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ABC-transporters as stem-cell markers in brain dysplasia/tumor epilepsies

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1. ABSTRACT

ABC-transporters prevent the access of antiepileptic drugs into brain parenchyma, which partly explains why seizures are frequently refractory to AEDs treatment. Overexpression of aABC-transporters and stem-cell markers including CD34, have been detected in malformations of cortical development (MCD) and brain tumors. ABC-transporters are constitutively expressed during maturation of normal progenitor stem-cells and cancer stem-cells. These abnormal/immature cells of MCD or brain tumors play an active role in the epileptogenesis but the precise nature of this phenomenon is unclear. Irrespective of their property in the pharmacoresistance, ABCB1-transporter P-glycoprotein also play a role in the membrane depolarization, suggesting that constitutive P-glycprotein overexpression in MCD and brain tumors could explain their epileptogeneic properties. MCD as wells as brain tumors arise from abnormal progenitor cells, where ABC-t together with others stem cell markers, could help to better identification of these abnormal progenitor cells and serve as biomarker of risk for seizure relapse after epilepsy surgery.

2. INTRODUCTION

Epilepsy is a common neurologic disorder, affecting over 60 million people worldwide, putting it among the most prevalent of neurological disorders (1-2% of the general population), and the highest incidence occurs in children under the age of 5 and in the elderly (1,2). Antiepileptic drugs (AEDs) are the first treatment of choice for patients with epilepsy, and although regular treatment with AEDs has been found to be useful for controlling seizures in many patients, ~35% of epileptic patients, remains having seizures that are resistant to AEDs, and they are consequently considered as presenting with "Refractory Epilepsy" (RE) (3). These patients failure to stop seizures after use of several and simultaneous AEDs, even at maximum tolerated doses administrated, and their chances of controlling seizures after a second (or more) AED administrated are really low (4,5).

Brain overexprtession of ABC-transporters as P-glycoprotein (P-gp), multidrug-resistance-associated proteins (MRPs) and breast cancer resistant protein (BCRP)

are directly related with the refractoriness in epilepsy, affecting AEDs pharmacokinetics and drug-interactions (6).

Epileptic syndromes are chronic neurological disorders, characterized by different forms of seizures, where prolonged and/or repetitive crisis may result in development of refractory epilepsy, and also neuronal death by apoptotic (programmed or 'active') or necrotic pathways. Gliosis and microglial activation inducing also not only histological changes but, alteration at the subcellular, synaptic and molecular levels are also observed. Several forms of brain damages (seizures, trauma, ischemia or hypoglycemia) can produces common alterations named as "dark neurons", whose mode of death is neither necrosis nor apoptosis, and these particular type of cells detected after injury, are capable of recovery (7), similarly to described at penumbra areas in stroke (8). On this area, active interventions would help to recovery of these particular modified-cells in transient-state between live and death, and repair the damaged tissue including the recovery of their normal function (9).

Interestingly, neuropathological alterations secondary to repetitive seizures, may be adaptive and reversible while others are permanent, and in others cases, similar brain alterations are the cause of convulsions playing a role in the epileptogenesis (10), a phenomenon in which various kinds of brain insults (e.g., traumatic brain injury, ischemia, infection, prolonged or repetitive seizures, etc.) trigger a cascade of events that eventually culminate in the occurrence of spontaneous seizures.

By definition, epileptogenesis refers to the biological processes that progressively alters neuronal excitability, establishes critical interconnections, perhaps requiring intricate structural changes developed during a not clearly defined silent period, between the initial insult and the occurrence of the first spontaneous seizure (11).

Epiletogenesis is also often associated with the development of symptomatic epilepsy related with an identifiable structural lesion in the brain; however it is also occurs in genetic epilepsies, in which failures in developmental programming of gene expression leading to abnormal neuronal discharge, aberrant circuitry during maturation, as well as abnormal process of neuronal migration and/or differentiation (12).

Effectively, epilepsy is a common direct consequence in many neurological diseases caused by a wide range of genetic factors as are seen in a long list of inherited epileptic channelopathies and others neurotransmitters receptors, as well as a common feature of metabolic diseases, where epileptogenesis is directly related with specific altered cellular and metabolic functions (13).

However, other cellular or tissue alterations would also explain the epileptogenesis. One approach to reveal potential novel epileptogenic mechanisms, is to understand why a mutation in a disease-causing gene or several insults without specific genetics alteration on ligands or voltage-gated ion channels that regulate neuronal excitability, are also associated with seizures or epilepsy, as observed in several brain tumors or brain developmental malformations.

As example, the cortical tubers from Tuberous Sclerosis Complex (TSC) are perhaps the most convincing evidence to support the idea of searching for novel epileptogenic mechanisms produced during the development of a disease, in which epilepsy is "just comorbidity". Recently, in an experimental genetic inactivation of TSC-1 gene (hamatin), it was demonstrated an induced hyperactivation of the mTOR pathway in the dorsal telencephalic neuroepithelium, that interfered with proper CNS development resulting in postnatal megalencephaly, cortical anomalies and epilepsy (14).

This altered mTOR pathway also could be present in other epileptic cortical malformations as cortical dysplasia Type IIB (CD-IIB), that share histopathologic features and the refractory epilepsy phenotype with cortical tubers, as well as in subependimal giant astrocytomas (SEGA) tumor cells from TSC, where all these characteristics would suggest the potential presence of similar epileptogenic mechanisms.

In a recent study, the morphological and electrophysiological properties of cortical cells in tissue from pediatric TSC (n=20) and CD-IIB (n=20) patients using whole cell patch clamp recordings and biocytin staining were compared (15). Cell morphology and membrane properties were similar in TSC and CD-IIB cases. Except for giant/balloon and intermediate cells, all neuronal cell types fired action potentials and displayed spontaneous postsynaptic currents. However, the frequency of spontaneous glutamatergic postsynaptic currents in normal pyramidal neurons and interneurons was significantly lower in CD-IIB compared with TSC cases and the GABAergic activity was higher in all neuronal cell types in CD-IIB. Further, acutely dissociated pyramidal neurons displayed higher sensitivity to exogenous application of GABA in CD-IIB compared with TSC cases. These results indicate that, in spite of similar histopathologic features and basic cell membrane properties, TSC and CD-IIB display differences in the topography of abnormal cells, excitatory and inhibitory synaptic network properties, and GABAA receptor sensitivity. The authors suggest that the mechanisms of epileptogenesis could differ in patients with TSC and CD-IIB (15).

The described differences do not explain the similar severity of seizures and the refractoriness of these epileptic syndromes. It is clear that epileprogenesis is not synonymous of pharmacoresistance, however, we suspect that some mechanisms directly related with both epileptogenesis and pharmacoresistance should be common between both diseases, such as the case of the high expression of multidrug resistant proteins, particularly P-glycoprotein (P-gp).

