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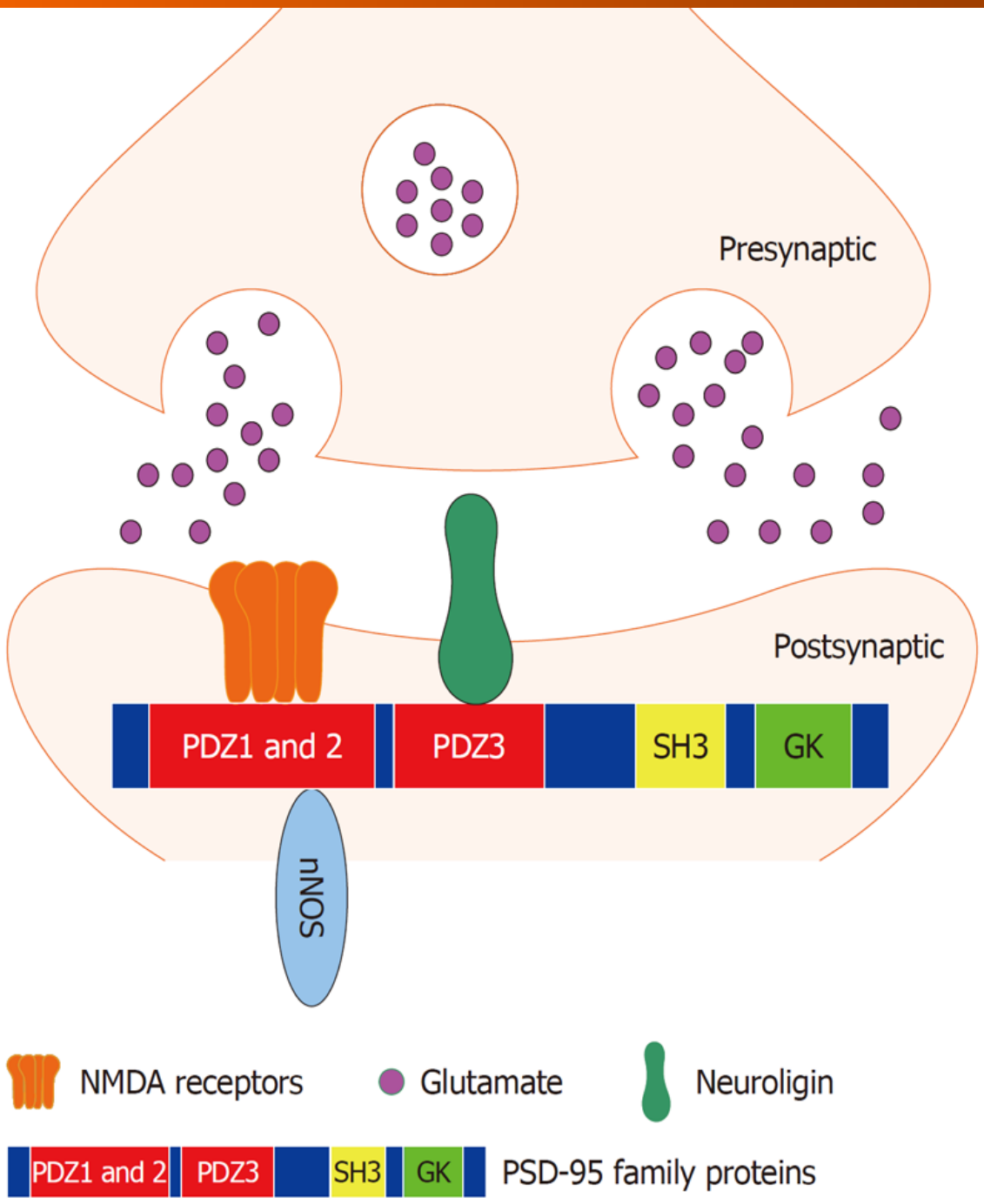

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EDITORIAL

What is the purpose of launching the *World Journal of Experimental Medicine*?

