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Proteomic profiling of epileptogenesis in a rat model: Focus on cell stress, extracellular matrix and angiogenesis

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ABSTRACT

Information about epileptogenesis-associated changes in protein expression patterns is of particular interest for future selection of target and biomarker candidates.

Bioinformatic analysis of proteomic data sets can increase our knowledge about molecular alterations characterizing the different phases of epilepsy development following an initial epileptogenic insult.

Here, we report findings from a focused analysis of proteomic data obtained for the hippocampus and parahippocampal cortex samples collected during the early post-insult phase, latency phase, and chronic phase of a rat model of epileptogenesis.

The study focused on proteins functionally associated with cell stress, cell death, extracellular matrix (ECM) remodeling, cell-ECM interaction, cell-cell interaction, angiogenesis, and blood-brain barrier function. The analysis revealed prominent pathway enrichment providing information about the complex expression alterations of the respective protein groups.

In the hippocampus, the number of differentially expressed proteins declined over time during the course of epileptogenesis. In contrast, a peak in the regulation of proteins linked with cell stress and death as well as ECM and cell-cell interaction became evident at later phases during epileptogenesis in the parahippocampal cortex. The data sets provide valuable information about the time course of protein expression patterns during epileptogenesis for a series of proteins. Moreover, the findings provide comprehensive novel information about expression alterations of proteins that have not been discussed yet in the context of epileptogenesis. These for instance include different members of the lamin protein family as well as the fermitin family member 2 (FERMT2). Induction of FERMT2 and other selected proteins, CD18 (ITGB2), CD44 and Nucleolin were confirmed by immunohistochemistry.

Taken together, focused bioinformatic analysis of the proteomic data sets completes our knowledge about molecular alterations linked with cell death and cellular plasticity during epileptogenesis. The analysis provided can guide future selection of target and biomarker candidates.

1. Introduction

Comprehensive cellular and network alterations characterize the process of epileptogenesis following an epileptogenic brain insult (Dichter, 2009; Pitkanen and Lukasiuk, 2011). Thereby, cell stress along with associated neuronal cell loss affecting different susceptible brain regions serve as an important trigger for subsequent remodeling of brain tissue and its cellular composition (Dingledine et al., 2014). Cellular plasticity is accompanied by intense molecular alterations in extracellular matrix (ECM) components, which in turn can play a crucial modulatory role following brain damage (Dityatev and Fellin, 2008; McRae and Porter, 2012; Pitkanen et al., 2014). Remodeling does not only involve neuronal and glial cells but also cells of the cerebrovascular system (Marchi and Lerner-Natoli, 2013). Enhanced

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angiogenesis and blood-brain barrier dysfunction are discussed as pro-epileptogenic factors contributing to the development of a hyper-excitable network (Marchi and Lerner-Natoli, 2013; Morin-Brureau et al., 2012).

From an overarching perspective, neuronal cell loss, ECM alterations and angiogenesis can be considered a linked triad of events, which can significantly contribute to the development of structural epilepsy. ECM remodeling can be a consequence of insult-associated neuronal damage as well as blood-brain barrier alterations with subsequent activation of astrocytes, which are a major source of components and modulators of the ECM (Soleman et al., 2013; B. R. Kim et al., 2016; S. Y. Kim et al.,2016). The other way round, various ECM molecules play an important role in the regulation of angiogenesis (Mongiat et al., 2016). In this context, we emphasize that targeting of both, ECM remodeling and angiogenesis, is discussed to reduce seizure susceptibility and epileptogenicity (B. R. Kim et al., 2016; S. Y. Kim et al.,2016; Pitkanen et al., 2014; Morin-Brureau et al., 2012).

Whereas some molecular alterations contributing to the respective cellular alterations have been studied in chronic epilepsy models, there is only limited information about the complex changes in the total protein expression patterns. Transcriptomic analysis in models with development of structural epilepsy provided information about the regulation of mRNAs associated with neuronal death, ECM modulation and angiogenesis (Hansen et al., 2014; Hunsberger et al., 2005; Okamoto et al., 2010). However, the findings from transcriptomic studies do not always translate into proteome alterations considering post-transcriptional and post-translational regulation mechanisms (Vogel and Marcotte, 2012).

As we have demonstrated previously, large-scale proteomic profiling can render comprehensive data sets with information about the temporal profile of proteome alterations during the course of epilepsy development (Keck et al., 2017), hypothesis-driven and focused analyses can in particular help to identify sets of proteins linked with a specific pathophysiological process (Keck et al., 2017; Walker et al., 2016). We capitalize on a previous proteomics profiling approach based on liquid chromatography tandem mass spectrometry (LC-MS/MS) using individual hippocampal (HC) and parahippocampal cortex (PHC) tissue samples from a rat post-status epilepticus model. The proteome profile confirmed on one hand previously reported proteins which are dysregulated during epileptogenesis, but also identified a series of interesting novel proteins associated with immunity and inflammation that proved to be co-regulated in different phases of epilepsy development (Walker et al., 2016).

