Array-CGH and FISH applications for diagnosis and study of gliomas

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Cancer

- Chromosome numerical aberrations / segmental alterations
- Restricted to tumor cells $\rightarrow$ selective advantage
  - Spécific aberrations ($bcr-abl$) or not (-3, +8, +7, ...)
  - Gains: oncogenes (amplification)
  - Losses: Tumor Supressor Genes
  - Translocations $\rightarrow$ gene overexpression / fusion genes
  ... mutations ...

Clinical impact: - Diagnosis
- Prognosis
- Treatment response
Gliomes

- Diagnostic histologique difficile

- Impact pronostic des co-délétions 1p36 et 19q13
  - Chimiosensibilité (réponse PCV)
  - Survie globale plus longue
Glioma

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Abstract | Gliomas are primary brain tumours that are thought to derive from neuroglial stem or progenitor cells. On the basis of their histological appearance, they have been traditionally classified as astrocytic, oligodendrogial or ependymal tumours and assigned WHO grades I–IV, which indicate different degrees of malignancy. Tremendous progress in genomic, transcriptomic and epigenetic profiling has resulted in new concepts of classifying and treating gliomas. Diffusely infiltrating gliomas in adults are now separated into three overarching tumour groups with distinct natural histories, responses to treatment and outcomes: isocitrate dehydrogenase (IDH)-mutant, 1p/19q co-deleted tumours with mostly oligodendrogial morphology that are associated with the best prognosis; IDH-mutant, 1p/19q non-co-deleted tumours with mostly astrocytic histology that are associated with intermediate outcome; and IDH wild-type, mostly higher WHO grade (III or IV) tumours that are associated with poor prognosis. Gliomas in children are molecularly distinct from those in adults, the majority being WHO grade I pilocytic astrocytomas characterized by circumscribed growth, favourable prognosis and frequent BRAF gene fusions or mutations. Ependymal tumours can be molecularly subdivided into distinct epigenetic subgroups according to location and prognosis. Although surgery, radiotherapy and alkylating agent chemotherapy are still the mainstay of treatment, individually tailored strategies based on tumour-intrinsic dominant signalling pathways and antigenic tumour profiles may ultimately improve outcome. For an illustrated summary of this Primer, visit: http://go.nature.com/1XY7Ri
Asolescents and young adults

Paediatrics

H3F3A K27
H3F3A G34

H3F3A G34 mutation
ATRX mutation
TP53 mutation
DNA hypomethylation

EGFR amplification
CDKN2A and CDKN2B deletion
PTEN mutation
TERT promoter mutation
+7q/-10q genotype

HIST1H3B mutation*
ACVR1 mutation*
ATRX mutation
TP53 or PPM1D mutation

PDGFRα amplification
TERT promoter mutation
+7q/-10q genotype

MGMT promoter methylation*

IMD wild-type gliomas

Glioblastoma (WHO grade IV)

RTK I
RTK II (classic)
Mesenchymal

IMD mutant gliomas

Astrocytoma (WHO grade II or III)

Oligodendroglioma (WHO grade II or III)

q-CIMP
ATRX mutation
TP53 mutation

CIC mutation

IDH-mutant glioblastoma (WHO grade IV)

q-CIMP
1p/19q co-deletion

CDKN2A and CDKN2B deletion
Chr. 10q loss

Adults > 50 years

Young adults

Improved outcome

Gliomas with diffusely infiltrative growth
Clinical impact of a-CGH analysis

Postoperative approach to astrocytic or oligodendroglial gliomas

- **IDH1 or IDH2 g-CIMP+**
  - Mutant or g-CIMP+
    - WHO grade II or III (or IV)
      - ATRX mutant
        - 1p/19q intact
          - RT or TMZ or PCV
        - 1p/19q co-deleted
          - RT/PCV or TMZ/RT → TMZ or TMZ or PCV
      - TERT mutant
        - WHO grade II or III
          - MGMT-
            - ≤65–70 years
              - RT
            - >65–70 years
              - TMZ/RT → TMZ
              - RT
    - WHO grade IV
      - MGMT-
        - ≤65–70 years
          - RT
        - >65–70 years
          - TMZ/RT → TMZ
          - RT
  - Wild-type or g-CIMP-
    - MAPK alteration
      - EGFR^{t}_{amp}, CDKN2A^{del} and +7q/-10q

- **WHO grade I**
Genome analysis

Resolution

Molecular biology

Gène moyen: 2.10⁴ pb

2000: CGH

2010: NGS

Exon: 50 à 1000 pb

Molecular cytogenetics

1970: High resolution Karyotype

1980: FISH

Sous-bande: 2.10⁶ pb

Conventional cytogenetics

1960: Karyotype

Chromosome: 2.10⁸ pb

Génome: 3.10⁹ pb

→ Which technique to be chosen?