Atsushi Mizoguchi

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Figure 1 Editor-in-Chief of the *World Journal of Experimental Medicine*. Atsushi Mizoguchi, MD, PhD, Associate Professor in Pathology, Harvard Medical School, Molecular Pathology Unit, Massachusetts General Hospital, CNY149-6024, 13th Steert, Charlestown, MA 02114, United States.

Abstract

The first issue of the *World Journal of Experimental Medicine* (*WJEM*), whose preparatory work was initiated on May 26, 2011, will be published on December 20, 2011. The *WJEM* Editorial Board has now been established and consists of 104 distinguished experts from 30 countries. Our purpose of launching the *WJEM* is to publish peer-reviewed, high-quality articles *via* an open-access online publishing model, thereby acting as a platform for communication between peers and the wider public, and maximizing the benefits to editorial board members, authors and readers.

Key words: Experimental medicine; Biomedical sciences; Peer-reviewed; Open-access; Journal

Peer reviewer: Anastasios K Markopoulos, DDS, MS, PhD, Professor, Department of Oral Medicine/Pathology, Aristotle University, School of Dentistry, University Campus, 54124 Thessaloniki, Greece

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INTRODUCTION

I am Atsushi Mizoguchi, MD, PhD, Associate Professor in Pathology, Harvard Medical School, Molecular Pathology Unit, Massachusetts General Hospital (Figure 1), together with De-Ling Kong, PhD, Professor, Institute of Molecular Biology, Nankai University, and Baohong Zhang, PhD, Assistant Professor of Biology, Department of Biology, East Carolina University, we will be the co-editor-in-chief for the *World Journal of Experimental Medicine* (*World J Exp Med*, *WJEM*, online ISSN 2220-315X, DOI: 10.5493). I am very pleased to announce that the first issue of *WJEM*, whose preparatory work was initiated on May 26, 2011, will be published on December 20, 2011. The *WJEM* is a bimonthly peer-reviewed, online, open-access, journal supported by an editorial board consisting of 104 experts in experimental medicine from 30 countries. It is my great honor to introduce the *WJEM* as a new forum for exchanging thoughts and experiences about any approaches to solving problems in research in

experimental medicine. Congratulations to the publisher, members of editorial board of the journal, all the authors and readers for this memorable event!

Experimental medicine is an essential part in medical research to provide solid rationale to cultivate novel, effective and safety measures for a wide spectrum of clinical purposes - prevention, diagnosis, prognosis, treatment, rehabilitation and nursing. In addition, recent advances in genetic and engineering technologies, such as medical devices and imaging, along with the expertise of experimental medicine have provided significant contributions for bringing novel concepts into diverse disease conditions that range from autoimmune diseases to cancer. Indeed, a growing body of evidence in the field of experimental medicine indicates more complicated mechanisms in the majority of diseases than previously predicted, suggesting the requirement of further extensive research. For example, many factors such as genetic, immune and environmental influences are all responsible for determining the induction as well as the progression of diseases. This fact clearly highlights the requirement of "personalized medicine" in the management of patients who have different genetic backgrounds and been exposed to different environmental factors. I believe that much more accumulation of data is needed from various fields within experimental medicine to successfully construct a database platform for such personalized medicine.

