatic cancer evolution based on genomic rearrangement patterns

- Mutations occur prior to polyploidy
- CNVs occur post-polyploidy



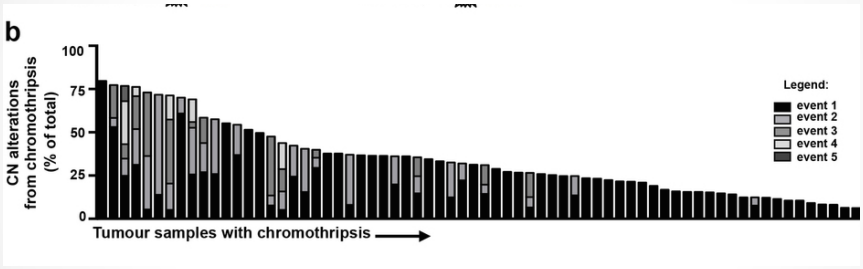
A renewed model of pancreatic cancer evolution based on genomic rearrangement patterns

- More copy number alterations post polyploidy



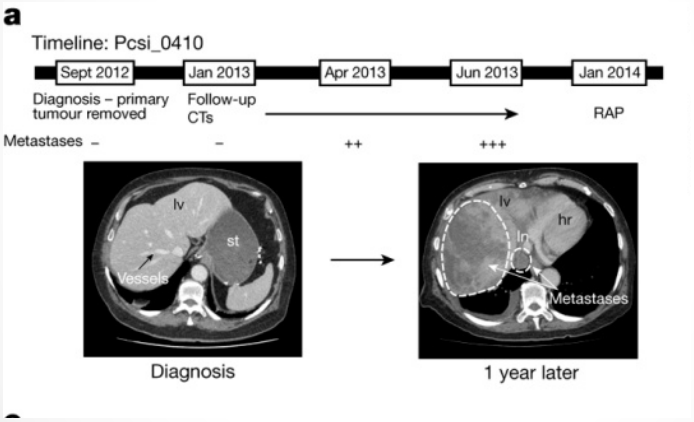
A renewed model of pancreatic cancer evolution based on genomic rearrangement patterns

- Most copy number changes from single chromothripsis event



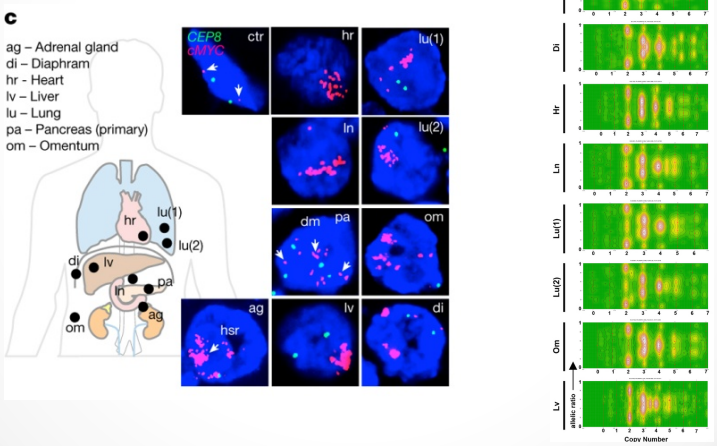
A renewed model of pancreatic cancer evolution based on genomic rearrangement patterns

- Chromothripsis and polyploidization in a patient with metastatic progression



A renewed model of pancreatic cancer evolution based on genomic rearrangement patterns

