VIRUSES AND NUCLEAR ORGANIZATION IN ONCOGENESIS

Yegor VASSETZKY, CNRS UMR 8126, Institut de Cancérologie Gustave Roussy
NUCLEAR ORGANIZATION:

A chemical reactor?

A precision mechanics?

Concentration
NUCLEAR STRUCTURES
CHROMATIN ORGANIZATION AND EPIGENETICS

DNA

NUCLEOSOMES

FIBERS

DOMAINS

CHROMOSOMES

- DNA methylation
- Histone modifications
- Histone variants
- Chromatin domains
- Chromosomal territories
The chromosomes are organized in the nucleus:

- In a tissue specific manner
- The organization is transmitted through the cell divisions
- This organization is evolutionarily conserved

The gene-rich regions occupy more central position in the nucleus
CHROMOSOME DYNAMICS

Schneider et al, 2007

Spector et al, 2003
**CHROMOSOME TERRITORIES: A UNIT OF NUCLEAR ORGANIZATION**

- Chromosomes have preferred position with respect to the center or periphery of the nucleus

- Variability between cell-types

- Non-random neighbors: purpose is to facilitate proper gene expression!

- Complex folded surface with active genes (red) extends (or loops) into the interchromatin space
HOW THE EXISTENCE OF CHROMOSOMAL TERRITORIES WAS PROVEN?

Cremer and Cremer, 2006
Dans les cellules CHO, les gènes DHFR (actif) et beta-globine (inctatif) n’ont pas de localisation préférentielle juste après le mitose, mais au début de phase S, le gène DHFR est rélocalisé à l’intérieur de noyau, et le beta-globine reste périphérique.
CHROMOSOMAL TRANSLOCATIONS

- Balanced chromosomal translocations
  - Chimeric genes
  - Loss or gain of genes

C A N C E R

Theodor Boveri (1862-1915)
BURKITT’S LYMPHOMA

- A non-Hodgkin Lymphoma

- Three forms:
  - An endemic form in Africa is 100% associated with EBV
  - A sporadic form in Europe and North America, rare and non-associated with EBV
  - A form associated with HIV is frequent in Europe and is found in up to 2% (!) of AIDS patients

- In ~90% of the cases BL is linked to the translocation t(8;14)(q24;q32) of the CMYC gene locus next to the IGH gene locus leading to activation of the CMYC gene.
C-MYC ACTIVATION IN BURKITT LYMPHOMA: IS IGH\(\mu\) ENHANCER REALLY INVOLVED?

- t(8 ; 14) translocation induces overexpression of \(c-my\)c in B-cells

![Diagram showing the location of IGH\(\mu\) Enhancer and the fusion region between 14q3 and 2, with a 200 Kb–1 Mb (!) range.]
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NUCLEAR SUBSTRUCTURES

- nuclear pore complex
- cleavage body
- heterochromatin
- PcG body
- Cajal body (red) and Gem (green)
- OPT domain
- RNA Pol II transcription factors
- nucleoli (red) nuclear lamina (green)
- perinucleolar compartment
- nuclear species
- chromosome territory
- ND10 or PML body
- SAM68 nuclear body

from: David L. Spector, J of Cell Science
NUCLEAR ARCHITECTURE
TRANSLOCATED \textit{c-myc} LOCUS IS LOCALIZED IN THE PERINUCLEOLAR REGION IN BURKITT LYMPHOMA

Normal B-lymphocytes

P3HR1 Burkitt cell line

RAJI Burkitt cell line

\textbf{C-myc} \quad \textbf{IgH} \quad \textbf{Nucleolus (anti-B23)}

\begin{align*}
\text{IGH 14} & \quad \text{cMYC 8} \\
\text{IGH der14} & \quad \text{cMYC der14}
\end{align*}

\begin{align*}
\text{Percentage of signals} & \quad \text{Radial position} \\
\text{Distance to nucleolus (µm)} & \\
\end{align*}
Nucleolin is one component of the B cell-specific transcription factor and switch region binding protein, LR1

**LR1 regulates c-myc transcription**

(MYC gene/Immunoglobulin/chromosome translocation)

