Revealing the mechanisms of epileptogenesis to design innovative treatments – what are the tools?

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Perspectives for novel treatments

Two groups of epilepsy patients who are severely affected and deserve novel treatments urgently:
- patients with pharmacoresistant focal epilepsies (prototype with adequate animal models: temporal lobe epilepsy)
- patients with epileptic encephalopathies, severe epilepsies of childhood often with mental decline and other symptoms

Approaches to find novel therapies:
- understand epileptogenesis to design preventive/disease-modifying treatments
- design novel drug screening assays, two examples:
  - promoter screening of relevant genes
  - zebrafish models as screening tools
- use bioinformatics to identify novel targets
Epileptogenesis

- gene defect
- febrile seizures
  - event/trigger/condition
- stroke
- trauma
- infection
- epigenetic effects
  - inflammation
  - many unknown factors
  - epilepsy
Examples for antiepileptogenic / disease-modifying treatments

Temporal lobe (and other focal) epilepsies:
- in many animal models, knock-out or pharmacological manipulation of relevant targets is able to reverse epileptogenesis
- so far no clinical examples

Genetic epilepsies / epileptic encephalopathies:
- ketogenic diet can improve cognitive function and epilepsy in patients with glucose transporter type 1 defects
- stiripentol does not only treat seizures but seems to slow disease progression in Dravet syndrome
Inflammatory mechanisms in epileptogenesis

Molecules:
- Toll-like receptor 4
- High mobility group box 1
- Interleukins

Vezzani et al. Nat Rev Neurol 2011;7:31-40

Potential future antiepileptogenic therapy:
- anti-inflammatory agents (existing and newly developed drugs)
Epigenetic mechanisms in epileptogenesis

Potential targets:
- reelin promoter
- ion channel promoters
  - $K_{V4.2}$
  - HCN1
  - $Ca_{V3.2}$


Potential future antiepileptogenic therapy:
HDAC inhibitors (valproate, newly developed drugs)
Manipulation of gene promoters

Influencing epileptogenesis in a model of temporal lobe epilepsy:
- \( \text{Ca}_v3.2 \) calcium channels are upregulated during epileptogenesis (presumably via upregulation of a transcription factor: Egr1)
- epileptogenesis is largely reduced in \( \text{Ca}_v3.2 \) knockout mice
- finding new therapeutic strategies:
  - establish promoter-reporter assays for high-throughput screening to find compounds suppressing \( \text{Ca}_v3.2 \) expression as antiepileptogenic therapy
  - viral transfer of Egr1 suppressors

Potential future antiepileptogenic therapies:
\( \text{Ca}_v3.2 \) promoter manipulations by small molecules or viral transfer → transferable to other promoters
Example of successful causative therapy in a genetic epilepsy and movement disorder: glucose transporter type 1 defects (GLUT1)

video child

Weber et al., J Clin Invest 2008
Defective glucose transport across the blood-brain barrier – pathophysiology and therapy

Pathophysiology:
- energy deficit in the basal ganglia after physical exertion induces involuntary movements
- permanent frontal metabolic deficit induces seizures

Translation into an existing therapy
ketogenic diet: circumvent glucose as energy carrier
→ remission of seizures and episodic involuntary movements
→ dramatic improvement of cognitive function
Advantages of zebrafish as an *in vivo* drug discovery model

- Genetic, physiologic and pharmacologic homologies to humans
- High fecundity and small size
- Fast development ex utero
- Optical transparency
- Only µg amounts of compounds needed
- Compounds readily absorbed (skin, GI tract, gills)

courtesy of Alex Crawford and Camila Esguerra
Zebrafish seizure assay

control  pentylenetetrazol-treated

*courtesy of Alex Crawford and Camila Esguerra*
High-throughput, *in vivo* CNS assays in zebrafish

Rapid development of larvae allows not only to screen for anti-seizure but also for **antiepileptogenic** activity of small molecules:
- establish chemoconvulsant models with epileptogenic phase of few days
- establish genetic models with epileptogenic/(pre-)treatment phase

**Rapid knockdown of genes using antisense morpholino oligomers (MO)**

courtesy of Alex Crawford and Camila Esguerra
Use of bioinformatics to search for novel candidate genes / targets (example Parkinson’s disease)

- Meta-analysis of public transcriptomics data
- Pathway visualization & enrichment
- Integration with BrainAtlas data
- Identify joint gene deregulation in aging and PD
- Phenolog candidates & GWAS SNPs
- Combine evidence to prioritize candidate genes
- Network & Machine learning analysis
- Build and interpret combinatorial marker models

Courtesy of Rudi Balling and Reinhard Schneider
Roadmap to find new therapeutic strategies: identification - selection - validation of novel target candidates

understanding epilepsy
identification & selection of targets
development and application of target modulation strategies: viral transfer, knockdown, knockout, knockin

Systems biology / bioinformatic modeling:
Cellular and molecular pathways, junction points, metabolic maps

Specific changes in genetic and acquired epileptogenesis:
- Genomics
- Epigenomics
- Metabolomics
- Transcriptomics
- Proteomics
- Inflammation

high-throughput medium-/low-throughput

rodent models
hiPSCs
human tissue

Clinical trials
Conclusion

New tools in experimental research provide a fantastic chance to be translated into novel treatment options for people with epilepsy