CHROMOSOMES THAT CAUSE CANCER:

Chromosomes with two centromeres and the breakage-fusion-bridge cycle

Ruth MacKinnon
@ruthnmackinnon
http://www.theleukaemiaproject.com/

Victorian Cancer Cytogenetics Service
Victorian Cancer Cytogenetics Service
St Vincent’s Hospital (Melbourne)

- Largest cancer cytogenetics lab in Australia
  - Director, Lynda Campbell
- Most adult cases in Victoria
  - Haematological malignancy
  - G-banding
  - FISH
- Specimen reception, lab maintenance
- Rosters/rotas for set-up, harvest and FISH
- After harvest, case is managed by a scientist
- Daily meeting of all for second check
AML, MDS

- bone marrow malignancy
- complex karyotype
  - Poor prognosis
- copy number aberrations
  - driver and passenger
  - unbalanced 20q translocations
    - 20q12 deletion (\( ?L3MBTL \))
dic(17;20)

- dicentric
  - 17 centromere, 20 centromere
- unbalanced translocation
  - → 17p, 20q deletions
- specific centromere probes used to screen metaphase chromosomes
  - --> some surprises

A new nonrandom unbalanced t(17;20) in myeloid malignancies

Crisoula Patsouris*, Patricia M. Michael, Lynda J. Campbell

Cancer Genet Cytogenet 2002 138:32
Characterisation of centromeres

- Interphase centromere FISH for aneuploidy detection
  - Metaphase centromere FISH uncommon
- Centromere identity assumed based on chromosome environment
  - Assumption not tested
- Repetitive sequences too hard / not transcribed
- No information from next gen sequencing, microarrays
Microarrays don’t tell us about
also translocations and translocation partners
centromeres, telomeres → chromosome stability
Dicentric project – unexplored territory

- Centromeres in unbalanced translocations (and telomeres)
- How they are very relevant to cancer genome evolution
  - (not just the breakage-fusion-bridge cycle)
Chromosome stability

Number and position of centromeres and telomeres are important for stability

- one centromere
- telomere at each end
  - prevent chromosomes from joining to each other
    - no "sticky ends"
- telomere degradation
  - End-to-end joining
  - 2 centromeres or ring
  - Breakage-fusion-bridge cycle

McClintock 1939 The Behavior in Successive Nuclear Divisions of a Chromosome Broken at Meiosis. PNAS 25:405-416
Isodicentric chromosome
- breakage-
fusion-
bridge
cycle

Ta et al, J Cancer Research, 2013
J Cancer Res 452809
Dicentric chromosome created from two different chromosomes
- breakage-
fusion-
bridge cycle

MacKinnon et al 2013
Evol Med Public Health 225
Dicentric chromosomes and the BFB cycle (click here)

The breakage-fusion-bridge cycle

An animation
Human erythroleukaemia cell line (HEL)

- Repetitive DNA used to work out sequence of evolution

FISH: NOR
- nucleolar organiser region
- acrocentrics (22p)
- highly repetitive

22p is on both: dic(9;22) came first

Human erythroleukaemia cell line (HEL)

+++JAK2
--CDKN2A

NOR - no information
Unstable dicentric chromosome

- Twist between centromeres at metaphase plate (>12-15 Mb)

Sullivan and Willard 1998. Stable dicentric X chromosomes with two functional centromeres
Nature Genet 20:227
Centromeres close together (<12-13 Mb)
- “twist” is less likely
- stable

“Dicentric” chromosomes aren’t always unstable

Unstable dicentric can produce stable derivatives

(1) Centromeres brought closer together

Deletion of material between centromeres
Unstable dicentric can produce stable derivatives

- (1) Centromeres brought closer together
- (2) One of centromeres removed

excision (ring)  deletion

G-band

M-BAND 20
M-BAND 17
Unstable dicentric can produce stable derivatives

- (1) Centromeres brought closer together
- (2) One of centromeres removed
- (3) One of centromeres inactivated (rare)

- MANY OF THESE LOOK MONOCENTRIC
Centromere loss in 11/29 unbalanced 20q translocations (dicentrics)

- Why hadn’t this been noticed before in cancer?
  - chromosome can look monocentric
  - centromere inactivation assumed
  - (c.f. inactivation in constitutional dicentrics – deletion is the exception)
  - or morphology unrecognisable after loss of constriction

\[
\begin{align*}
\text{dic}(17;20) & \rightarrow \\
\text{add}(17p);(17;20)
\end{align*}
\]

How do we know a monocentric is derived from a dicentric?

- breakpoints
  - material from both sides of both centromeres (SNP array)
- using these criteria
  - 21/24 unbalanced 20q translocations were dicentric (at least)
    - high incidence
    - including secondary monocentrics
    - many don’t look dicentric
Another surprise: 20q12 deletion is separate from translocation

dic(20;21)

looks like a del(20q)
SNP or CGH array
8/8 unbalanced 20q translocations retained the most telomeric probes

- Deletion physically separate from translocation
- How common is subtelomere retention in unbalanced 20q translocations?

MacKinnon et al. 2010 Genes Chr Cancer 49:998
Telomere fusion in AML/MDS?