SCOPE

In order to achieve this long-term goal, the aim of the *WJEM* is to rapidly report new theories, methods and techniques for prevention, diagnosis, treatment, rehabilitation and nursing in the field of experimental medicine. The *WJEM* covers topics concerning clinical laboratory medicine (applied and basic research in hematology, body fluid examination, cytomorphology, genetic diagnosis of hematological disorders, thrombosis and hemostasis, and blood typing and transfusion), biochemical examination (applied and basic research in laboratory automation and information systems, biochemical methodology and biochemical diagnostics), clinical microbiology (microbiological laboratory quality control and management; microbiological specimen collection and its influencing factors; conventional, automatic or molecular detection of clinical microorganisms; monitoring of bacterial and fungal drug resistance, drug resistance mechanisms and rational application of antibiotics; monitoring and control of nosocomial infections), immunodiagnostics (laboratory diagnosis of infectious diseases, tumor markers and their application, laboratory diagnosis of autoimmune diseases and immunotechnology), clinical laboratory management (laboratory quality control and management, traceability and calibration, information management systems and laboratory automation, and laboratory biosafety management) and experimental medicine-related traditional medicine, integrated Chinese and Western medicine. The journal also publishes original articles and reviews that report the results of experimental medicine-related applied and basic research in fields such as immunology, physiopathology, cell biology, pharmacology, medical genetics and pharmacology of Chinese herbs.

CONTENTS OF PEER REVIEW

In order to guarantee the quality of articles published in the journal, *WJEM* usually invites three experts to comment on the submitted papers. The contents of peer review include: (1) whether the contents of the manuscript are of great importance and novelty; (2) whether the experiment is complete and described clearly; (3) whether the discussion and conclusion are justified; (4) whether the citations of references are necessary and reasonable; and (5) whether the presentation and use of tables and figures are correct and complete.

COLUMNS

The columns in issues of the *WJEM* will include: (1) Editorial: to introduce and comment on the substantial advance and its importance in the fast-developing areas; (2) Frontier: to review the most representative achievements and comment on the current research status in the important fields and propose directions for the future research; (3) Topic Highlight: this column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: to update the development of old and new questions, highlight unsolved problems and provide strategies on how to solve the questions; (5) Guidelines for Clinical Practice: to provide guidelines for clinical diagnosis and treatment; (6) Review: to systematically review the most representative progress and unsolved problems in the major scientific disciplines, comment on the current research status and make suggestions on the future work; (7) Original Articles: to originally report the innovative and valuable findings in experimental medicine; (8) Brief Articles: to briefly report the novel and innovative findings in experimental medicine; (9) Case Report: to report a rare or typical case; (10) Letters to the Editor: to discuss and reply to the contributions published in the *WJEM*, or to introduce and comment on a controversial issue of general interest; (11) Book Reviews: to introduce and comment on quality monographs of experimental medicine; and (12) Guidelines: to introduce consensuses and guidelines reached by international and national academic authorities worldwide on research in experimental medicine.

We welcome any articles that set a challenge to current concepts in translational, pre-clinical or clinical fields. Our editorial board members are looking forward to your submissions and anticipate working with you to achieve our mission.

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EDITORIAL

Early anesthetic exposure and long-term cognitive impairment

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Abstract

Several lines of evidence from clinical cohort studies and animal studies have shown that early exposure to anesthetics is a significant risk factor for later development of learning disabilities. However, the underlying molecular mechanism is unclear. Recent studies have indicated that hippocampal neurogenesis and synaptogenesis may be involved in the mechanisms by which early anesthetic exposure produces long-term cognitive impairment. It is possible that synaptic scaffolding protein postsynaptic density-95 (PSD-95) PDZ (PSD 95/Discs large/Zona occludens-1) domain-mediated protein-protein interactions are involved in the regulation of neurogenesis and synaptogenesis in the central nervous system. PDZ domain-mediated protein-protein interactions are disrupted by clinically relevant concentrations of inhaled anesthetics. It will help us understand the molecular mechanism underlying anesthetic-induced long-term cognitive dysfunction if we can demonstrate the role of synaptic PDZ interactions in early anesthetic exposure-produced long-term cognitive impairment.

