Molecular Pathology of Glial Tumors

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Outline

• I-Infiltrating astrocytomas/Glioblastoma
  – MGMT methylation
  – Gene Expression analysis
  – IDH1/2 mutations
• II-Oligodendroglial Neoplasms
  – 1p19q
• III-Pediatric gliomas
  – BRAF/MAPK alterations
  – H3K27 mutations


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Gliomas

WHO Classification

• I-Astrocytic Tumors
  – Pilocytic Astrocytoma (WHO grade I)
  – Pleomorphic Xanthoastrocytoma (WHO grade II)
  – Diffuse Astrocytoma (WHO grade II)
  – Anaplastic Astrocytoma (WHO grade III)
  – Glioblastoma (WHO grade IV)
• II-Oligodendroglial tumors
  – Oligodendroglioma and oligoastrocytoma (WHO grade II)
  – Anaplastic Oligodendroglioma and OA (WHO grade III)
• III-Ependymal Tumors

I-Infiltrating astrocytomas/Glioblastoma
Infiltrating astrocytomas

Diffuse Astrocytoma (gr II)

Anaplastic Astrocytoma (gr II)

Glioblastoma

“Butterfly glioma”  Multicentric GBM

Glioblastoma (grade IV)

Pseudopalisading  Non-pseudopalisading

Glioblastoma

Microvascular Proliferation

Primary vs Secondary Glioblastoma

• Primary (or de novo) GBM
  – Short clinical history and symptom development
  – Older patients
  – Majority of GBM (>90%)
• Secondary GBM
  – Longer clinical history/lower grade precursor
  – Younger patients

Indistinguishable Pathologically, but Different Genetic Profiles
GBM variants
Small cell astrocytoma

- Monotonous oval cells
- May have perinuclear halos
- Often GFAP negative
- Older patients
- Aggressive behavior
- Main differential diagnosis: anaplastic oligodendroglioma

GBM variants
Small Cell astrocytoma

EGFR amplification (70%)  Chr 10/10q loss (97%)

GBM variants
Giant Cell Glioblastoma

- Rare GBM subtype
- Almost always of the primary subtype
- High frequency of TP53 mutations
- Main differential diagnosis: Pleomorphic xanthoastrocytoma

Current Molecular Markers in GBM
MGMT methylation

- O6-methylguanine-DNA methyltransferase (MGMT)
- DNA repair protein that removes alkyl adducts from the O6 position of guanine
- Most commonly inactivated by epigenetic (e.g. methylation) than genetic mechanisms (approximately 45%)

Fig 1. The DNA repair process mediated by O6-methylguanine methyltransferase (MGMT)

Current Molecular Markers in GBM
MGMT methylation

Esteller M et al. NEJM 2000

Hegi M et al. NEJM 2005
MGMT Immunohistochemical Expression and Promoter Methylation in Human GBM

MGMT  CD68
GFAP  CD31

Rodriguez FI, Thibodeau SN, Jenkins RB et al. AIMM 2008

MGMT gene methylation testing
Why perform it?
• Oncologists and patients want it
• Pre-requisite for clinical trial enrollment
• May guide management in the setting of recurrent high grade glioma vs. pseudoprogression

Glioblastoma
Recurrent/progressive GBM

3/2007
5/2009

Glioblastoma
Pseudoprogression
• May be the expression of treatment-induced necrosis
• Daily temozolomide may represent a potent radiosensitizing regimen
• Significantly (positively) correlated with MGMT methylation status

Glioblastoma
Radiation Changes

Comprehensive genomic characterization defines human glioblastoma genes and core pathways

An Integrated Genomic Analysis of Human Glioblastoma Multiforme

Science 2008
An integrated Genomic Analysis of Human GBM

Parsons et al. Science 2008

Current Molecular Markers in GBM

**IDH1/2**

- “Isocitrate dehydrogenase” (IDH)
  - IDH1: cytosolic form
  - IDH2: mitochondrial form
- Converts isocitrate to α-ketoglutarate
- Mutation impairs normal function
  - Gains ability to convert α-ketoglutarate to 2HG
- Mutations frequent in diffuse gliomas, rare in non-CNS tumors

Current Molecular Markers in GBM

**IDH1 Immunohistochemistry**

- Recognizes most frequent mutation (R132H)
- Works well in formalin fixed tissues
- Useful **diagnostically** (gliosis vs. infiltrating glioma)
- Useful **prognostically** (improved prognosis in positive high grade gliomas)

Infiltrating astrocytomas

**Summary**

- **MGMT** methylation: High grade gliomas
- **IDH1/2** mutation testing
  - High grade gliomas: Prognostic
  - Diagnostically: tumor vs. reactive gliosis
- EGFRvIII, EGFR amplification, PTEN loss?
II-Oligodendroglial Neoplasms

