Improving central nervous system tumor diagnostics through methylation profiling

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Disclosures

• I have no relevant financial relationships to disclose
Learning Objectives

1. Describe the general principle of genomic DNA methylation profiling
2. Explain why genomic DNA methylation profiling works as a diagnostic tool
3. List some of the limitations of genomic DNA methylation profiling and why complementary molecular tests like next generation sequencing are still needed
And now for something completely different...
methylation fingerprinting

- DNA methylation is a "fingerprint" that shows cell-of-origin
- helps classify difficult tumors
- generates invaluable data for research
IDH\textsuperscript{wt} glioblastoma

IDH\textsuperscript{mut} astrocytoma

posterior fossa ependymoma type B
DNA methylation–based classification of central nervous system tumours

A list of authors and their affiliations appears in the online version of the paper.
classifier development

- ~2800 CNS tumors
- Infinium 450K methylation
- random forest algorithm
  - combines many weak classifiers to make a strong one
- results
  - sensitivity = 0.989
  - specificity = 0.999
  - overall error rate = 1.14%
case 1:
if it looks like a duck and methylates like a duck...
case 1

- 65 year-old man
- left brain mass
synaptophysin
NGS results

- TP53 mut
- MET amp
- CDK6 amp
Epithelial and Pseudoepithelial Differentiation in Glioblastoma and Gliosarcoma

A Comparative Morphologic and Molecular Genetic Study

Frequent characteristics:

1. Varying degrees of CAM5.2 immunoreactivity
2. High Ki67 proliferation index
3. Strong p53 IHC
4. TP53 mutations and CDKN2A deletions
-diagnosis

• glioblastoma, IDH wild-type, WHO grade 4, with pseudoepithelial differentiation
• died several weeks later
case 2: Unidentified Metastatic Object
case 2

• 67 y/o F
• right frontoparietal mass
cytokeratin 7

cytokeratin 20
preliminary diagnosis

• metastatic carcinoma of unclear origin
• mutations in
  – PIK3CA
  – ARID1A
  – KRAS
  – GNAS
450k Classifier Scores (mnpprediction version 0.1.9)

<table>
<thead>
<tr>
<th>Score</th>
<th>Abbreviation</th>
<th>Tumor subclass</th>
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<tbody>
<tr>
<td>0.3266</td>
<td>MET_LUNG</td>
<td>NA</td>
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<tr>
<td>0.1411</td>
<td>MET_BRCA</td>
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<tr>
<td>0.0691</td>
<td>CPH_PAP</td>
<td>Papillary craniopharyngioma</td>
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<tr>
<td>0.0293</td>
<td>EWS</td>
<td>Ewing sarcoma</td>
</tr>
</tbody>
</table>
• large RLL pleural-parenchymal tumor with hilar lymphadenopathy
• nothing abnormal in the biliary tree, liver, spleen, or pancreas
final diagnosis

• metastatic lung adenocarcinoma
case 3: the Swiss Cheese model in action
case 3

• 40 year-old man
• right frontal tumor
• diagnosed as “high grade glioma” at OSH
NGS results

• ZFTA fusion
• homozygous deletion of $CDKN2A/B$
Infinium 850K methylation profile
revised diagnosis

• ependymoma, *ZFTA* fusion-positive, WHO grade 3
case 4: correcting past mistakes
case 4

- 47 year-old man
- left temporal lobe tumor
- diagnosed as GBM at OSH
two years later
chest wall and spine
chest wall mass
GFAP  chest wall mass  olig2
NGS

brain tumor
• BRAF V600E
• TERT promoter mutant
• SETD2 mutant

chest wall mass
• BRAF V600E
• TERT promoter mutant
• SETD2 mutant
• TP53 mutant
Infinium 850K methylation profile
revised diagnosis

- pleomorphic xanthoastrocytoma, WHO grade 3, with metastases to the chest wall and spine
- responded to combination of BRAF and MEK inhibitors
- still alive 3+ years later
Association of Pleomorphic Xanthoastrocytoma with Cortical Dysplasia and Neuronal Tumors