**APRIL BRYŠ† AND NANCY MAIZELS†**

Department of Molecular Biophysics and Biochemistry

Identification of Nucleolin as an AU-rich Element Binding Protein Involved in bcl-2 mRNA Stabilization*

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TRANSCRIPTION FACTORIES ARE LOCATED IN THE PERINUCLEOLAR REGION

NUCLEOLUS (Anti-B23) Phosphorylated (active) RNA polymerase II NUCLEUS (DAPI)
NUCLEOLIN, CTCF AND TRANSCRIPTIONAL REGULATION OF THE CMYC LOCUS IN BURKITT’S LYMPHOMA

Allinne et al., Blood, 2014
INTRANUCLEAR RELocalization AND GENE ACTIVATION IN CANCER
Ectopic overexpression of nucleolin leads to its delocalization in the nucleus and overexpression of endogenous $CCND1$, $c$-myc and $bcl$-2 oncogenes
VIRAL THEORY OF CANCER: UPS AND DOWNS

Peyton Rouss
1911: discovery of RSV
1966: Nobel Prize

Denis Burkitt
1957: discovery of Burkitt’s lymphoma

Anthony Epstein
1964: discovery of EBV in Burkitt’s lymphoma samples

Yvonne Barr
A non-Hodgkin Lymphoma

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**HIGH OCCURRENCE OF BURKITT’S LYMHOMA IN HIV PATIENTS: WHY?**

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Frequency in the general population</th>
<th>Frequency in AIDS patients</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burkitt’s Lymhoma</td>
<td>1:200 000</td>
<td>1:4000</td>
<td>50</td>
</tr>
<tr>
<td>Mantle Cell Lymphoma</td>
<td>1:200 000</td>
<td>1:200 000</td>
<td>1</td>
</tr>
</tbody>
</table>

- gp-120 can interact with CD21 expressed on B cells (Moir et al. 2000).
- HIV-1 causes B-cell hyperactivation (Schnittan et al., 1984).
- Elevated class switch in B lymphocytes induces B cell to proliferate (Nair MPN and al. 1988).
- Causes B cell abnormal response
- The production of autoantibodies
- DNA double strand breaks repair *via NHEJ* (Abeyesinghe et al., 2003).
- Spatial proximity (colocalization) of the two translocation partners. (Nikiforova et al., 2000; Misteli, 2003).

Three events are necessary to produce a translocation:

- DNA double strand breaks (Vilenchik et Knudson, 2003).
- Double strand breaks repair *via NHEJ* (Abeyesinghe et al., 2003).
- Spatial proximity (colocalization) of the two translocation partners. (Nikiforova et al., 2000; Misteli, 2003).
EBV AND BURKITT’S LYMPHOMA

→ EBV is 100% associated with the endemic form in Africa
   → Malaria and the use of latex-producing plants are additional risk factors in Africa

→ EBV is an innocent passenger in tumour cells?

→ EBV plays a role in initial transformation?
   → EBNA1 Stabilizes B-lymphocytes

→ A role or EBV in sustenance of the tumour?
   → A role of non-coding RNAs (EBER)?

→ Tumour formation due to other cellular changes
   → Does EBV infection affect the nuclear architecture?
HIV: A ROLE IN INTRANUCLEAR REORGANIZATION AND IN GENERATION OF SPECIFIC TRANSLOCATIONS

HIV Tat $\rightarrow$ NFkB $\rightarrow$ RAG $\rightarrow$ DSB $\rightarrow$ NHEJ $\rightarrow$ CMYC relocalization

Tat C22
Transcription

RAGi
Mirin
NU7026
AN EXPERIMENTAL SYSTEM FOR INDUCTION OF TRANSLOCATIONS IN LCL USING TALEN/CRISPRs

Chromosome 8  Cen  Tel
High frequency induction of translocations
Chromosome 14  Cen  Tel

HIV, EBV, Tat, other factors

Detection by FISH/qPCR
Analysis of translocation frequency
Analysis of gene expression

<table>
<thead>
<tr>
<th>Talen</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>MW 1</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

___Talen____ Control___
1    2           3   MW 1   2           3

1  2  3

MW 1  2  3
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