Despite all advances to treat cancer in the periphery, any progress has been made in the therapy of

brain cancer and the CNS diseases, where access of chemotherapy is restricted. Several ABC-transporters including P-gp, MRPs and BCRP, are highly expressed in brain tumors, in the tumor-supplying vasculature, and in the parenchymal tissue surrounding the tumor limiting the drugs access, and as example, at date, the diagnosis of glioblastoma is still a death sentence.

Primary brain tumors can also be associated with epilepsy, causing typically focal and secondary generalized seizures in 20–45% of patients. In all these cases, seizures are also refractory to antiepileptic drug treatment probably secondary to the mentioned high expression of the ABC-transporters, but the nature of epileptogenesis which is suggested as multifactorial in all of them, remains unclear. One proposed mechanism of epileptogenesis is the different tumor types with changes in the properties of tumor´s cell-membranes, which could generate action potentials and thus affect neuronal excitability (16). Other studies have also identified aberrant expression of glutamatergic neurotransmitter receptors in dysplastic neurons, and intracerebral electrodes implanted to localize seizure onset, identified early ictogenic discharges from cells of gangliogliomas (17,18).

Taking together, these observations could be according with a differential role of P-glycorotein related with a direct effect on membrane depolarization, described initially in cultured tumor cells (19). Such property would support a hyperexcitable neuronal-tumor component functionally integrated into excitatory circuitries. In bases of the mentioned data, more recently was experimentally demonstrated how after repetitive seizures, neuronal overexpression of P-glycoprotein contributes to cell-membrane depolarization of hippocampus and neocortex, and this particular P-gp activity, was suggested as a potential novel mechanism of epileptogenesis (20).

3. BRAIN TUMORS AND MALFORMATIONS OF CORTICAL DEVELOPMENT

Several malformations of genetically determined cortical development have been associated with epileptic syndromes and/or developmental delay, and the main brain abnormalities identified are heterotopic gray matter, polymicrogyria, agyria-pachygyria, schizencephaly, hemimegalencephaly, transmantle dysplasia, focal cortical dysplasia, or cortical tubers, and all of them are characterized by phamacoresistant phenotype (21).

Tuberous sclerosis is a multisystemic disorder caused by an inactivating mutation, in either the TSC1 or TSC2 genes, which encodes hamartin and tuberin, respectively, affecting some organs including the central nervous system. The proteins encoded by these two genes form a tumor-suppressor complex acting through the Ras homologue enriched in brain protein (RHEB) to limit activation of the mammalian target of rapamycin (mTOR) complex 1. When either TSC1 or TSC2 is deficient, mTOR complex 1 is constitutively upregulated, leading to abnormal cellular growth, proliferation, and protein synthesis (22,23).

The most common brain lesions described as cortical tubers are important features of the disease where abnormal cells are the hallmark. Cortical tubers are frequently epileptogenic and refractory to antiepileptic drugs treatment and patients could be considered for surgical treatment of their refractory epilepsy (24,25).

Subependymal giant cell astrocytoma (SEGA) observed in 10% to 20% of patients with tuberous sclerosis complex (TSC), is a benign and slowly growing tumor also associated with seizures (26,27). In spite that clinically, SEGA is frequently characterized by increased intracranial pressure after misdiagnosed tumor growth, or intratumorous haemorrhage and high risk of death (28), some reports describe SEGA presenting only with worsening of seizures and developing refractory epilepsy (29).

Epilepsy is common in patients with brain tumors and can substantially affect daily life, even if the tumor is under control. In all cases, prophylactic use of antiepileptic drugs is not recommended, and potential interactions between antiepileptic and chemotherapeutic agents persuades against the use of enzyme-inducing antiepileptic drugs. Furthermore, multidrug-ABC-transporters expressed at blood brain barrier, brain tumor cells and tumor-surrounding cells, prevent the access of antiepileptic drugs into brain parenchyma, which partly explains why seizures are frequently refractory to treatment. Slow-growing tumours (ie, mainly low-grade gliomas) are the most epileptogenic (30,31), and several ABC-transporters are overexpressed in these tumors and in the two major causes of medically intractable epilepsy as focal cortical dysplasia and glioneuronal tumors (32).

4. ABC-TRANSPORTERS AND STEM-CELL MARKERS IN MCD AND BRAIN TUMORS

Early reports indicates that P-glycoprotein and others ABC-transporters, are over-expressed in malformations of cortical development and brain cortical tubers explaining the refractory epilepsy phenotype (33-36), as well as in SEGA, where ABC-transporters and CD34 stem cell markers were also detected (37). Similarly, co-expression of CD34 and neurofilament were described in epilepsy-associated glioneuronal tumors (38). Interestingly, the products of genes ABCB1 and ABCG2, corresponding to ABC-transporters P-glycoprotein and BCRP respectively, can be transiently expressed as markers of CD34-stem-cells or in earlier progenitor cell (39,40).

In normal brain, CD34 occurs only transiently during neurulation (41) and recently CD34 immunoreactivity was also detected as a subpopulation of balloon cells confined to the white matter but not observed in neocortical layers from brain specimens of Taylor's focal cortical dysplasia (FCD) (42). Balloon cells from FCD or tuberous sclerosis, can be ambiguous in their staining patterns, expressing markers of both immature and mature glial cells as well as neuronal markers, indicating interference with differentiation (43,44). Such is the case of FCDs that constitute the major class of cortical

malformations associated with intractable epilepsies, and most balloon cells retain "immature" cell markers such as vimentin, CD34 and nestin (45).

Cortical dysplasia often coexists with glioneuronal tumors in 40–80% of cases and in FCD, differential expression of early progenitor cell marker expression can be observed, that could distinguishes Type II from Type I FCD (46). Furthermore, cortical tubers that are frequently epileptogenic and refractory to antiepileptic drugs treatment, also express embryonic neuronal markers (47).

Tissues from therapeutic resections of several refractory epilepsies, as dysembryoplastic neuroepithelial tumors (DNT), FCD and hippocampal sclerosis (HS), as well as several brain tumors as glial and glioneuronal tumors, are associated with pharmacoresistant epilepsy, having a much poorer pharmacological outcome compared to other symptomatic epilepsies (48). In all of them, an overexpression of ABC-transporters as P-glycoprotein, MRPs and BCRP were described (49-51). Furthermore, markers for immature cell types as Otx1, Pax6 and Tbr2, were noted in varied of pathologies related with cortical dysplasias, and activation of progenitor cell populations which could contribute to the pathophysiology of these lesions, was suggested (52).

All these observations suggests that MDR-proteins, and others stem-cells markers detected in abnormal cell from epileptogenic brain areas, could build a new biomarkers profile to give predictive information for long-term follow-up, after surgical treatments of refractory epilepsy and/or related with the risk of relapse of seizures, even before that a new brain structural anomaly can be detected.