Oligodendroglial Neoplasms Pathology
- Includes oligodendrogliomas (WHO grade II-III)
- Grading based on brisk mitotic activity/endothelial changes/necrosis (grade III)
- Improved prognosis and treatment sensitivity compared to infiltrating astrocytomas

Oligodendroglial Neoplasms
Low grade (II)

Oligodendroglial Neoplasms
Anaplastic (WHO grade III)

Oligodendroglial Neoplasms
1p19q codeletion
- Present in the majority of oligodendrogliomas (up to 90% of grade II, 60% of grade III)
- Strongly associated with “classic” oligodendroglial histology
- Associated with improved prognosis and responsiveness to treatment

1p19q in Oligodendroglial Neoplasms
Testing methods
- Fluorescence in situ hybridization (FISH)
- Microsatellite Analysis
- Copy number array analysis
  - Comparative genomic hybridization (CGH)
  - Single nucleotide polymorphism (SNP)
1p19q in Oligodendrogial Neoplasms

Problems in current assays (FISH, STR)

- The probes or primers interrogate only small portions of the chromosomes.
  - Small deletions can be missed or misinterpreted as involving the whole arm.
- STR loci are not always informative; i.e. some of them could be germ line homozygous.
  - STR analysis of normal tissue of the same patient helps to resolve the problem, but not always available.
- FISH can detect only deletions but not copy neutral-LOH
- STR cannot distinguish CN-LOH from deletions

1p19q in Oligodendrogial Neoplasms

FISH

www.abbottmolecular.com

1p19q in Oligodendrogial Neoplasms

Copy Number Determination

- B allele frequency (BAF) / Allele Ratio
  - Discrimination between the A and B alleles performed by a single nucleotide extension step using two dye chemistry
- Copy Number
- Log R Ratio (LRR)
  - The sum of the measured intensities compared to normal controls

10-260 1p19q deletion

1p19q in Oligodendrogial Neoplasms

Mechanism


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Molecular Analysis of Pediatric Oligodendrogliomas Highlights Genetic Differences with Adult Counterparts and Other Pediatric Gliomas

Specific Genetic Predictors of Chemotherapeutic Response and Survival in Patients With Anaplastic Oligodendrogliomas

1p19q in Oligodendroglial Neoplasms
Diagnostic Usefulness

- Anaplastic oligodendroglioma vs. small cell astrocytoma
- Oligodendroglioma vs. morphologic mimics (DNET, clear cell ependymoma, central neurocytoma)
  - Exception: extraventricular neurocytoma

Kaplan-Meier estimates of overall survival by treatment for patients with 1p/19q-codelleted anaplastic oligodendroglioma (AO)/anaplastic oligoastrocytoma (AOA).

Overall survival in both treatment arms for (A) the patients with 1p/19q-codelleted tumors (n = 80) and (B) the patients with non-1p/19q-codelleted tumors (n = 236).
**1p19q in Oligodendrogial Neoplasms**

**Summary**

- Historically, one of the strongest prognostic molecular markers in neuropathology
- Strongly associated with classic oligodendroglial histology
- Caveat: 1p19q co-deletion uncommon in pediatric oligodendroglioma

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**Diffuse Gliomas**

Recent advances in molecular classification

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**ATRX alterations in high grade gliomas**

**Telomere specific FISH**

**ATRX**

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**Distribution of ATRX, TP53, IDH, CIC, and FUBP1 mutations, grade II-IV gliomas**

363 brain tumors

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**Altered Telomeres in Tumors with ATRX and DAXX Mutations**

Heaphy C et al. Science 2011

**Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and pediatric glioblastomas**

01/2012

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**Mutations in CIC and FUBP1 Contribute to Human Oligodendroglioma**

Science 2011

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**Oligodendroglial Neoplasms**

**Recent advances in molecular classification**
Comprehensive, Integrative Genomic Analysis of Diffuse Lower Grade Gliomas

- 3 molecular subtypes of lower grade gliomas with clinical implications
- More robust than schemes based on histopathologic analysis alone
- 1-Tumors with IDH mutations and 1p19q co-deletion (best prognosis, closely associated with oligodendroglioma histology, and also containing CIC, FUBP1, NOTCH1 and TERT promoter mutations)

Comprehensive, Integrative Genomic Analysis of Diffuse Lower Grade Gliomas

- 2-IDH and TP53 mutations (intermediate prognosis, closely associated with astrocytic histology)
- 3-IDH wild type tumors (worse prognosis, similar to glioblastoma)

Mutational Landscape of Somatic Alterations in Lower-Grade Glioma.