Key words: Anesthetics; Cognitive dysfunction; PDZ interactions; Neurogenesis; Synaptogenesis

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INTRODUCTION

A recent population-based cohort study by Wilder *et al*[1] has shown that early exposure to anesthetics is a significant risk factor for later development of learning disabilities (LD). They found, in particular, that the risk for LD increased with longer cumulative duration of anesthesia exposure and that their data cannot reveal whether anesthesia itself may contribute to LD or whether the need for anesthesia is a marker for other unidentified factors that contribute to LD. Animal studies have also shown that exposure to a variety of commonly used intravenous and volatile anesthetics produces cognitive and social behavioral deficits that last into adulthood[2,3]. Thus, the possibility that general anesthetics might have long-lasting effects on the developing brain has gained traction with anesthesiologists, popular media and parents. Although cause-effect relationships have not been established, these cognitive and behavioral dysfunctions have been attributed to the suppression of neurogenesis or widespread neuroapoptosis after anesthetic exposure in the early postnatal period[2,4]. In addition, anesthetic exposure fundamentally alters dendritic spine formation and therefore synaptic function[5]. Taken together, these results strongly suggest that anesthetics can produce synaptic remodeling in the developing brain that could potentially produce long-term cognitive impairment.

In the central nervous system, the hippocampus is a critical region for learning and memory. *N*-methyl-D-aspartate (NMDA) receptors are present at the postsynaptic membrane of the hippocampal synapses and

are involved in the synaptic mechanism of learning and memory. The functional activities of NMDA receptors are regulated by postsynaptic density-95 (PSD-95) family proteins, which contain three PDZ (PSD 95/Discs large/Zona occludens-1) domains in their N-terminus (Figure 1). Through the first two PDZ domains (mainly PDZ2), PSD-95 interacts with NMDA receptor NR2 subunit and neuronal nitric oxide synthase (nNOS)[6,7] to regulate NMDA receptor-mediated synaptic plasticity[6-8]. PSD-95 PDZ3 domain-mediated PSD-95/neuroligin signaling at the postsynaptic site of the hippocampal synapses modulates the presynaptic release probability of glutamate vesicles in a retrograde manner[9]. In addition to its effect on synaptic function, PSD-95 has also been proposed to affect synapse maturation and stabilization[10-12]. PSD-95 promotes synaptogenesis and deletion of the PSD-95 PDZ2 domain specifically prevents multi-innervated spine formation[13]. Our previous studies have demonstrated that PSD-95 PDZ domain-mediated protein-protein interactions are disrupted by clinically relevant concentrations of inhaled anesthetics and inhaled anesthetic binding results in chemical shift changes of PSD-95 PDZ2[14], and that disrupting PSD-95 PDZ domain-mediated protein interactions reduces the threshold for inhalational anesthesia[15]. Moreover, hippocampal neurogenesis can be regulated by glutamate release and subsequent NMDA receptor activation[16]. Therefore, it is possible that synaptic PDZ interactions are involved in the regulation of neurogenesis and synaptogenesis in the hippocampus and disruption of the PDZ interactions is one of the mechanisms underlying anesthetic-induced cognitive dysfunction.