5. STEM-CELLS AND EPILEPSY

Tumor itself may be the seizure focus, or the tumor may cause secondary several perilesional tissue alterations, thereby triggering seizure activity. Tumor-associated seizures that typically manifest as focal crisis with secondary generalization are commonly refractory not only to classic chemotherapy, but also to antiepileptic drug treatment (53), suggesting that some common mechanism should be in between multidrug resistance in cancer and drug-resistant epilepsies.

Cells with self-renewal capacity and the ability to generate all differentiated lineages (multipotentiality) are defined as Stem Cells (SCs), and tissue-specific stem cells have been identified in multiple embryonic and adult organs, as well as specific cancer stem cells (CSCs).

Both SCs and CSCs are also described have resistance to apoptosis, a feature also related with multidrug resistance to conventional cancer chemotherapy (54), due to overexpression of MDR-proteins (55). Furthermore, a hypothesis indicating that CSCs that have similar properties to normal SCs, was first described by Rudolf Virchow and Julius Conheim in the 19th century, suggesting that cancer arises from activation of "dormant cells" presents in normal mature tissue, which are remainders of embryonic cells (56).

According with these concepts, is was reported that ABC-transporters are transiently expressed at high levels in human neural stem/progenitor cells (hNSPCs) but are downregulated in differentiated hNSPCs, and in normal conditions, they are expressed in a highly regulated manner, with the highest expression in primitive cells and subsequent downregulation following commitment to differentiation (57). These observations are in according with the main feature of non-fully mature cells as hallmark of MCD where persistent expression of ABC-transporters has been reported (51). Furthermore, more recently it was experimentally demonstrated in mice that the constitutive deficiency of ABC-transporters leads to distinct impairments in neural stem/progenitor cells maturation and adult neurogenesis *in vivo*, indicating a functional role of ABC-transporters in stem cell maintenance and differentiation (58).

All these data clearly suggests that presence of ABC-transporters, in abnormal and immature brain cells as well as in immortalized tumor cells, are clearly a hallmark of a non-fully differentiated progenitor cell.

Additionally to the properties described, a natural special tissue niche is necessary, to regulate the differentiation and proliferation of SCs, and some special neighboring types of cells as macrophages or immunocompetent cells are also needed. These specific areas observed in different organs, as crypts in the gut, bone marrow or hippocampus in the brain, may act as selective niches of different SCs for each specific tissue. After particular stimulations, SCs participate in several processes as tissue growth and differentiation during development, normal cell turnover of adult tissues, and the repair of damaged organs including CNS (59). So, SCs or CSC may remain inside their niche in "silent-state", waiting the activation by stress (seizures included) or specific growth factors and cytokines, as well as start again their proliferation after time-programmed of latency.

Perhaps, this particular cell behavior, could explain the seizures relapse observed in a several refractory epilepsy patients, long-time after epilepsy surgery. According with these descriptions, epileptogenesis is often divided into three stages: the acute event (the triggering insult or initial seizure), a latent period clinically silent, and later spontaneous seizures. Perhaps in this process, some particular type of cells are stem cells or could be acting as such, and after a silent long period of latency, these cells could return to be actives developing the relapse of epilepsy. If it is the case, obviously, the primary defect of the genetic abnormality on these cells, is not modified by the epilepsy surgery (Figure 1).

6. SEIZURE RELAPSE AFTER EPILEPSY SURGERY

Remaining those procedures on cancer, epilepsy surgery has been established as an effective treatment option in pharmacoresistant epilepsies (60,61). However, in one study of long-term outcome in 325 people having

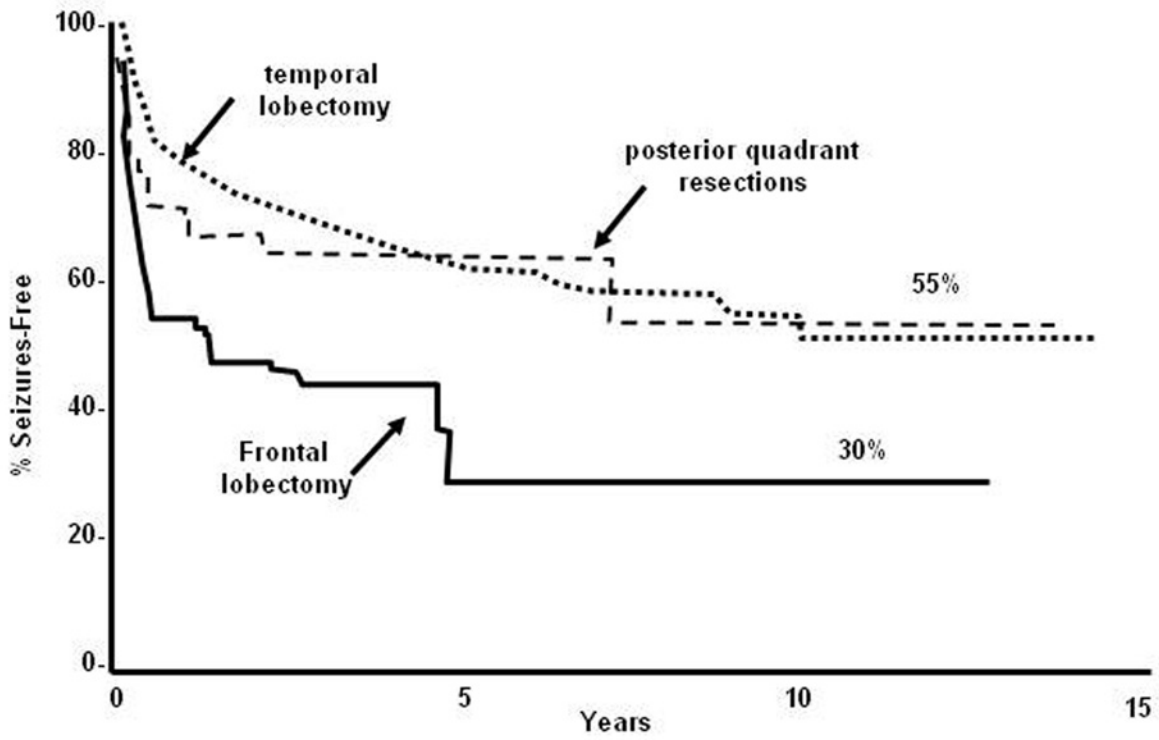

Figure 1. Normal Stem-cells proliferation, migration and differentiation to reach an also normal location as specialized mature neurons vs abnormal stem-cells or microenviroment, inducing persistence of depolarized membranes of these non-differentiated cells associated with an aberrant location and epileptogenesis**.**

anterior temporal resection, the rate of seizure freedom was 41% at 10 years. Patients who were seizure free 2 years postoperatively had a 74% probability of seizure freedom by 10 postoperative years. Late recurrence after initial seizure freedom was not uncommon, and risk factors associated with such recurrence are unknown (62).

