Epigenetic Markers of Epileptogenesis

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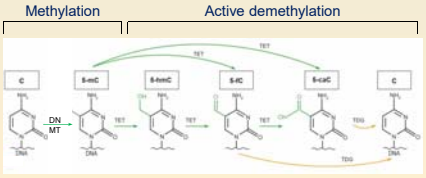
DNA Modifications

- DNA methylation
  - CpG versus non-CpG
- DNA hydroxymethylation



Modified from: https://www.epigentek.com/catalog/complete-guide-to-methylcytosine-mc-derivatives-n-13.html
DNMT: DNA methyltransferase
TET: Ten-eleven translocation
TDG: thymine DNA glycosylase

Disclosures

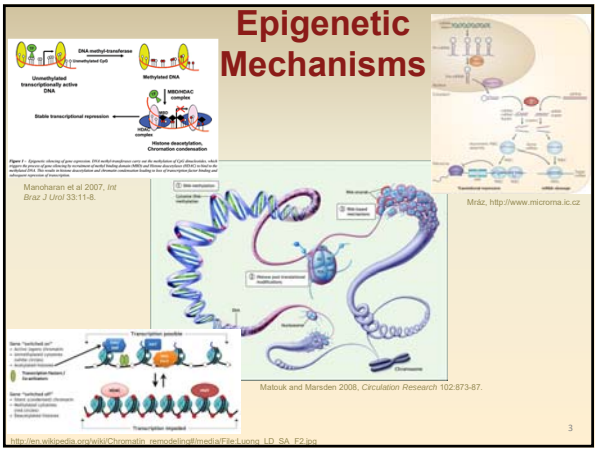
- None

DNA Methylation in Epilepsy

- DNA methyltransferases (*DNMT1*, *DNMT3A*) upregulated in epileptic brain;[1] *TET1* decreased in hippocampus after seizure[2]
  - Another study indicated no change in gene expression of *DNMT1*, *DNMT3A* or *DNMT3B* in experimentally induced epilepsy (rats)[3]
- Altered methylation in *RELN*, *CPA6*, *Bdnf* , *Grin2b*, *Gria2*, *ZNF638*, *CYP3A43*, *PYY*, *ADCY5*, *HOXD11*, *ZNF257*, *Cpne6*, *Gtf2i*, and *Casp4* in human and rodent studies of epilepsy[4-9]
  - But, no consistency across studies
  - Gene ontology implicates growth regulation, skeletal development and morphogenesis, anion binding, drug metabolism neuron differentiation, axon guidance, protein kinase activity, DNA binding, transcriptional regulation, actin binding, sodium channel activity[8-11]
- In general, hypermethylation is more prevalent than hypomethylation, except at promoter regions[8,9,11]

1. Zhu *et al* 2012, *J Mol Neurosci* 46:420-6.
2. Kaas *et al* 2013, *Neuron* 79:1086-93.
3. Kobow *et al* 2013, *Acta Neuropathol* 126:741-56.
4. Kobow *et al* 2009, *J Neuropathol Exp Neurol* 68:356-64.
5. Belhedi *et al* 2014, *Epilepsy Research* 108:144-8.
6. Ryley Parrish *et al* 2013, *Neuroscience* 248:602-19.
7. Machnes *et al* 2013, *PLoS One* 8:e76299.
8. Long *et al* 2017, *Sci Rep* 7:43810.
9. Miller-Delaney *et al* 2012, *J Neurosci* 32:1577-88.
10. Xiao *et al* 2018, *Mol Neurobiol* 55:793-803.
11. Kobow *et al* 2013, *Acta Neuropathol* 126:741-56.

Epigenetic Mechanisms



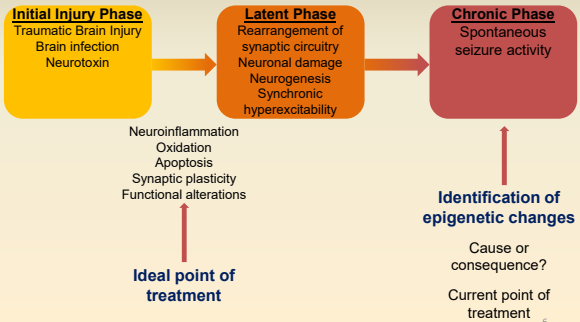
Manoharan et al 2007, *Int Braz J Urol* 33:11-8.