- All mets polyploid and have myc amplification



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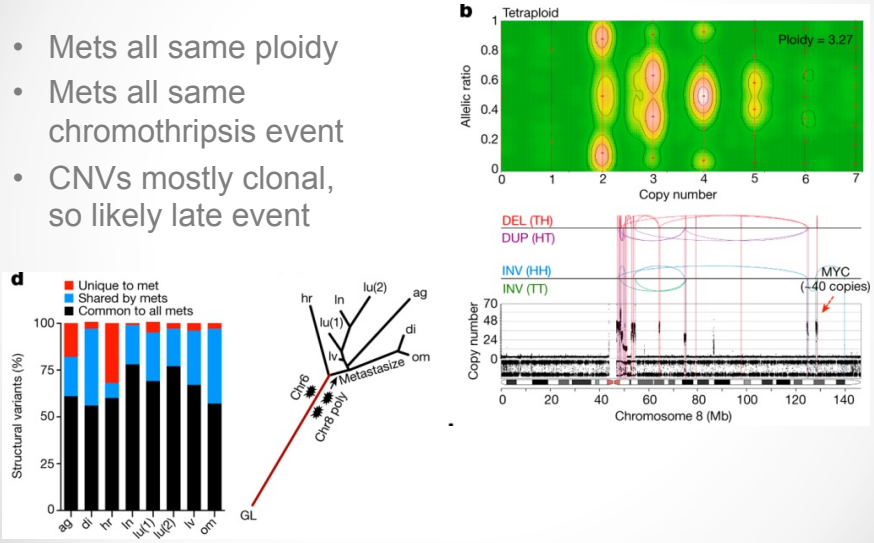
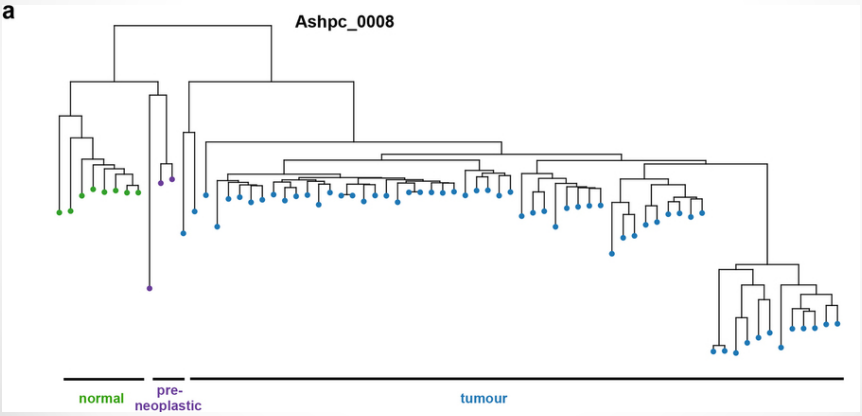
- Mets all same ploidy
- Mets all same chromothripsis event
- CNVs mostly clonal, so likely late event



A renewed model of pancreatic cancer evolution based on genomic rearrangement patterns

- Examine 96 single cells from one patient
- Single event knocked out CDKN2A and SMAD4
- 16% of all PancCa, all 4 drivers altered simultaneously



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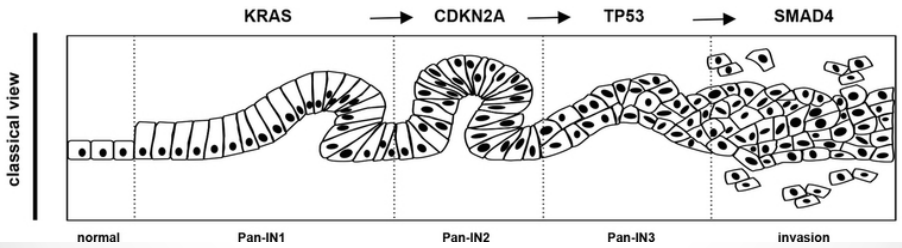
- Sequential hit model “a la Vogelstein”



A renewed model of pancreatic cancer evolution based on genomic rearrangement patterns

- Back to problem 1: Vogelstein pathway is no more than a rough outline…

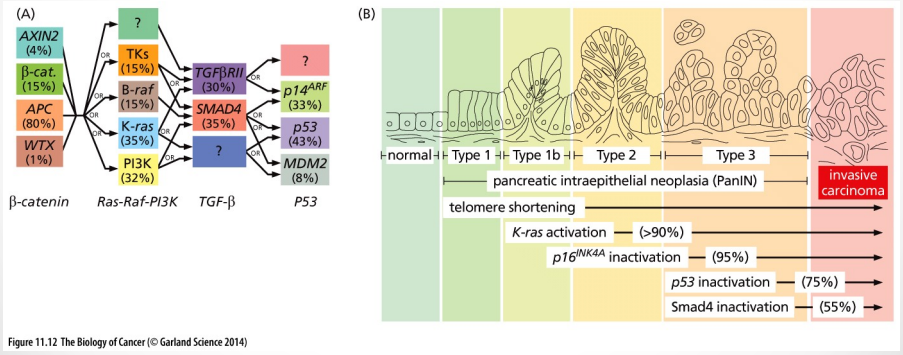
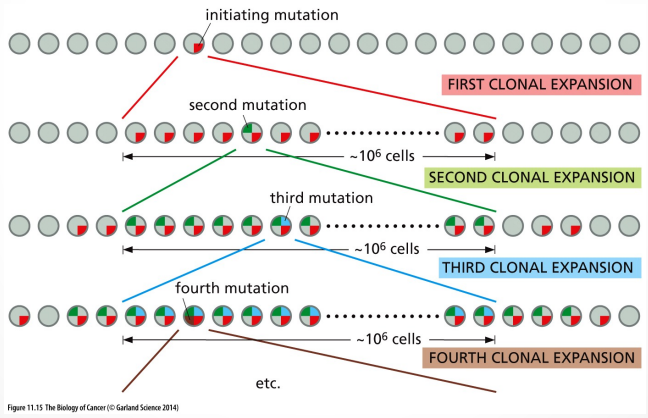


Figure 11.12 The Biology of Cancer (© Garland Science 2014)

Early Molecular Model of Tumor Progression

- Problem 2: How do you accumulate all of these mutations in one cell?