More recently, the long-term outcome of surgery for epilepsy in 615 adults, indicated that although most patients showed a substantial reduction in seizures, only 40% entered long-term remission by virtue of having no seizures from time of surgery, and only 28% of those who were seizure free at last follow-up had discontinued antiepileptic drugs and could therefore be regarded as being cured (63). Similar results were also described by other authors (64,65).

For patients with discrete lesions associated with high seizure rates, such as cavernous malformations and dysembryoplastic neuroepithelial tumors, patients seizure-free were only 40 to 45 percent after 10-year, and at date, the available evidence demonstrates that long-term seizure freedom is achieved in about 60% of patients who undergo temporal lobe surgery, and in 30–40% of those who undergo extratemporal surgery (66) (Figure 2)

All these results indicate that in spite of the success obtained at early follow-up after epilepsy surgery, relapse of seizures remains to be a problem at long-term, and new biomarkers as well as further improvements in pre-surgical assessment and surgical treatment of people with chronic epilepsy, are needed (67).

7. STEM-CELL THERAPY AND/OR NEUROTROPHIC FACTORS AS INDUCERS OF STEM-CELS

In this regards it is known that in cerebral cortex, two main classes of neurons exist, the pyramidal cells with glutamatergic projection, and the GABAergic interneurons containing gamma-aminobutyric acid that only represent ~25% of the neurons in the cortex. However, they play an important role in the regulation of cortical function. These interneurons modulate and synchronize the activity of pyramidal cells, which is critical for normal cortical functioning. Multiple lines of evidence directly implicate cortical interneuron dysfunction in several neurological and psychiatric illnesses, including, autism, schizophrenia and epilepsy (68). Thus far, efforts to differentiate human pluripotent stem cells to cortical neurons have primarily focused on generating pyramidal cells (69). Instead, generation of specific functionally mature cortical interneurons using methods that recapitulate the normal development of this important population of neurons, have been recently obtained from human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) (70,71). According with this reports, cell transplantation strategies have received significant attention as an alternative therapy for temporal lobe epilepsy (TLE) in pre-clinical studies (72,73).

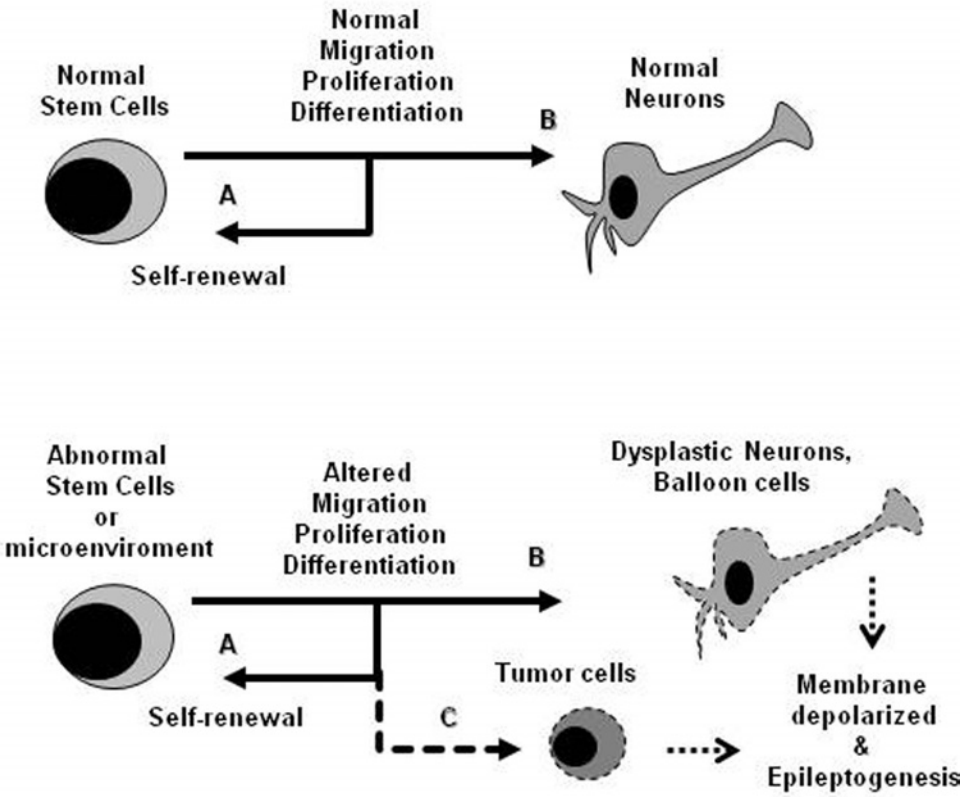

Figure 2. Percentage of seizures-free patients at long time follow-up after epilepsy surgery. Modified from reference (65).

Particularly neural stem cells (NSCs) are one of the donor cell candidates considered for grafting in the domain of cell-based therapy for TLE because these cells can be expanded in culture for extended periods from diverse sources such as the fetal, postnatal and adult brain, human embryonic stem (ES) cells, and human induced pluripotent stem (iPS) cells suggested to treatment of Dravet syndrome (74,75). Several evidences indicate that therapeutic benefits can be obtained by transplanting fetal GABAergic progenitors into the dentate gyrus in rodents with TLE. Recent progress in cell transplantation using the TLE model were well described (76) and, fetal hippocampal cell grafts, neuronal precursor cells from medial ganglionic eminence, and other neural stem cells (NSCs) have been tested as efficacious transplantable cells able to replace damaged hippocampal neurons in epilepsy models (77,78).

Prior to transplantation, perhaps we must to know the optimal degree of differentiation of a somatic stem cell to be used for a particular disease, and not let to free evolution *in situ* of pluripotent stem cells. So, implanting cells pre-committed *ex vivo* to yield a uniform mature neural cell-type as motor neurons or oligodendrocytes, would certainly maximize not only the number, but also their ability to engraft, their properties to respond to varying environmental cues, to migrate, to integrate networks, as well as to provide other needed neural cell types. Consequently, specifics biomarkers are needed to better selections and stimulation of these progenitor cells.

A recent but non-epileptic study, demonstrated that stem/progenitor cells positive for c-kit increased the expression level of glutamate transporter GLT-1 in the surrounding astrocytes that produced a subsequent lowering of extracellular glutamate level reducing cell damage to surrounding tissue (79). In this context, stem cell therapy for epilepsy, may supplement the damaged brain with pro-survival growth factors inducing a subsequent improve the host microenvironmental niche; an important interest could also have the recent development of use of pluripotent stem cells for neurodevelopmental disorders (80,81).

A highly efficient method to induce human pluripotent stem cells (iPS) into primitive neural stem cells in 7 days was reported, and subsequently, these cells achieved the differentiation of neural stem cells into region-specific neuronal subtypes, such as GABAergic, dopaminergic, and motor neurons.