Mráz, http://www.microrna.ic.cz

Matouk and Marsden 2008, *Circulation Research* 102:873-87.

http://en.wikipedia.org/wiki/Chromatin_remodeling#/media/File:Luong_LD_SA_F2.jpg

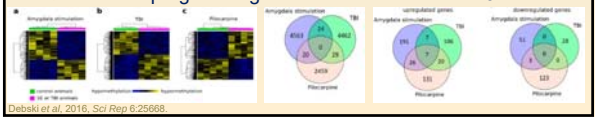
Epileptogenesis



Rat Models of Epileptogenesis

When does epileptogenesis ≠ epileptogenesis?

- Three different rat models of epileptogenic injury
  - Focal amygdala stimulation
  - Systemic pilocarpine injection
  - Lateral fluid-percussion induced traumatic brain injury
- Each model identified differentially methylated regions associated with epilepsy (hippocampus)
  - No consensus of across all three models
    - Hypermethylation gene bodies; hypomethylation non-genic areas
  - But.... 7 upregulated genes were common to all 3 models



Debski *et al*, 2016, *Sci Rep* 6:25668.

Potential Therapeutics

- DNA methyltransferases
  - Decitabine (approved to treat leukemia) and zebularine (preclinical trials for cancer) have been shown to inhibit long-term memory potentiation in the hippocampus[1]
  - DNMT inhibitor RG108 upregulates *Gria2*, and blocks seizure inducing effects of kainic acid in the hippocampus[2]
- Adenosine augmentation
  - Endogenous regulator of DNMT (i.e., high levels of adenosine inhibit methyl donor transfer)
  - Adenosine augmentation significantly reduces DNA methylation in status epilepticus and completely suppressed seizure incidence (incidence was reduced for at least three months after therapy)[3]
  - Inhibits mossy fiber sprouting in hippocampus, and prevented progression of epilepsy for up to three months[4]

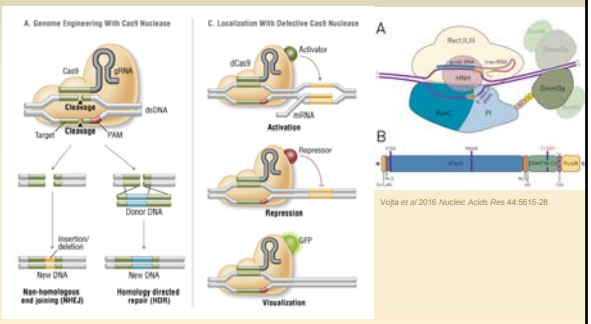
1. Levenson *et al* 2006, *J Biol Chem* 281:15763-73.
2. Machnes *et al* 2013, *PLoS One* 8:e76299.
3. Williams-Karnesky *et al* 2013, *J Clin Invest* 123:3552-63.
4. Boison 2016, *Frontiers Mol Neurosci* 9:1-15.

Fetal Programming

- Resetting of important physiological parameters by environmental events that can endure into adulthood
  - Influenced by DNA methylation and histone modifications
- Evidence of fetal programming in several neuropsychiatric disorders
  - Schizophrenia[1], depression[2], autism[3]
  - Differential methylation seen in children (buccal cells) with fetal alcohol spectrum disorder[4]
    - Gene ontology showed enrichment of neurodevelopmental processes and diseases, such as anxiety, epilepsy and autism spectrum disorders
  - Gestational diabetes increases incidence of neuropsychiatric disease in offspring[5]
    - Incidence of infantile spasms increased (not significant)

1. Pidsley *et al* 2014, *Genome Biol* 15:483.
2. Nemoda *et al* 2015, *Transl Psychiatry* 5:e545.
3. Schroeder *et al* 2016, *Mol Autism* 7:51.
4. Portalas-Casama *et al* 2016, *Epigenetics Chromatin* 9:28.
5. Nahum Sacks *et al* 2016, *Ob J Gynecol* 215:380e1-7.