- 1087 gliomas screened for selected alterations
- Gliomas classified into five main groups based on molecular alterations
- Independently associated with outcome
- Distinct clinical associations
- Associated with specific germline variants
III-Pediatric Glioma

Neurofibromatosis type 1

- Genetic tumor-predisposing syndrome
- ~1/3000
- Caused by germline mutations in the NF1 gene located at 17q11.2
- Predisposed to peripheral and CNS tumors
- Distinctive predilection to involve the optic nerve, chiasm, and hypothalamus.

NF1-associated glioma

Pilocytic astrocytoma most frequent subtype

Pilocytic Astrocytoma

WHO Grade I

“Piloid Area”

Microcystic area

Pilocytic Astrocytoma

Rosenthal Fibers

EGBs

Pilocytic Astrocytoma

BRAF duplication

- Tandem duplication of the BRAF kinase domain resulting in KIAA1549:BRAF fusion
- Multiple independent publications in 2008:
  - Bar, E.E., et al., JNEN 2008

Tandem duplication at 7q34 produces a fusion gene between KIAA1549 and BRAF

Jones et al. 2008

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**KIAA1549-BRAF fusions in paraffin FISH strategy**

- Fusion schematic of the KIAA1549 gene in relation to the BRAF gene on a normal chromosome 7 as well as the fusion product on the abnormal chromosome 7.

**BRAF duplication in paraffin FISH strategy**

**BRAF fusion in paraffin FISH strategy**


**BRAF duplication/fusion in PA**


**BRAF point mutations**

**BRAF^{V600E}**

- Frequent in papillary thyroid carcinoma and melanoma
- Absent to extremely rare in GBM, oligodendroglial tumors, ependymomas
- Present in a subset of low grade/pediatric gliomas (Schindler G et al. 2011)
  - 66% of pleomorphic xanthoastrocytomas
  - 18% of gangliogliomas
  - 9% of pilocytic astrocytomas

**BRAF point mutation**

Wild Type: BRAF^{V600E}

- T A GCT ACA GT G AAA TC
- AGCTACAGAGAAATCTCG
Pediatric low-grade glioma
Whole genome/exome sequencing

- BRAF V600E IHC

- Recurrent somatic alterations of FGFR1 and NTRK2 in pediatric astrocytomas
  - MAPK pathway alterations in all tumors analyzed
  - PA predominantly single pathway disease

- WGS of 95 pilocytic astrocytomas
  - FGFR1, PTPN11 and NTRK2 fusions
  - mTOR pathway alterations

- 12 PXAs
  - BRAF mut (6/12)
  - TP53 (3/12)
  - mTOR pathway genes (NF1, PIK3R1, TSC2) (4/12)
  - BRAF mut and mTOR pathway mut mutually exclusive

- 39 PLGA and glioneuronal tumors
  - Single non-silent somatic mut in 62%
  - FGFR1 or MYB alterations in grade II diffuse gliomas

- 44 FFPE diffuse PLGG
  - 13.1 gain (28% of DA)
  - Partial MYBL1 duplication with truncation of C-terminal regulatory domain
  - Similar MYB alteration in 2 angiocentric gliomas
Glioblastoma

1 week old male

Pediatric Glioblastoma

Diffuse Intrinsic Pontine Glioma

• Specific clinicopathologic subset of infiltrating astrocytoma
• Uniform poor prognosis
• Preoperative biopsy not always performed in the past, but more often performed in the context of clinical trials

Diffuse Intrinsic Pontine Glioma

Morphology

Full spectrum of diffuse WHO grades represented
1 Grade II, 1 grade II-III
4 (17%) Grade III
18 (75%) Grade IV

5mC
H3K27me3

Pediatric Glioma
Epigenetics

DIPG
pGBM

Ballester L et al. AJSP 2014
Epigenetic and Biologic Subgroups of GBM

Pediatric Infiltrating Astrocytoma
Alterations in Chromatin Remodeling Proteins

NEW MODEL OF GLIOMAGENESIS
Specific mutations lead to global alterations in chromatin/epigenetics responsible for specific brain tumor phenotypes

Conclusions

• Genetic landscape of glioblastoma increasingly clarified in the past several years
• Molecular subgroups of diffuse gliomas with distinct biology and prognosis
• Gliomas in children and adults are biologically and clinically distinct

Conclusions

• Required markers in most neurooncology centers
  – MGMT methylation and IDH mutation (high grade astrocytomas)
  – 1p19q and IDH mutation (oligodendrogliomas)
  – BRAF mutation/duplication (pediatric gliomas)
Questions?