In this regards, the use of neuroprotective agents, as many neurotrophic factors could have therapeutic effects against brain damages observed in several epileptic syndromes. In this regards, direct administration insulin-like growth factor 1 exerts neuroprotective and anti-inflammatory effects on KA-induced animal models of TLE which coincided with improved cognitive function (82). Or the mammalian target of rapamycin (mTOR) signaling pathway, which led to a novel pharmacological modulation of epileptogenesis by use of rapamycin (83), not only for tuberous sclerosis complex but also for

acquired epilepsy, as experimentally demonstrated (84,85). Rapamycine, inhibits mTOR complex 1, correcting the specific molecular defect causing the tuberous sclerosis complex. Several case reports suggest that mTOR inhibition leads to shrinkage or stabilization of renal angiomyolipomas, lymphangioleiomyomatosis facial angiofibromas, and subependymal giantcell astrocytoma (86).

Interestingly, in an experimental model of kainite induced status epilepticus, the mTOR inhibitor rapamycin, administered before kainate, blocked both the acute and chronic phases of seizure-induced mTOR activation and decreased kainate-induced neuronal cell death, neurogenesis, mossy fiber sprouting, and the development of spontaneous epilepsy. Late rapamycin treatment, after termination of status epilepticus, blocked the chronic phase of mTOR activation and reduced mossy fiber sprouting and epilepsy but not neurogenesis or neuronal death. These findings indicate that mTOR signaling mediates mechanisms of epileptogenesis in the kainate rat model and that mTOR inhibitors have potential antiepileptogenic effects in this model (87). Furthermore, several glioneuronal tumors have expressed the Pi3K-mTOR signaling, that could be inhibited by rapamycine (88). Molecular-biological studies identified the insulin-growth factor receptor cascade to be involved in the pathogenesis of FCD type IIB, and have indicated accumulation of distinct allelic variants of TSC1 also in FCDIIB suggesting that TSC1 represents a key factor in the phosphatidylinositol 3-kinase (PI3K) signaling (89), a pathway directly related with mTOR activity, that also could be inhibited by rapamycine. According with the potential role of P-gp en epileptogenesis described above and these mentioned data on rapamycne, recent experimental study demonstrated that P-glycoprotein can be induced by mTOR pathway activation (90).

8. SUMMARY AND PERSPECTIVE

Several observations described here, suggests that ABC-transporters, as P-glycoprotein and BCRP, could be interpreted as stem-cell markers presents in several brain cortical malformations, being constitutive components of immature not-fully differentiated cells, as observed in dysplastic neurons and ballooned cells or brain tumor cells. Interestingly, all these abnormal cells play a role in the epileptogenesis, have high expression of ABC-transporters and are also refractory to AEDs.

Irrespective to the properties of ABC-transporters related with pharmacoresistance, the functional effects of P-pg on membrane depolarization associated to epileptogenesis, and its constitutive expression in non-differentiated cell, should be more investigated in brain specimens from this epileptic syndromes surgically treated.

So, ABC-transporters and other stem-cell markers, if they are presents in those mentioned abnormal cells, could contribute to build a risk score or prognostic profile for long-time seizures relapse. Under this conditions, new therapeutics tools as iSC and/or neurotrophic factors or rapamycin, could improve the clinical evolution of these severe refractory epilepsy syndromes, avoiding the seizures relapse or the reiterative use of surgical procedures.

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10. REFERENCES

1. National Institutes of Health. Consensus conference: Surgery for epilepsy. *JAMA*;264:729-33(1990)

2. Theodore, W.H., S.S. Spencer, S. Wiebe, J.T. Langfitt, A. Ali, P.O. Shafer, A.T. Berg and B.G. Vickrey. Epilepsy in North America: a report prepared under the auspices of the global campaign against epilepsy, the International Bureau for Epilepsy, the International League Against Epilepsy, and the World Health Organization. *Epilepsia* 47:1700–1722 (2006)

3. Berg, A.T. Identification of pharmacoresistant epilepsy. *Neurol Clin* 27:1003–13 (2009)

4. Kwan, P. and J.W. Sander. The natural history of epilepsy: an epidemiological view. J Neurol Neurosurg Psychiatry 75:1376–1381(2004)

5. Kwan P, Brodie MJ. Early identification of refractory epilepsy. *New Engl J Med* 342:314–319 (2000)

6. Lazarowski, A. and L. Czornyj. Potential role of multidrug resistant proteins in refractory epilepsy and antiepileptic drugs interactions. *Drug Metab Drug Interact* 26:21–26 (2011)

7. Gallyasa, F., V. Kiglicsb, P. Baracskayb, G. Juhászb and A. Czurkób. The mode of death of epilepsy-induced "dark" neurons is neither necrosis nor apoptosis: An electron-microscopic study. *Brain Res* 1239:207–215(2008)

8. del Zoppo, G.J. Focus on research stroke and neurovascular protection. *N Engl J Med* 6:553–555(2006)

9. Merelli, A., L. Czornyj and A. Lazarowski. Erythropoietin: a neuroprotective agent in cerebral hypoxia, neurodegeneration, and epilepsy. *Curr Pharm Des* 19:6791-801(2013)

10. Blanc, F., L. Martinian, I. Liagkouras, C. Catarino, S.M. Sisodiya and M. Thom. Investigation of widespread neocortical pathology associated with hippocampal sclerosis in epilepsy: a postmortem study. Epilepsia 52(1):10-21(2011)

11. Di Maio, R. Neuronal mechanisms of epileptogenesis. Front Cell Neurosci.;8:29 (2014)

12. Pitkänen, A. and K. Lukasiuk. Mechanisms of epileptogenesis and potential treatment targets. *Lancet Neurol* 10:173–86 (2011)

13. Lazarowski, A. and L. Czornyj. "Genetics of Epilepsy and Refractory Epilepsy". Colloquium Series on: *The Genetic Basis of Human Disease*. Editor: Michael Dean, Ph.D. Copyright © by Morgan & Claypool Life Sciences (2013)

14. Magri, L., M. Cambiaghi, M. Cominelli, C. Alfaro-Cervello, M. Cursi, M. Pala, A. Bulfone, J.M. García-Verdugo, L. Leocani, F. Minicucci, P.L. Poliani and R. Galli. Sustained activation of mTOR pathway in embryonic neural stem cells leads to development of tuberous sclerosis complex-associated lesions. *Cell Stem Cell* 9:447–462 (2011)

15. Cepeda, C., V.M. André, J.S. Hauptman, I. Yamazaki, M.N. Huynh, J.W. Chang, J.Y. Chen, R.S. Fisher, H.V. Vinters, M.S. Levine and G.W. Mathern. Enhanced GABAergic network and receptor function in pediatric cortical dysplasia Type IIB compared with Tuberous Sclerosis Complex. *Neurobiol Dis* 45:310–321(2012)

16. van Breemen, M., E.B. Wilms and C.J. Vecht. Epilepsy in patients with brain tumours: epidemiology, mechanisms, and management. *Lancet Neurol* 6: 421–30 (2007)