CRISPR/Cas9 Editing



Vojta *et al* 2016 *Nucleic Acids Res* 44:5615-28.

https://www.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology

Neurodevelopment

(5-hydroxymethylcytosine, 5hmC)

- Increased 5hmC in NSCs compared to iPSCs ($p<2.2x10^{-16}$).
- Significant enrichment of genes involved in focal adhesion ($p=4.11x10^{-2}$) and lamellipodium (component of the focal adhesion architecture, $p=4.47x10^{-2}$), suggest an important role for 5hmC in neuronal development
- Near-significant association for post-synaptic density ($p=6.57x10^{-2}$) suggests important role for 5hmC in synaptic plasticity, which is disrupted in several neuropsychiatric diseases, including epilepsy
- Global levels of 5hmC are reduced in the hippocampus (dentate gyrus) of a rodent kainate model of status epilepticus[1]

1. Zybura-Broda *et al*, 2016, *PLoS One* 11:e0159745.

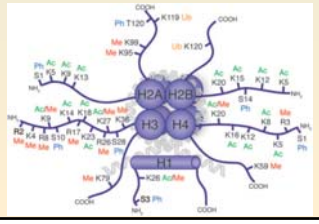
DNA Methylation

Take Home Message

- DNA methylation is important in epilepsy, but the implications of this are highly variable, depending on.....
  - Model used: human versus rat versus mouse
  - Type of epilepsy: partial versus generalized; severity of seizures
  - Source of tissue: blood versus brain
  - Detection method: array (promoter versus genome-wide) versus sequencing (what sort)
  - Etiology: how do seizures arise
- Until studies consistently implicate candidate genes, there is limited clinical utility of DNA methylation profiles

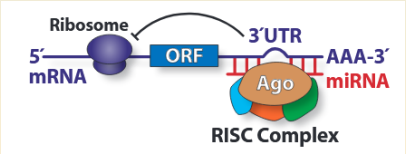
Protein Modifications

- Chromatin consists of histone and non-histone proteins bound to DNA
  - Histone modifications strongly regulate transcription, in conjunction with DNA methylation
    - E.g., methylation/demethylation, acetylation/deacetylation, phosphorylation, ubiquitination



RNA Modifications

- Focus on miRNAs, most widely studied RNA-based epigenetic modification in epilepsy



https://www.activemotif.com/documents/1944.pdf

Histone Modifications in Epilepsy

Model	Region	Target	Modification
Rat	CA3	BDNF	↑ H4 acetylation[1]
	CA3	GluR2	↓ H3, H4 acetylation[1]
	Hippocampus	n/a	↑ H4 acetylation[2]
	Dentate gyrus	n/a	↑ H4 phosphorylation[2]
	Hippocampus	Mmp9	↑ H3 Ser10 phosphorylation[3]
	Hippocampus	n/a	↓ H3 Lys27 trimethylation[3]
	Hippocampus	n/a	↑ H2A Lys119 ubiquitination[3]
Mouse	Hippocampus	n/a	↑ H3 Ser10 phosphorylation[4]
		Gad67/Gad65	↓ acetylation in GABAergic neurons (↓ expression of GAD67/GAD65); increase in histone deacetylases (HDACs) post seizure[5]
Human	Temporal neocortex		↓ H3, H4 acetylation[5]

Table modified from: Hauser *et al* 2017, *Neuroscientist*, Epub ahead of print

1. Huang *et al* 2002, *J Neurosci* 22:8422-8.
2. Sng *et al* 2006, *Eur J Neurosci* 23:1269-82.
3. Zybura-Broda *et al* 2016, *PLoS One* 11:e0159745.
4. Crosio *et al* 2003, *J Cell Sci* 116:4905-14.
5. Wang *et al* 2016, *Neurochem Res* 41:1751-60.