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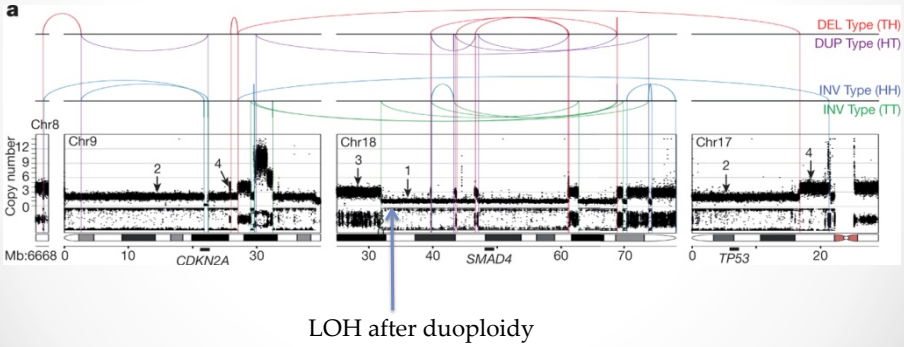
- Complex genomic rearrangement of cancer driver genes



LOH after duoploidy

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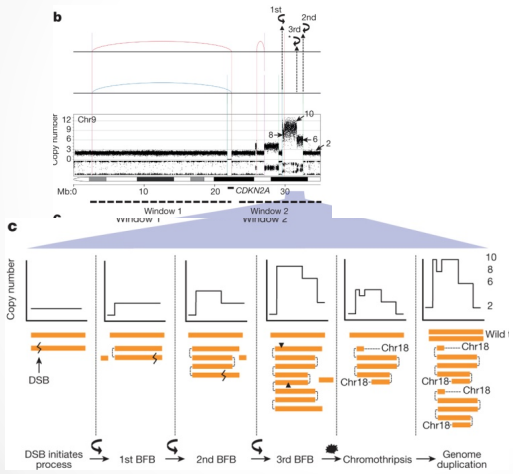
- 3 copy number drops are evidence of BFB



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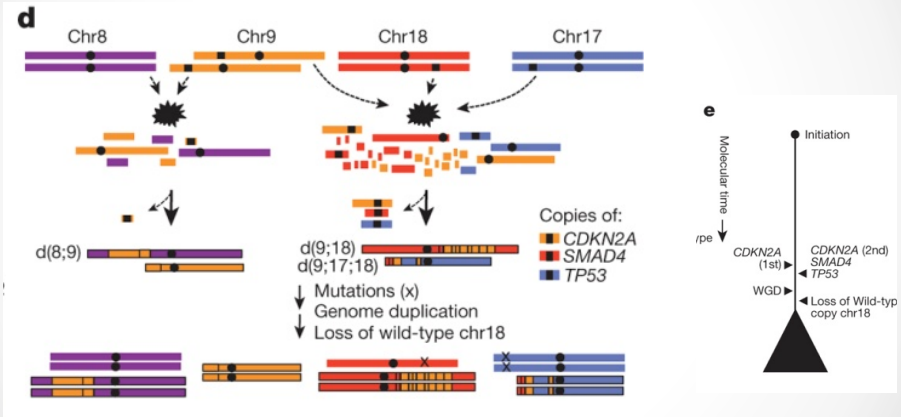
- Simultaneous knockout of pancreatic cancer driver genes



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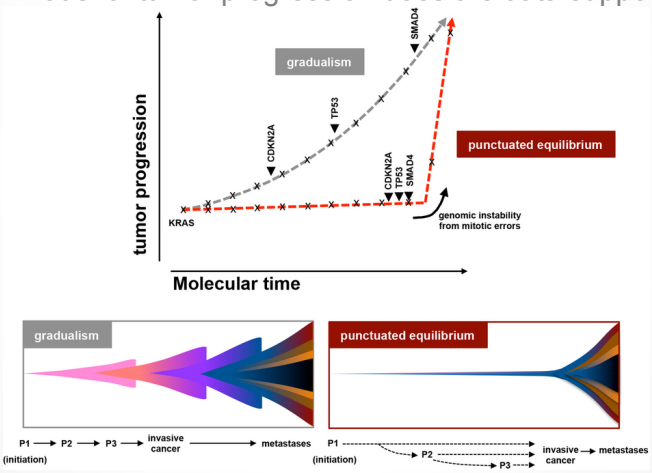
- Which model of tumor progression does the data support?



Questions/Outline

- What is Genomic instability?
- What factors contribute to genome integrity?
- How we identify these aberrations?

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- Notta, et al, Nature October 12, 2016
- **doi:10.1038/nature19823**