17. Ferrier, C.H., E. Aronica, F.S. Leijten, W.G. Spliet, A.C. van Huffelen, P.C. van Rijen, and C.D. Binnie. Electrocorticographic discharge patterns in glioneuronal tumors and focal cortical dysplasia. *Epilepsia* 47:1477–86 (2006)

18. Blümcke, I. Neuropathology of focal epilepsies: A critical review. *Epilepsy Behav* 15:34–39 (2009)

19. Wadkins, R.M. and P.D. Roepe. Biophysical aspect of P-glycoprotein mediated multidrug resistance. *Int Rev Cytol* 171: 121-65 (1997)

20. Auzmendi, J.A., S. Orozco-Suárez, I. Bañuelos-Cabrera, M.E. González-Trujano, E.C. González, L. Rocha and A. Lazarowski. P-glycoprotein contributes to cell membrane depolarization of hippocampus and neocortex in a model of repetitive seizures induced by pentylenetetrazole in rats. *Current Pharm Desing* 19,38:6732–6738 **(**2013)

21. Brodtkorb, E., G. Nilsen, O. Smevik and PA Rinck. Epilepsy and anomalies of neuronal migration: MRI and clinical aspects. *Acta Neurol Scand* 86:24–32(1992)

22. Huang, J. and B.D. Manning. The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. *Biochem J* 412:179-90 (2008)

23. Chan, J.A., H. Zhang, P.S. Roberts, S. Jozwiak, G. Wieslawa, J. Lewin-Kowalik, K. Kotulska, and D.J. Kwiatkowski. Pathogenesis of tuberous sclerosis subependymal giant cell astrocytomas: biallelic inactivation of TSC1 or TSC2 leads to mTOR activation. *J Neuropathol Exp Neurol* 63:1236-42 (2004)

24. Avellino, A.M., M.S. Berger, R.C. Rostomily, C.M. Shaw and G.A. Ojemann. Surgical management and seizure outcome in patients with tuberous sclerosis. *J Neurosurg* 87:391-6 (1997)

25. Crino, P.B., H. Miyata and H.V. Vinters. Neurodevelopmental disorders as a cause of seizures: Neuropathologic, genetic, and mechanistic considerations. *Brain Pathol* 12:212-33 (2002)

26. Louis, D.N., H. Ohgaki, O.D. Wiestler, W.K. Cavenee, P.C. Burger, A. Jouvet, B.W. Scheithauer, P. Kleihues. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 114:97-109 (2007) Review.

27. Kim, S.K., K.C. Wang, B.K. Cho, H.W. Jung, Y.J. Lee, Y.S. Chung, J.Y. Lee, S.H. Park, Y.M. Kim, G. Choe and J.G. Chi. Biological behavior and tumorigenesis of subependymal giant cell astrocytomas. *J Neurooncol* 52:217–225(2001)

28. Frèrebeau, P., J. Benezech, F. Segnarbieux, H. Harbi, A. Desy and C. Marty-Double. Intraventricular tumors in tuberous sclerosis. *Childs Nerv Syst* 1:45–8 (1985)

29. Shepherd, C.W., B. Scheithauer, and M.R. Gomez. Brain tumors in tuberous sclerosis: a clinicopathologic study of the Mayo clinic experience. *Ann N Y Acad Sci* 615:378–9 (1991)

30. Riva, M. Brain tumoral epilepsy: a review. *Neurol Sci* 26(suppl 1):S40–S42 (2005)

31. Calatozzolo, C, M. Gelati, E. Ciusani, F.L. Sciacca, B. Pollo, L. Cajola, C. Marras, A. Silvani, L. Vitellaro-Zuccarello, D. Croci, A. Boiardi and A. Salmaggi. Expression of drug resistance proteins Pgp, MRP1, MRP3, MRP5 and GST-pi in human glioma. *J Neurooncol* 74:113–21 (2005)

32. Aronica, E., J.A. Gorter, G.H. Jansen, C.W. van Veelen, P.C. van Rijen, S. Leenstra, M. Ramkema, G.L. Scheffer, R.J. Scheper and D. Troost. Expression and cellular distribution of multidrug transporter proteins in two major causes of medically intractable epilepsy: focal cortical dysplasia and glioneuronal tumors. *Neuroscience* 118:417–29 (2003)

33. Tishler, D., K. Weinberg, D. Hinton, N. Barbaro, A. Geralyn and R. Corey. MDR1 gene expression in brain of patient with medically intractable epilepsy. *Epilepsia* 36:1-6 (1995)

34. Sisodiya, S.M., J. Heffernan and M.V. Squier. Over-expression of P-glycoprotein in malformations of cortical development. *Neuroreport* 10(16):3437-41 (1999)

35. Lazarowski, A., G. Sevlever, A.L. Taratuto and M. Massaro, A. Rabinowicz. Tuberous sclerosis associated with MDR1 gene expression and drug-resistant epilepsy. *Pediatr Neurol* 21(4):731-4 (1999)

37. Lazarowski, A., F. Lubieniecki, S. Camarero, H. Pomata, M. Bartuluchi, G. Sevlever and A.L. Taratuto,

Multidrug resistance proteins in tuberous sclerosis and refractory epilepsy. *Pediatr Neurol* 30:102-106 (2004)

37. Lazarowski, A, F. Lubieniecki, S. Camarero, V.Cuccia and A.L.Taratuto. Stem-cell marker CD34, multidrug resistance proteins P-gp and BCRP in SEGA. *Receptor Clin Invest 1, Ahead of prin* (2014)

38. Fassunke, J., M. Majores, C. Ullmann, Ch.E. Elger, J. Schramm, O.D. Wiestler and A.J. Becker. *In situ*-RT and immunolaser microdissection for mRNA analysis of individual cells isolated from epilepsy-associated glioneuronal tumors. *Lab Invest* 84,1520–1525 (2004)

39. Goodell, M.A., K. Brose, G. Paradis, A.S. Conner and R.C. Mulligan. Isolation and functional properties of murine hematopoietic stem cells that are replicating *in vivo*. *J Exp Med* 183:1797-1806 (1996)

40. Bunting, K.D. ABC Transporters as Phenotypic Markers and Functional Regulators of Stem Cells. *Stem Cells* 20:11-20 (2002)

41. Reali, C., F. Scintu, R. Pillai, S. Cabras, F. Argiolu, M.S. Ristaldi, M.A. Sanna, M. Badiali and V. Sogos. Differentiation of human adult CD34+ stem cells into cells with a neural phenotype: role of astrocytes. *Exp Neurol* 197(2):399-406 (2006)

42. Fauser, S., A. Becker, A. Schulze-Bonhage, M. Hildebrandt, I. Tuxhorn, H.W. Pannek, R. Lahl, J. Schramm and I. Blumcke. CD34-immunoreactive balloon cells in cortical malformations. *Acta Neuropathol* 108:272-8 (2004)