miRNAs in the Pathology of Epilepsy

miRNA	Model	Region	Implication
miR-34a	Mice Rat	CA1, CA3 CA1	↑ in model of status epilepticus[1] ↑ in model of temporal lobe epilepsy[2]
miR-128	Mice	Germline	↓ expression results in fatal epilepsy[3]
miR-132	Mice Rat	CA3 Hippocampus	↑ in status epilepticus[4] ↑ in chronic temporal lobe epilepsy[5]
miR-134	Mice Rat	CA3 Hippocampus	↑ in status epilepticus[6] ↑ in chronic temporal lobe epilepsy[5]
miR-184	Mice	CA1	↑ following seizure preconditioning; inhibiting expression results in increased seizure-induced neuronal death; may be involved in tolerance[7]

** Selection of significant results.

- Expression of let-7a and miR-23a/b is dysregulated from immediately after status epilepticus until 50 days later
  - Not consistent direction; variable at different time points[5]

Table modified from Younus and Reddy 2017, *Pharmacology and Therapeutics* 177:108-22.

1. Sano *et al* 2012, *Cell Death and Disease* 3:e287.
2. Hu *et al* 2012, *BMC Neuroscience* 13:115.
3. Tan *et al* 2013, *Science* 342:1254-8.
4. Jimenez-Mateos *et al* 2011, *Am J Pathol* 179:2519-32.
5. Song *et al* 2011, *Brain Research* 1387:134-40.
6. Jimenez-Mateos *et al* 2012, *Nat Med* 18:1087-94.
7. McKiernan *et al* 2012, *Experimental Neurology* 237:346-54.

Therapies

- Histone deacetylase inhibitors (HDACi)
  - Benzamides (e.g., entinostat, mocetinostat)
  - Hydroxymates (e.g., vorinostat, trichostatin A)
  - ** Aliphatic fatty acids: can cross blood-brain barrier
    - Valproic acid: approved for treatment of epilepsy since 1967; ↑ H3 acetylation in brain, facilitates DNA demethylation[1,2]
    - Sodium butyrate: ↑ H3 and H4 acetylation, decreases development of epileptogenesis (delayed onset of severe epilepsy/seizures)[3]
- Histone acetyltransferases (HATs)
  - Curcumin modulates HATs[4] and can attenuate seizures in a kainate-induced model of epilepsy[5]
  - None are in clinical trials for brain disorders
- Current approaches exert global epigenetic effects
  - Specificity may help: epigenetic editing for targeted modifications

1. Eleuteri *et al* 2009, *Neurotoxicity Res* 15:127-32.
2. Dong *et al* 2007, *PNAS* 104:4676-81.
3. Deutsch *et al* 2008, *Eur Neuropsychopharmacol* 18:565-8.
4. Huang *et al* 2016, *Pharmacol Res* 114:1-12.
5. Kiasalari *et al* 2013, *Pharm Biol* 51:1572-8.

miRNA Biomarkers in Epilepsy

- EpimiRBase: list of miRNAs associated with epilepsy[1]
- Serum miRNAs have been associated with epilepsy[2]
  - Upregulation: let-7d-5p, miR-106-5p, miR-130a-3p, miR-146a-5p
  - Downregulation: miR-15a-5p, miR-194-5p
  - miR-106b-5p best diagnostic value (80.3% sensitivity, 81.2% specificity)
- Serum miRNA profiles show differences in patients with controlled versus refractory seizures[3]
  - Dysregulation in drug-resistant epilepsy: miR-194-5p, miR-301a-3p, miR-30b-5p, miR-342-5p and miR-4446-3p
- miR-219 expression decreased in CSF of patients with temporal lobe epilepsy (n=8)[4]
- Electrochemical detection of miR-134 may serve as a diagnostic tool, showing increased plasma miR-134 levels in epileptics versus controls[5]
  - miR-134 ↑ in epilepsy and ↓ following intake of valproic acid[6]

1. Mooney *et al* 2016, *Bioinformatics* 32:1436-8.
2. Wang *et al* 2015, *Sci Rep* 5:9522.
3. Wang *et al* 2015, *Sci Rep* 5:10201.
4. Zheng *et al* 2016, *Mol Neurobiol* 53:1-7.
5. Spain *et al* 2015, *RSC Advances* 5:90071-8.
6. Wang *et al* 2017, *Oncotarget* 8:72748-54.

