Absolute quantification of somatic DNA alterations in human cancer
Overview

1) Inference of tumor purity / ploidy, copy-numbers per cell (ABSOLUTE)

1) Analysis of somatic point-mutations using ABSOLUTE

1) Analysis of genome doublings in human cancer development
High throughput characterization of cancer genomes

Purity = fraction of tumor cells

Aliquot of mixed tumor and normal DNA

70%

T N

Ploidy = mass of DNA in units of normal haploid genome mass. Here ~2.7.

Observed copy-number signal is proportional to locus concentration, both for sequencing and hybridization methods: dependant on sample purity and ploidy.

Illumina sequencing

SNP-array hybridization
Inference of purity and ploidy (ABSOLUTE)
Validation

**Purity**

Cancer / normal mixing experiment

**Ploidy**

FACS analysis of primary OvCa samples
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Purity and ploidy determine power to detect mutations

Clonal Subclonal

[Graphs showing detection power vs. copy number and sequence coverage for clonal and subclonal mutations]
Identification of subclonal point-mutations by sequencing

E.g., sequencing results in $x$ A’s and $y$ G’s at a mutated locus: allelic- fraction is $x / (x+y)$

Discrete allelic-fractions are obscured by tumor purity and local copy-number.

Resolved with ABSOLUTE: change units to *cellular multiplicity* (integral allelic-count)
Common mechanism for clonal / subclonal mutations

Equivalent nucleotide substitution frequencies for clonal and subclonal point-mutations. Rules out contamination.
Tumor suppressors are often homozygous. \((P = 0.006)\)

Oncogenes are not. \((P = 0.012)\)
Identification of $TP53$ as early event in ovarian cancer

$TP53$ mutations occur prior to gain of chr17
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Bimodal distribution of ploidy in human cancer

Mitelman data (Storchova et al. 2008)

Cytogenetics (SKY)

ABSOLUTE

Tumor-derived DNA (SNP arrays)

e.g. 57 chromosomes
Visualizing absolute allelic copy-numbers

Example: High-grade serous ovarian carcinoma

Low-copy homologues

High-copy homologues

Samples

Ploidy

Genome
Inference of genome doubling

High ploidy samples evolved via a genome doubling event

Inflection point

Ploidy

Genome
Frequent whole genome doublings in human cancers
Genome doubling occurs after aneuploidy

Similar frequencies of arm-level deletion (LOH) with and without genome doubling

Simplest explanation: LOH precedes doubling

Tetraploidization is not an initiating oncogenic event in ovarian cancer
Genome doubling occurs after aneuploidy
Genome doubling occurs after aneuploidy
Genome doubled samples have more copy alterations

Linear fit to log length vs. log frequency: power law scaling with exponent \(\sim 0.71\), regardless of genome doubling
Genome doubled ovarian cancer evolves differently

- Clonal het: P = 1.216e-09
- Clonal het / ploidy: P = 0.8852
- Clonal hom: P = 1.553e-08
- Clonal amplified: P = 7.552e-26

HGS-OvCa genome doublings:
- 0
- 1 (93)
- > 1 (28)

- HGS-OvCa: P = 3.3e-09

Mutations per sample

Homozygous dels per sample
13/15 mutations in \textit{NF1} occurred in non-doubled samples, in which case they were homozygous ($P = 0.002$)

Selection acts specifically on \textit{recessive} inactivation of \textit{NF1}.

No \textit{amplified} mutations in \textit{NF1} were observed in doubled samples; \textit{NF1} mutators \textit{do not progress via genome doubling}. In contrast to p53
Clinical correlations with genome doubling

Ovarian carcinoma

\( P = 1.5 \times 10^{-7} \)

\( P = 0.055 \)

\( P = 0.0084 \)
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