43. De Rosa, M.J., D.L. Secor, M. Barsom, R.S. Fisher and H.V. Vinters. Neuropathologic findings in surgically treated hemimegalencephaly: immunohistochemical, morphometric, and ultrastructural study. *Acta Neuropathol* 84:250-60 (1992)

44. Farrell, M.A., M.J. DeRosa, J.G. Curran, D.L. Secor, M.E. Cornford, Y.G. Comair, W.J. Peacock, W.D. Shields and H.V. Vinters. Neuropathologic findings in cortical resections (including Hemispherectomies) performed for the treatment of intractable childhood epilepsy. *Acta Neuropathol* 83:246–259 (1992)

45. Blümcke, I., H.V. Vinters, D. Armstrong, E. Aronica, M. Thom and R Spreafico. Malformations of cortical development and epilepsies: neuropathological findings with emphasis on focal cortical dysplasia. *Epileptic Disord* 11:181-93(2009)

46. Orlova, K.A., V. Tsai, M. Baybis, G.G. Heuer, S. Sisodiya, M. Thom, K. Strauss, E. Aronica, Ph.B. Storm and P.B. Crino. Early Progenitor Cell Marker Expression Distinguishes Type II from Type I Focal Cortical Dysplasias. *J Neuropathol Exp Neurol* 69:850–863 (2010)

47. Crino, P.B., J.Q. Trojanowski, M.A. Dichter and J. Eberwine. Embryonic neuronal markers in tuberous sclerosis: single-cell molecular pathology. *Proc Natl Acad Sci USA*. 93(24):14152-7(1996)

48. French, J.A. Refractory epilepsy: clinical overview. *Epilepsia* 48 (Suppl. 1):3–7(2007)

49. Sisodiya, S.M., W.R. Lin, B.N. Harding, M.V. Squier, M. Thom. Drug Resistance in Epilepsy: Expression of Drug-resistance Proteins in Common Causes of Refractory Epilepsy. *Brain* 125(Pt 1):22–31(2002)

50. van Breemen, M.S., E.B. Wilms, C.J. Vecht. Epilepsy in patients with brain tumours: epidemiology, mechanisms, and management. *Lancet Neurol* 6:421-30 (2007)

51. Aronica, E., S.M. Sisodiya and J.A. Gorter. Cerebral expression of drug transporters in epilepsy. *Adv Drug Deliv Rev* 64:919-29(2012)

52. Hadjivassiliou, G., L. Martinian, W. Squier, I. Blumcke, E. Aronica, S.M. Sisodiya and M. Thom. The application of cortical layer markers in the evaluation of cortical dysplasias in epilepsy. *Acta Neuropathol* 120:517–528 (2010)

53. Rajneesh, K.F. and D.K. Binder. Tumor-associated epilepsy. *Neurosurg Focus* 27(2):E4 (2009)

54. Clarke, M.F. and M. Fuller. Stem cells and cancer: two faces of eve. *Cell* 124:1111–1115(2006)

55. Dean, M., T. Fojo and S. Bates. Tumor stem cells and drug resistance. *Nat Rev Cancer* 5:275–284 (2005)

56. Huntly, B.J. and D.G. Gilliland. Leukemia stem cells and the evolution of Cancer Stem Cells. *Nat Rev Cancer* 5:311–321 (2005)

57. Lin, T., O. Islam and K. Heese. ABC transporters, neural stem cells and neurogenesis – a different perspective. *Cell Res* 16:857-871(2006)

58. Schumacher, T., M. Krohn, J. Hofrichter, C. Lange, J. Stenzel, J. Steffen, T. Dunkelmann, K. Paarmann, C. Fröhlich, A. Uecker, A.S. Plath, A. Sommer, T. Brüning, H.J. Heinze, and J. Pahnke. ABC transporters B1, C1 and G2 differentially regulate neuroregeneration in mice. *PLoS One.*7(4):e35613(2012)

59. Gritti, A., A.L. Vescovi and R. Galli. Adult neural stem cells: plasticity and developmental potential. *J Physiol* 96,81-90 (2002)

60. Engel, J.Jr, S. Wiebe, J. French, M. Sperling, P. Williamson, D. Spencer, R. Gumnit, C. Zahn, E. Westbrook, B. Enos, Quality Standards Subcommittee of the American Academy of Neurology; American Epilepsy Society and American Association of Neurological Surgeons. Practice parameter: temporal lobe and localized neocortical resections for epilepsy: report of the Quality Standards Subcommittee of the American Academy of Neurology, in association with the American Epilepsy

Society and the American Association of Neurological Surgeons. *Neurology* 60:538−547(2003)

61. Lhatoo, S.D., J.K. Solomon, A.W. McEvoy, N.D. Kitchen, S.D. Shorvon and J.W. Sander. A prospective study of the requirement for and the provision of epilepsy surgery in the United Kingdom. *Epilepsia* 44:673−676 (2003)

62. McIntosh, A.M., R.M. Kalnins, L.A. Mitchell, G.C. Fabinyi, R.S. Briellmann and S.F. Berkovic. Temporal lobectomy: long-term seizure outcome, late recurrence and risks for seizure recurrence. *Brain* 127:2018–30 (2004)

63. de Tisi, J., G.S. Bell, J.L. Peacock, A.W. McEvoy, W.F.J. Harkness, J.W. Sander and J.S. Duncan. The long-term outcome of adult epilepsy surgery, patterns of seizure remission, and relapse: a cohort study. *Lancet* 378:1388–95(2011)

64. Janszky, J., H.W. Pannek, I. Janszky, R. Schulz, F. Behne, M. Hoppe and A. Ebner. Failed surgery for temporal lobe epilepsy: predictors of long-term seizure-free course. *Epilepsy Res* 64:35–44 (2005)

65. Jehi, L., R. Sarkis, W. Bingaman, P. Kotagal and I. Najm. When is a postoperative seizure equivalent to "epilepsy recurrence" after epilepsy surgery? *Epilepsia* 51:994–1003 (2010)

66. Wiebe, S. Epilepsy: Outcome patterns in epilepsy surgery—the long-term view. *Nat Rev Neurol* 8:123-124 (2012)

67. Hirsch, L.J. Long-Term Outcome after epilepsy surgery: relapsing, remitting disorder? *Epilepsy Curr* 12,4:140–142 (2012)
68. Marín, O. Interneuron dysfunction in psychiatric disorders. *Nat Rev Neurosci* 18,13:107-20 (2012)

69. Espuny-Camacho, I., K.A. Michelsen, D. Gall, D. Linaro, A. Hasche, J. Bonnefont, C. Bali, D. Orduz, A. Bilheu, A. Herpoel, N. Lambert, N. Gaspard, S. Péron, S.N. Schiffmann, M. Giugliano, A. Gaillard and P Vanderhaeghen. Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits *in vivo*. *Neuron* 77:440-56 (2013)