Here, we now focused the bioinformatics analysis of the proteomics dataset on proteins and pathways linked with neuronal death, ECM changes and angiogenesis. Prominent and complex epileptogenesis-associated alterations were identified for all three-protein groups. The analysis provides valuable information about the time course of expression patterns for various protein subgroups and individual proteins. The findings received further confirmation by immunohistochemical demonstration of the hippocampal induction of FERMT2 and other selected proteins in the latency phase.

2. Materials and methods

2.1. Animals

The investigation has been approved by the responsible government (reference number 55.2154-2532-94-11) and has been conducted in compliance with the German Animal Welfare act and the EU directive 2010/63/EU. Experiments were performed in female Sprague Dawley rats (n = 59; Harlan Laboratories, now Envigo, Udine, Italy) at 10–11 weeks age (200–224 g) after at least one week of acclimation. Animals were housed with a constant light/dark cycle of 12/12 h (lights on at 7:00 a.m.–lights off at 7:00 p.m.), under controlled temperature (20–24 °C) and humidity (45–65%) conditions. Animals received nesting material and free access to standard food and tap water in their home cage. Every effort was made to avoid or reduce pain or discomfort and to minimize the number of animals used in the study.

2.2. Post-status epilepticus model

Rats underwent stereotactic implantation of the combined recording and stimulation electrode in the right anterior basolateral amygdala as previously described (Walker et al., 2016). At least six weeks after electrode implantation, a status epilepticus (SE) was induced as described by Ongerth et al. (2014). Only animals exhibiting a generalized SE (85%) were included in the subsequent experiments. Groups of five electrode-implanted control animals and five SE rats were euthanized by intraperitoneal pentobarbital injection (500 mg/kg; Narcoren, Sigma-Aldrich GmbH, Munich, Germany) two days, ten days, and eight weeks after SE induction. After removing the brain, hippocampus and PHC (containing the entorhinal, perirhinal und posterior-piriform cortex) were rapidly dissected on ice, for the PHC the ventrocaudal cortex was cut ventral to the rhinal fissure until approximately 5 mm posterior to bregma. Followed dissection the ipsilateral and contralateral tissue was pooled. Tissue samples were transferred to ice cold phosphate-buffered saline (PBS, pH 7.2). The subsequent mass spectrometry analysis was performed using tissue samples of the hippocampus and the parahippocampal cortex. Animals belonging to the group with tissue sampling eight weeks following SE induction underwent a continuous video- and EEG-monitoring (24 h per day/seven days a week over 19 days) starting 6 weeks following SE with a combined EEG- and video-detection system as previously described (Pekcec et al., 2008). Animals that did not exhibit spontaneous generalized seizures were excluded from further analysis.

2.3. Mass spectrometry, label-free quantification and protein identification

The dataset analyzed here was previously published (Walker et al., 2016). It was generated by label-free LC-MSMS-based proteomics as described (Walker et al., 2016). Briefly, after tissue extraction, proteolysis and LC-MSMS, MS spectra were used for quantification by the Progenesis LC-MS software (Version 2.5, Nonlinear Dynamics) for label-free quantification and MS/MS spectra were used for protein identification with Mascot as described ((Hauck et al., 2010; Hauck et al., 2012; Walker et al., 2016). For quantification, only unique peptides of an identified protein were used and the total cumulative normalized abundance was measured by summarizing the abundances of all peptides assigned to the respective protein. Further analysis was performed with proteins quantified with a minimum of two peptides. The relation of protein abundances between control and SE animals (fold change) was determined to measure differential expression. All proteins exhibiting a fold change ≥ 1.5 (up- or downregulated) with a corresponding p-value (ANOVA) < 0.05 were considered differentially expressed.

2.4. Pathway analysis

Pathway analysis was performed as described previously by Walker et al. (2016). Briefly, following LC-MS/MS, gene symbols for all quantified proteins were searched in the Ensemble database (http://www.ensembl.org/Rattus_norvegicus/; version 69; 32,971 sequences). If a rat genome annotation was not present, the human orthologue was used. Pathway enrichment analysis was performed using both a licensed (Genomatix Pathway System (GePS), Genomatix Software GmbH, Munich, Germany) and a publically available (ConsensusPathDB over-representation tool, Kamburov et al. (2011); Kamburov et al. (2009)) pathway tool for a combined pathway analysis.

The respective background lists for the three different time points consisted of the respective sets of total quantified hippocampal or parahippocampal proteins. Only enriched pathways linked with cell