miRNA Expression in Epileptic Brains

- miR-181a expression increased in temporal lobe epilepsy in children (surgical resection for intractable epilepsy, n=25)[1]
- miR-203 expression increased in hippocampal post-mortem tissue of epileptics (n=6)[2]
- 20 miRNAs dysregulated in the hippocampus of mesial temporal lobe epilepsy with hippocampal sclerosis (n=33, control n=9)[3]
  - Potentially regulate targets and pathways implicated in epilepsy (potassium channels, γ-aminobutyric acid, neurotrophin signaling, axon guidance)
- miR-124 expression reduced in patients with refractory epilepsy (n=12, temporal neocortex)[4]

1. Ren *et al* 2016, *Genet Mol Res* 15:15017798.
2. Lee *et al* 2016, *Mol Neurobiol 54:3300-8.*
3. Bencurova *et al* 2017, *Epilepsia* 58:1782-93.
4. Wang *et al* 2016 , *Exp Rev Mol Med* 18:e4.

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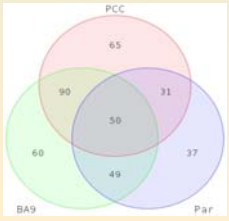
miRNAs: Blood-Brain Correlations

- Investigated correlations between peripheral blood and brain miRNA expression in a baboon model
  - Identified 50 miRNAs that showed at least moderate correlation between blood and three brain regions involved in the **default mode network**



Potential Therapies

- 14 miRNAs functionally interrogated, 12 show beneficial effect on EEG, seizures or histopathology[1]
  - AntagomiR (miRNA inhibitor) reduces status epilepticus or protected hippocampus (miRNA upregulated in model)
    - miR-34,[2] miR-132,[3] miR-134,[4,5] miR-181a,[6] miR-199a,[7] miR-210[8]
  - AntagomiR spontaneous recurrent seizures
    - miR-134,[4] miR-203[9]
  - AgomiR (miRNA mimic) reduces status epilepticus or protected hippocampus (miRNA downregulated in model)
    - miR-128,[10] miR-219,[11] miR-23b,[12] miR-124[13]
  - AgomiR reduces spontaneous recurrent seizures
    - miR-22-3p[14]

1. Henshall *et al* 2016, *Lancet Neurol* 15:1368-76.
2. Hu *et al* 2012, *BMC Neuroscience* 13:115.
3. Jimenez-Mateos *et al* 2011, *Am J Pathol* 179:2519-32.
4. Jimenez-Mateos *et al* 2012, *Nat Med* 18:1087-94.
5. Jimenez-Mateo *et al* 2015, *Brain Struct Func* 220:2387-99.
6. Ren *et al* 2016, *Genet Mol Res* 15:15017798.
7. Wang *et al* 2016, *Epilepsia* 57:706-16.
8. Chen *et al* 2016, *Neuropsych Dis Treat* 12:1731-7.
9. Lee *et al* 2016, *Mol Neurobiol 54:3300-8.*
10. Tan *et al* 2013, *Science* 342:1254-8.
11. Zheng *et al* 2016, *Mol Neurobiol* 53:1-7.
12. Zhan *et al* 2016, *Neuroreport* 27:764-8.
13. Wang *et al* 2016 , *Exp Rev Mol Med* 18:e4.
14. Jimenez-Mateos *et al* 2015, *Sci Rep* 5:e17486.

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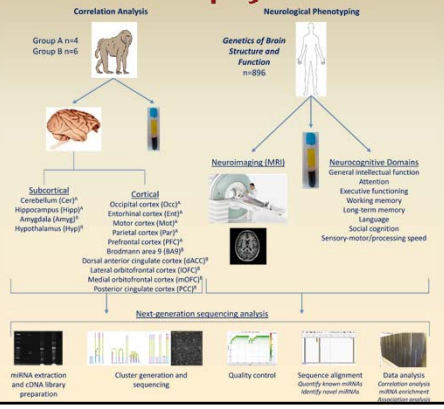
miRNAs: Enrichment in Neurological Processes

- miRNA Enrichment Analysis and Annotation (miEAA) used to define pathways enriched for miRNA target genes

