Non-coding genes: promising targets for molecular biomarkers and disease modifying epilepsy treatments

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Topic 4: New targets for innovative diagnostics and treatment

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Pathogenesis of epilepsy

Treatment:
- AEDs (symptomatic only)
- Surgery
- Other (diet, brain stimulation)

Key goals:
- Better understanding of epileptogenesis
- Disease-modifying treatments
- Biomarkers of epileptogenesis

Neuronal death
Gliosis
Inflammation
Plasticity (axonal, dendritic)
Neurogenesis
Extracellular matrix
Metabolism

Animal trials
- Glutamate antagonists (excitability, neuroprotection)
- Neuropeptides (inhibition)
- Growth factors (various)
- Celecoxib (inflammation)
- Rapamycin (structure, metabolism)

Large-scale changes in gene expression

Epileptic seizures
[protein-coding] gene targets in epilepsy

Despite major insights and progress……. errors in protein-coding genes do not explain the majority of cases of epilepsy

Disappointing anti-epileptogenesis trials in animals

<table>
<thead>
<tr>
<th>Seizure modification</th>
<th>Decrease in frequency</th>
<th>Decrease in duration</th>
<th>Milder seizure type</th>
<th>Prevention of progressive increase in seizure frequency</th>
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<tbody>
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<td>Yes</td>
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Pitkanen & Lukasiuk  Lancet Neurol (2011)
Non-coding RNA – a new gene focus in epilepsy

The amount of non-coding RNA increases as a function of organism complexity

Taft et al. Bioessays 2007
Non-coding RNA – forms and functions

Long non-coding RNA (>200 nucleotides)

Largest class of ncRNA in the human genome (100,000+), interact with DNA, RNA & protein

Functions: Epigenetic (guiding methylation); transcriptional/translational interference

LncRNA functions
- Transcriptional and post-transcriptional regulation
- Genomic site-specific epigenetic reprogramming
- Long-range genomic interactions
- Scaffolds for nuclear macromolecular assemblies
- Maintenance of stoichiometry and molecular titration

Qureshi & Mehler, Nat Rev Neurosci 2012
LncRNA; brain enrichment, emerging functions

IncRNAs display specific subfield expression

IncRNAs regulate synapse formation

...and specific subcellular localization

...and seizure thresholds
Short non-coding RNA

Includes **microRNA** (miRNA) - small non-coding RNAs (~22 nt); ~1500 in humans

Function to fine-tune **protein output**

“meta” controllers of gene expression – single miRNA may target ~200 mRNAs

- Messenger RNA encodes protein
- MicroRNAs bind to matching parts of the messenger RNA, reducing protein production
Altered miRNA levels after *status epilepticus* and in epilepsy

Schratt et al. *Nat Rev Neurosci* 2009

Critical for excitatory signals

Schratt et al. *Nat Rev Neurosci* 2009
Targeting miRNAs

Locked nucleic acid-modified DNA antisense oligonucleotides

- Antagomirs bind to miRNAs, allowing protein production to resume

Antagomir

messenger RNA

protein

miR-134

UGUGACUGGGUGACACGACGGG

ACACTGACCAACTGCT

Chol-TEG

Veh / KA

Scr / Ant-134

Ventriple

Amygdala


Treatment of HCV Infection by Targeting MicroRNA


Silencing miR-134 reduces seizures

92% reduction in epileptic seizures

miRNAs and seizures

Increasing numbers of miRNAs linked to seizures and/or epilepsy pathology
miRs 21, 34a, 132, 138, 134, 146a, 155, 184…..

<table>
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<tr>
<th>Silenced miR</th>
<th>Brain role</th>
<th>EEG</th>
<th>Damage</th>
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<tbody>
<tr>
<td>miR-34a</td>
<td>apoptosis</td>
<td>—</td>
<td>✓</td>
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<tr>
<td>miR-132</td>
<td>spines</td>
<td>—</td>
<td>✓</td>
</tr>
<tr>
<td>miR-134</td>
<td>spines</td>
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<tr>
<td>miR-184</td>
<td>?</td>
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New ways to target miRNAs
Viral approaches?
Mesenchymal stem cell ADK-targeting miRNAs reduce seizures

Which are the best targets?
How can we “medicinize”?
– Are they safe?
– Administration routes
– Effects on established epilepsy?

Other future focuses on non-coding RNA in epilepsy

**miRNA as diagnostic biomarkers?**
- Stable in biofluids, pH and freeze-thaw resistant
- Detectable in multiple biofluids (blood, CSF)
- Signal-carrying paracrine functions
- Identify at risk patients, track epileptogenesis, support prognosis

**Brain and blood microRNA expression profiling of ischemic stroke, intracerebral hemorrhage, and kainate seizures**

MicroRNA Let-7i is a Promising Serum Biomarker for Blast-Induced Traumatic Brain Injury

**Non-coding RNA variation in human epilepsy?**
- Sequence variation in ncRNA including miRNA may function as a risk factor
Future

1. Non-coding RNA is a largely unknown contributor in epileptogenesis
   Which short and long ncRNAs are pathogenic vs adaptive/useful/not important?
   What is their mechanism? What controls their expression?

2. Genetics of non-coding RNA
   Do ncRNA variants have a role in explaining disease risk?

3. Sources of molecular biomarkers
   Are miRNAs disease biomarkers? Do different precipitating injuries elicit unique signatures?
   What is the best biofluid source and ncRNA “pool”?

4. Next generation of disease-modifying treatments?
   Are drugs acting on ncRNA safe for use in the CNS? How can they be delivered?
   Do AEDs or other drugs have any ncRNA-modifying effects?