- FISH
  - 19 unbalanced 20q translocations
    - with 20q12 deletion
  - 12/19 retained subtelomeric BAC

MacKinnon et al. 2011 Cancer Genet 204:153
Telomere fusion in AML/MDS?

- Are these telomere fusion events?
  - cf t-AML/t-MDS – chemotherapy, toxins

- Which came first?
  - del(20q) → telomere fusion
  - or telomere fusion → BFB → deletion
Telomere FISH

- four unbalanced 20q translocations
  - 4/4 retained 20qter (SNP array and/or BAC FISH)
  - 3/4 had telomere signal at translocation site

20q BAC LNA telomere probe (Exiqon)
Why hadn’t we noticed this before?

- These chromosomes don’t look like telomere fusions
  - distance between centromeres ≠ length of arms
  - translocation produces loss of tumour suppressor gene(s)
  - assume exchange → tumour suppressor genes deleted
  - array pattern (telomere retained) but don’t know it’s a translocation

\[ \text{dic}(17;20) \]
Hypothesis

- Unbalanced translocations are usually produced via the formation of a dicentric chromosome
- (21/24 unbalanced 20q translocations)
- telomere fusion
- or
- exchange of chromosome material that produces a dicentric and an acentric chromosome.
How do we think of unbalanced translocations?

If they look monocentric they’re assumed to be monocentric?

- **Step 1.** Translocation (chromosomes swap ends)
  - $\rightarrow$ 2 monocentric chromosomes
How do we think of unbalanced translocations?

If they look monocentric they’re assumed to be monocentric?

- **Step 2.** One chromosome lost at mitosis
  - \( \rightarrow \) 1 monocentric chromosome

- This would mean there are **2 causative events**
Step 1. telomere fusion
- Initially no copy number aberration
- Then automatic BFB → gain and loss

Only one causative event required
Dicentric chromosome formed by translocation

- Step 1. translocation

dicentric

acentric

Automatic loss of acentric
- can’t segregate
• Are dicentric chromosomes more common than we think?

• If so then
  • predominant role in unbalanced translocations?
  • DNA breakage-repair, more than mitotic error?
More roles for repetitive DNA

Original Article
Cytogenet Genome Res 2010;129:365–374
DOI: 10.1159/000315887
Accepted: January 25, 2010
by M. Schmid
Published online: July 6, 2010

Telomere Capture as a Frequent Mechanism for Stabilization of the Terminal Chromosomal Deletion Associated with Inverted Duplication

S. Yu  W.D. Graf
Children’s Mercy Hospitals and Clinics and University of Missouri-Kansas City School of Medicine, Kansas City, Mo., USA

- Telomere capture
- Uncapped piece of chromosome
  - e.g. inverted duplication
  - stabilises chromosome
- Centromere capture?
• **HEL Human Erythroleukaemia cell line**
Centromere capture

MacKinnon et al. 2013 Evol Med Publ Health 2013:25
Centromere capture

- Another complex karyotype AML

Paracentric gain
- 11 centromere?

11 centromere
16p and 20p (neither as far as centromere) and 11 centromere
Chromothripsis

- Stable anachromosome
  - centromere and two telomeres
  - or lost/unstable?
- Lost fragments may have been repaired but also need a centromere

MacKinnon and Campbell 2013 Cancer Genet 206:28
Centromere capture

1 2 5 14 17 20

MacKinnon and Campbell 2013 Cancer Genet 206:28
Centromere capture

- Common in complex karyotypes?
- Mechanism for rescuing broken chromosome fragments?
- To identify: identification of multiple centromeres in complex abnormal chromosomes
Summary – centromeres in cancer research

- Identity and location of centromeres may give us clues to cancer genome evolution
- Dicentric chromosomes
  - Common?
  - Centromere deletion
  - Dicentrics as predominant cause of unbalanced translocations?
    - (DNA breakage and repair a given; but mitotic error?)
- Centromere capture?
- Telomere fusion?
Acknowledgements

Cancer Council of Victoria
Leukaemia Foundation of Australia
Leukaemia Foundation of Victoria
ANZ Trustees
  James and Vera Lawson Philanthropic Trust
Perpetual Trustees
  Derham Green Fund
State Trustees Australia Foundation
Equity Trustees
  Harold and Cora Brennen Benevolent Trust
Eirene Lucas Foundation
Rebecca Cooper Medical Research Foundation
St Vincent’s Hospital Research Endowment Fund

VCCS including
  Lynda Campbell
  Bruce Mercer
  Lan Ta
  Meg Wall
  Adrian Zordan
  Pina D’Achille
  Cris Batzios

Deakin University
  Hendrika Duivenvoorden

Dept of Medicine (St Vincent’s Hospital / University of Melbourne)
  Harshal Nandurkar
  Carly Selan
  Nisha Narayan
  Matthew Ku

Pathwest (King Edward Memorial Hospital, Perth)
  Liz Baker
  Joanne Peverall
Paper Thin

Coming soon
A leukaemia awareness film (and fundraiser!)
Written, directed and produced by Elizabeth Duong
Based on the story of Sadako and the Thousand Paper Cranes
by Eleanor Coerr

[click here](#) for behind the scenes video