Category	FDR-adjusted p-value	Expected number of miRNAs	Observed number of miRNAs
GABA receptor signaling	0.002	3.8	13
Alzheimer's disease	0.006	7.2	16
Serotonin receptor signaling	0.006	2.9	10
Glutamate receptor group	0.011	4.2	11
Acetylcholine receptor signaling	0.022	4.9	11
Axon guidance	0.046	3.6	8

miRNAs in Neuropsychiatric Disease



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Blood-Brain Correlated miRNAs in Neuropsychiatric Disease

- In humans, blood-brain correlated miRNAs are associated with brain structural variation

miRNA	Phenotype	P-value
miR-144-3p	Fusiform thickness	1.98×10^{-5}
	Hippocampal volume	5.86×10^{-5}
	Amygdala volume	8.50×10^{-5}
miR-181a-2-3p	Fusiform thickness	1.13×10^{-4}
miR-155-5p	Parahippocampal volume	1.61×10^{-5}
miR-29a-5p	Rostral anterior cingulate volume	4.87×10^{-5}

  - miR-144-3p shows high correlation between blood and amygdala (r=1.000) and hippocampus (r=0.66)
  - miR-144-3p is implicated in fear extinction (hippocampus modulates this) and action of mood stabilizers

Future of Epilepsy Therapies

- Optimal time window
  - Reliable biomarkers to predict epilepsy onset (prior to structural neuronal damage by seizures)
- New molecular tools
  - Organoids/mini-brains
  - CRISPR
  - miRNA delivery to neuronal cells
  - DREADDs (designer receptors exclusively activated by designer drugs)
    - Remotely control neuronal signaling
    - Allows temporal and spatial control of G-protein signaling (i.e., not an epigenetic therapy, but could target genes that are epigenetically modified in epilepsy)

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Our Ultimate Goal

- Use of cellular models (human) and epigenetic tools to identify novel treatments for epilepsy
  - Targeted approaches: we need to know what genes/miRNAs are dysregulated
  - We need to understand what phenotypes are useful markers of disease
- New animal models to establish treatment efficacy and safety
  - Epilepsy occurs naturally in our cohort of baboons
    - Baboons are more similar to humans (behavior, genetics, physiology, brain structure) than rodents
    - Are the same genes/miRNAs responsible?

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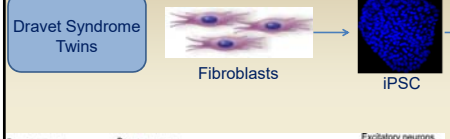
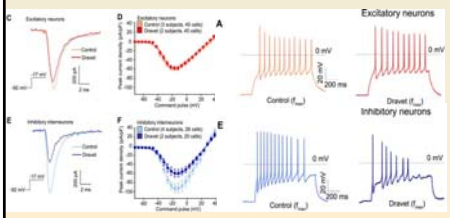
Cells in a Dish

Dravet Syndrome Twins



Neurons



Amplitude of voltage-dependent sodium currents and action potential firing is reduced in inhibitory neurons from Dravet patients

Sun *et al* 2016, *eLife* 5:e13073.

Questions?

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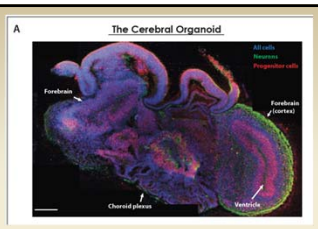
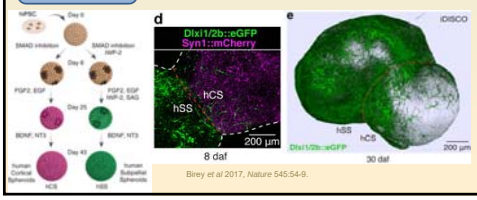
Brains in a Dish

Timothy Syndrome 3 patients



Lancaster *et al* 2013, *Nature* 501:10; http://sitn.hms.harvard.edu/flash/2013/brains-in-a-dish-establish-new-tool-for-developmental-neuroscience/



Birey *et al* 2017, *Nature* 545:54-9.

Cortical interneurons display cell-autonomous migration defect (move more frequently but less efficiently) in Timothy syndrome