70. Maroof, A.M., S. Keros, J.A. Tyson, S.W. Ying, Y.M. Ganat, F.T. Merkle, B. Liu, A. Goulburn, E.G. Stanley, A.G. Elefanty, H.R. Widmer, K. Eggan, P.A. Goldstein, S.A. Anderson and L. Studer. Directed differentiation and functional maturation of cortical interneurons from human embryonic stem cells. *Cell Stem Cell* 12:559-72 (2013)

71. Nicholas, C.R., J. Chen, Y. Tang, D.G. Southwell, N. Chalmers, D. Vogt, C.M. Arnold, Y.J. Chen, E.G. Stanley, A.G. Elefanty, Y. Sasai, A. Alvarez-Buylla, J.L. Rubenstein and A.R. Kriegstein. Functional maturation of hPSC-derived forebrain interneurons requires an extended timeline and mimics human neural development. *Cell Stem Cell* 12:573-86 (2013)

72. Richardson, R.M., N.M. Barbaro, A. Alvarez-Buylla and SC Baraban. Developing cell transplantation for temporal lobe epilepsy. *Neurosurg Focus* 24:E17(2008)

73. Naegele, J.R., X. Maisano, J. Yang, S. Royston and E. Ribeiro. Recent advancements in stem cell and gene therapies for neurological disorders and intractable epilepsy. *Neuropharmacology* 58:855–864 (2010)

74. Inoue, H., N. Nagata, H. Kurokawa and S. Yamanaka. iPS cells: a game changer for future medicine. *EMBO J.* 33(5):409-17 (2014)

75. Jiao, J., Y. Yang, Y. Shi, J. Chen, R. Gao, Y. Fan, H. Yao, W. Liao, X-F. Sun and S. Gao. Modeling Dravet syndrome using induced pluripotent stem cells (iPSCs) and directly converted neurons. *Hum Mol Genet.* 22(21):4241-52 (2013)

76. Shetty, A.K. Progress in cell grafting therapy for temporal lobe epilepsy. *Neurotherapeutics* 8:721–735 (2011)

77. Gallego, J.M., F.J. Sancho, S. Vidueira, L. Ortiz, U. Comez-Pinedo and J.A. Barcia. Injection of embryonic median ganglionic eminence cells or fibroblasts within the amygdala in rats kindled from the piriform cortex. *Seizure* 19:461–466 (2010)

78. Calcagnotto, M.E., I. Zipancic, M. Piquer-Gil, L.E. Mello and M Alvarez-Dolado. Grafting of GABAergic precursors rescues deficits in hippocampal inhibition. *Epilepsia* 51 (Suppl. 3), 66–70 (2010)

79. Corti, S., M. Nizzardo, M. Nardini, C. Donadoni, S. Salani, C. Simone, M. Falcone, G. Riboldi, A. Govoni, N. Bresolin and G.P. Comi. Systemic transplantation of c-kit+ cells exerts a therapeutic effect in a model of amyotrophic lateral sclerosis. *Hum Mol Genet* 19, 3782–3796 (2010)

80. Chailangkarn, T., A. Acab and AR. Muotri. Modeling neurodevelopmental disorders using human neurons. *Curr Opin Neurobiol* 22,785–790 (2012)

81. Yan, Y., S. Shin, B.S. Jha, Q. Liu, J. Sheng, F. Li, M. Zhan, J. Davis, K. Bharti, X. Zeng, M. Rao, N. Malik and M.C. Vemuri. Efficient and rapid derivation of primitive neural stem cells and generation of brain subtype neurons from human pluripotent stem cells. *Stem Cells Transl Med* 2:862–870 (2013)

82. Miltiadous, P., A. Stamatakis, P.N. Koutsoudaki, D.G. Tiniakos and F. Stylianopoulou. IGF-I ameliorates hippocampal neurodegeneration and protects against cognitive deficits in an animal model of temporal lobe epilepsy. *Exp Neurol* 231,223–235 (2011)

83. Russo, E., R. Citraro, A. Constanti and G. De Sarro. The mTOR signaling pathway in the brain: Focus on epilepsy and epileptogenesis. *Mol Neurobiol* 46,662–681 (2012)

84. Canpolat, M., H. Per, H. Gumus, A. Yikilmaz, E. Unal, T. Patiroglu, L. Cinar, A. Kurtsoy and S. Kumandas. Rapamycin has a beneficial effect on controlling epilepsy in children with tuberous sclerosis complex: results of 7 children from a cohort of 86. *Childs Nerv Syst* 30:227-40 (2014)

85. Huang, X., H. Zhang, J. Yang, J. Wu, J. McMahon, Y. Lin, Z. Cao, M. Gruenthal and Y. Huang. Pharmacological Inhibition of the Mammalian Target of Rapamycin Pathway Suppresses Acquired Epilepsy. *Neurobiol Dis* 40:193–199 (2010)

86. Krueger, D.A., M.M. Care, K .Holland, K. Agricola, C. Tudor, P. Mangeshkar, K.A. Wilson, A. Byars, T. Sahmoud, D.N. Franz. Everolimus for Subependymal Giant-Cell Astrocytomas in Tuberous Sclerosis. *N Engl J Med* 363:1801-11(2010)

87. Zeng, L.H., N.R. Rensing and M Wong. The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. *J Neurosci* 29:6964–6972 (2009)

88. Boer, K., D. Troost; W. Timmermans,P.C. van Rijen, W.G.M. Spliet and E. Aronica. 4Pi3K-mTOR Signaling and AMOG Expression in Epilepsy-associated Glioneuronal Tumors. *Brain Pathol* 20:234–244 (2010)

89. Schick, V., M. Majores, A. Koch, C.E. Elger, J. Schramm, H. Urbach, A.J. Becker. Alterations of phosphatidylinositol 3-kinase pathway components in epilepsy-associated glioneuronal lesions. *Epilepsia* 48 Suppl 5:65-73 (2007)

90. Wang, S.F., Y.C. Chou, N. Mazumder, F.J. Kao, L.D. Nagy, F.P. Guengerich, C. Huang, H.C. Lee, P.S. Lai and Y.F. Ueng. 7-Ketocholesterol induces P-glycoprotein through PI3K/mTOR signaling in hepatoma cells. *Biochem Pharmacol* 86:548-60 (2013)

Abbreviations: Antiepileptic drugs, AEDs; ABC-transporters, ABC-*t*; breast cancer resistant protein, BCRP; focal cortical dysplasia, FCD; human neural stem/progenitor cells, hNSPCs; MCD, malformations of cortical development; multidrug resistance, MDR; multidrug-resistance-associated proteins, MRPs; P-glycoprotein, P-gp; subependimal giant astrocytoma, SEGA; refractory epilepsy, RE; tuberous sclerosis complex, TSC.

Key Words: ABC-transporters, MCD, Brain Tumors, Stem Cell Markers, Epileptogenesis, Review

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