1 – Hybridation in situ en fluorescence : FISH

Principe de la FISH
Hybridation d’une séquence d’ADN cible par des sondes ADN marquées par un fluorochrome

1 - Marquage de la sonde

2 - Dénaturation de l’ADN et de la sonde

3 - Hybridation

4 - Lecture
FISH analysis

Acta Neuropathologica 2006

Del 1p

Del 19q
FISH analysis

- Routine screening

Acquisition station
Slide charger

Automatic nuclei segmentation

Image analysis
BioView’s Solution

BioView’s Solution

H&E / IHC region of interest selection can be performed from any location via standard web browsers on a digital slide.

H&E digitally review and marked using BioView’s SoloWeb application.
BioView’s Solution

Integrated solution to allow matching between H&E/IHC and FISH slides prepared on parallel sections

FISH analysis is performed according to pathologist guidance
Comparative Genomic Hybridization (CGH)

Metaphasis (Kallioniemi et al., 1992)

**Technical steps**

1. **DNA extraction**
2. **Labelling**
3. **Hybridization (metaphasis)**
4. **Scanning**
5. **analysis**

**Resolution**: 10 – 20 Mb
Comparative Genomic Hybridization (CGH)

Tumoral DNA → Labelling Cy5 → Labelling Cy3 → Reference DNA

Labelling DNA mix → Hybridization on array CGH → Scan & Data acquisition

Data interpretation Bioinformatics tools

Copy number Amplification

Genome coordinates Deletion

Two genomic aberrations are detected.

http://www.helixio.fr/contenu/support-fr/technologies-fr

High resolution
Resolution: number and distribution of clones

- Metaphasis: Karyotype bands (5-10 Mb)
- BACs: ~ 170 kb
- Oligonucleotides

180K: 30-50kb resolution
200x higher than karyotype

Data analysis
Array-CGH for tumor classification in routine

- Genomic copy number profiles can distinguish distinct subgroups within histologically defined disease entities
- Tumor type specific copy number patterns can be used for efficient tumor classification and patient stratification
Array-CGH: Limitations

Technical

Spot Finding of the Four Corners of the Array

Grid Normal

Outlier Numbers with Spatial Distribution

236 rows x 215 columns

Technical Background

<table>
<thead>
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<th>Metric Name</th>
<th>Value</th>
<th>UpLim</th>
<th>LowLim</th>
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<tr>
<td>g_SignalIntensity</td>
<td>212.21</td>
<td>NA</td>
<td>50.00</td>
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<td>g_GCONoise</td>
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<td>g_SignalIntensity</td>
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</tr>
</tbody>
</table>

Histogram of Signals Plot (Red)

Histogram of Signals Plot (Green)

Background

Waves (GC)
Array-CGH: Limitations

**Technical**

Samples:

- Fresh: Blood, BM, other liquids
- Frozen tissues (cancer)
Analysis

- DNA origin (% of tumoral cells, reactive cells)
- Balanced chromosomal aberrations: translocations, inversions
- Subclones, ploidy
- Driver chromosomal aberrations \rightarrow \text{Genomic databases}
- Constitutional abnormalities (predisposition genes), mutations
- Chromosomal mechanism: FISH, karyotype
# FISH et ACPA : analyse comparative

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<tr>
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<th>FISH</th>
<th>ACPA</th>
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<td></td>
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<tr>
<td><strong>Mise en œuvre</strong></td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td><strong>Tissus fixés</strong></td>
<td>+++</td>
<td>+/-</td>
</tr>
<tr>
<td><strong>Analyse ciblée des C tumorales</strong></td>
<td>+++</td>
<td>-</td>
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<tr>
<td><strong>Infiltrat tumoral</strong></td>
<td>10%</td>
<td>&gt; 30%</td>
</tr>
<tr>
<td><strong>Automatisation</strong></td>
<td>+++</td>
<td>+/-</td>
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<td><strong>Coût / test</strong></td>
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<tr>
<td><strong>Etude pangénomique</strong></td>
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<tr>
<td><strong>Résolution</strong></td>
<td>+</td>
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</table>
Clinical impact of Array-CGH analysis

1p / 19q co-deletion

CDKN2A / CDKN2B deletion

10q loss / 7q gain

PDGFRA or EGFR amplification

Tumor classification

Prediction of patient outcome

Post surgical approach
Conclusion

• Diagnostic et pronostic individuel, choix du traitement

• ACPA :
  ➢ Tissu frais, congelé (→ FFPE samples)
  ➢ Analyse pangénomique de haute résolution

• ACPA : limites
  ➢ Taille du prélèvement, % de C tumorales, clones, ploïdie, hétérogénéïté tumorale ...
  ➢ FISH en 2ème intention ou pour confirmation
  ➢ Réseau (CHU Angers)

➢ Facturation : RiHN / B
Acknowledgments

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- Pathology Department
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- PEGMC