A Report of Three Cases

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Neil Duggal, M.D.2
Vasco F. DaSilva, M.D.3
Brien G. Benoit, M.D.3

A rare clinical presentation: a pleomorphic xanthoastrocytoma presenting with intracerebral haemorrhage and metastasizing vigorously—case report and review of the literature

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Metastatic pleomorphic xanthoastrocytoma in the scalp

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Spinal Pleomorphic Xanthoastrocytoma: Case Report and Literature Review

Darius Tan1, Leon T. Lai1,2, Christopher D. Daly1, Vu Tran1, Julian Maingard3,4, Craig Timms1

Anaplastic pleomorphic xanthoastrocytoma with leptomeningeal dissemination responsive to BRAF inhibition and bevacizumab

A Clinical Observation

Complete Remission of an Extracranially Disseminated Anaplastic Pleomorphic Xanthoastrocytoma With Everolimus: A Case Report and Literature Review

Amanda J. Saraf, DO4,5, Ghada Elhawary, MBChB6, Jonathan L. Finlay, MD3, Suzanne Scott, APRN6, Randal Olshefski, MD4, Mark Halverson, MD5, Daniel R. Boue, MD, PhD4, Mohamed S. AbdelBaki, MD4,5

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journal homepage: www.elsevier.com/locate/pnu
Using methylation profiling to diagnose systemic metastases of pleomorphic xanthoastrocytoma

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case 5: nobody’s perfect

DUDE, NOBODY’S PERFECT, MAN
case 5

• 30 y/o M

• mass in the pineal region

• original tumor resected 6 years ago, didn’t recur until 6 years later

• consult from OSH—no radiology
original tumor
recurrent tumor 6 years later
recurrent tumor 6 years later

Ki67
molecular results

- NGS
  - H3-3A K27M
  - BRAF K601N
- oncoscan
  - gain of 6p
  - loss at 1p
  - CN-LOH on 1q and 17q
- methylation profiling
  - “diffuse midline glioma, H3K27M mutant”
Personalized oncology and $BRAF^{K601N}$ melanoma: model development, drug discovery, and clinical correlation

Brian A. Keller$^{1,2,3}$, Brian J. Laight$^1$, Oliver Varette$^{1,2}$, Aron Broom$^4$, Marie-Ève Wedge$^{1,5}$, Benjamin McSweeney$^1$, Catia Cemeus$^1$, Julia Petryk$^1$, Bryan Lo$^{1,3,6}$, Bruce Burns$^3$, Carolyn Nessim$^{1,7}$, Michael Ong$^{1,8}$, Roberto A. Chica$^4$, Harold L. Atkins$^{1,2,9}$, Jean-Simon Diallo$^{1,2}$, Carolina S. Ilkow$^{1,2}$, John C. Bell$^{1,2}$
A long-term survivor of pediatric midline glioma with \textit{H3F3A K27M} and \textit{BRAF V600E} double mutations

Yoshiko Nakano$^{1,2}$ · Kai Yamasaki$^{1,2}$ · Hiroaki Sakamoto$^3$ · Yasuhiro Matsusaka$^3$ · Noritsugu Kunihiro$^3$ · Hiroko Fukushima$^4$ · Takeshi Inoue$^4$ · Mai Honda-Kitahara$^1$ · Junichi Hara$^2$ · Akihiko Yoshida$^5$ · Koichi Ichimura$^1$

\textbf{Co-occurrence of histone H3 K27M and BRAF V600E mutations in paediatric midline grade I ganglioglioma}

Mélanie Pagès$^{1,2,3}$, Kevin Beccaria$^{4}$, Nathalie Boddaert$^5$, Raphaël Saffroy$^6$, Aurore Besnard$^1$, David Castel$^{7,8}$, Frédéric Fina$^9$, Doriane Barrets$^{10}$, Emilie Barret$^{7,8}$, Ludovic Lacroix$^{11}$, Franck Bielle$^{12}$, Felipe Andreiuolo$^1$, Arnault Tauziède-Espariat$^1$, Dominique Figarella-Branger$^{10,13}$, Stéphanie Puget$^4$, Jacques Grill$^{7,8}$, Fabrice Chrétien$^{1,2,14}$, Pascale Varlet$^{1,2,3}$

\textbf{Scientific correspondence}

Evidence for \textit{BRAF V600E} and \textit{H3F3A K27M} double mutations in paediatric glial and glioneuronal tumours
diagnosis

• midline glioma with H3-3A K27M and BRAF mutations (see comment)
Variant allelic frequency of driver mutations predicts success of genomic DNA methylation classification in central nervous system tumors

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1. *if* a driver mutation is present in virtually 100% of tumor cells...
2. *...and* nearly always exists in a heterozygous state...
3. *...and* there are no copy number variations at that gene locus...
4. *...then* Variant Allelic Fraction x 2 = % tumor cellularity
if you don’t get a match, insufficient tumor cellularity might be the problem
case 6:
uncharted territory
case 6

• 2 year-old boy
• cerebral tumor
block 2: “choroid plexus tumor”

block 1: “ATRT”
block 1: ATRT

probable hypodiploid genomes

block 2: choroid plexus tumor
NGS results

ATRT (block 1)
• TP53 p.Arg282Trp
• TSC2 p.Arg1477Glyfs*46
• KMT2D p.Pro2354Ser

choroid plexus tumor (block 2)
• TP53 p.Arg282Trp
• TSC2 p.Arg1477Glyfs*46
• KMT2D p.Pro2354Ser
diagnosis

- high grade neoplasm with divergent embryonal (ATRT) and CPC subclonal evolution
methylation profiling fosters the discovery of new tumor types

High-grade glioma with pleomorphic and pseudopapillary features (HPAP): a proposed type of circumscribed glioma in adults harboring frequent TP53 mutations and recurrent monosomy 13

Drew Pratt1, Zied Abdullaev1, Antonios Papanicolau-Sengos1, Courtney Ketchum1, Pavalan Panneer Selvam1, Hye-Jung Chung1, Ina Lee1, Mark Raffeld1, Mark R. Gilbert2, Terri S. Armstrong2, Peter Pytel3, Ewa Rorys4, Joshua M. Klonoski5, Matthew McCord6, Craig Horbinski6, Daniel Brat6, Arie Perry7, Charles Eberhart8, Caterina Giannini9, Martha Quezado9, Kenneth Aldape1

Glioneuronal tumor with ATRX alteration, kinase fusion and anaplastic features (GTAKA): a molecularly distinct brain tumor type with recurrent NTRK gene fusions

Expanded analysis of high-grade astrocytoma identifies an epigenetically and clinically distinct subtype associated with neurofibromatosis type 1

Patrick J. Cimino1, Courtney Ketchum2, Rust Turakulov2, Omkar Singh2, Zied Abdullaev2, Caterina Giannini2, Peter Pytel2, Giselle Yvette Lopez2, Howard Colman6, MacLean P. Nasrallah7, Marilana Sant1, Igor Lima Fernandes9, Jeff Nirschl9, Sonika Dahlia11, Stewart Nell12, David Solomon13, Ellis Perez14, David Capper14, Haresh Mani15, Darío Cacamo16, Matthew Ball17, Michael Badrduddin18, Rati Chkhelidze19, Sandra Camelo-Piragia20, Joseph Fulmer21, Sandra Alexandrescu22, Gabrielle Yee22, Charles Eberhart24, Maria Martinez-Lage25, Jie Chen26, Leor Zach27, B. K. Kleinschmidt-DeMasters28, Marco Hefti29, Maria-Beatriz Lopes30, Nicholas Nuechterlein31, Craig Horbinski32, Fausto J. Rodriguez33, Martha Quezado2, Drew Pratt2, Kenneth Aldape2
methylation profiling is now an **indispensable** part of neuropathology
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Any questions?