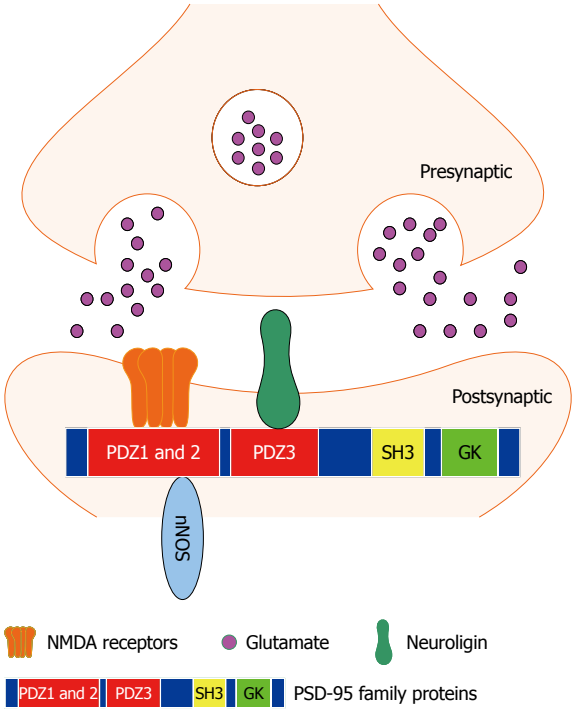

Figure 1 *N*-methyl-D-aspartate receptor signaling and protein interactions mediated by PDZ domains of postsynaptic density-95 family proteins at postsynaptic density in the central nervous system. Through the first two PDZ domains (mainly PDZ2), postsynaptic density-95 (PSD-95) family proteins interact with *N*-methyl-D-aspartate (NMDA) receptor NR2 subunit and downstream molecule neuronal nitric oxide synthase to regulate NMDA receptor-mediated synaptic plasticity. PSD-95 PDZ3 domain-mediated PSD-95/neuroligin interaction at the postsynaptic site of synapses modulates the presynaptic release probability of glutamate vesicles in a retrograde manner.

HIPPOCAMPAL NEUROGENESIS AND ANESTHETIC-INDUCED COGNITIVE DYSFUNCTION

Neurogenesis in both the developing and adult dentate gyrus is important for hippocampal function, specifically learning and memory[17]. Stratmann *et al*[4] showed that in postnatal day (PND) 60 rats, 4 h of isoflurane exposure at 1 minimum alveolar concentration increased early neuronal differentiation in the dentate gyrus, decreased progenitor proliferation for 1 d, and subsequently increased progenitor proliferation 5-10 d after anesthesia. Identical isoflurane treatment in PND 7 rats did not induce neuronal lineage selection but decreased progenitor proliferation until at least 5 d after anesthesia[4]. They also showed that isoflurane exposure improved spatial reference memory of PND 60 rats long-term but caused a delayed-onset, progressive, persistent hippocampal deficit in PND 7 rats in fear conditioning and spatial reference memory tasks[4]. Thus, isoflurane differentially affects both neurogenesis and long-term neurocognitive function in PND 60 and PND 7 rats. In another study[18] that subjected PND 14 and PND 60 rats to repeated isoflurane exposure (1.7%, 35 min daily for 4 successive days), object recognition and reversal learning were significantly impaired in isoflurane-treated young rats, whereas adult animals were unaffected, and these deficits became more pronounced as the animals grew older. The memory deficit was paralleled by a decrease in the hippocampal stem cell pool and persistently reduced neurogenesis, subsequently causing a reduction in the number of dentate gyrus granule cell neurons in isoflurane-treated rats[18]. These results suggest that hippocampal neurogenesis might mediate the long-term neurocognitive dysfunction after isoflurane exposure in neonatal rats.

Given that (1) hippocampal neurogenesis can be regulated by glutamate release and subsequent NMDA receptor activation[16] and (2) PSD-95 PDZ domain-mediated protein-protein interactions modulate presynaptic glutamate release[9] and regulate postsynaptic NMDA receptor function[6-8], it is well-founded that PSD-95 PDZ domain-mediated protein-protein interactions may play an important role in hippocampal neurogenesis and then contribute to early anesthetic exposure-produced long-term cognitive impairment.

HIPPOCAMPAL SYNAPTOGENESIS AND ANESTHETIC-INDUCED COGNITIVE DYSFUNCTION

The developing brain is most vulnerable during the pe-

riod of rapid synaptogenesis[2]. Administration with a combination of drugs commonly used in pediatric anesthesia (midazolam, nitrous oxide and isoflurane) in doses sufficient to maintain a surgical plane of anesthesia for 6 h causes widespread apoptotic neurodegeneration in the developing brain of PND 7 rats, deficits in hippocampal synaptic function and persistent memory/learning impairments[2]. Therefore, synapse development is a critical element in the progress of anesthetic neurotoxicity. Head *et al*[19] reported a substantial loss of dendritic spines and a reduction in synapses in cultured neurons and the hippocampus of PND 7 rodents exposed to isoflurane (1.4%, 4 h). However, isoflurane (1.5%, 2 h) exposure on PND 16 significantly increased dendritic spine density in the rat medial prefrontal cortex during synaptogenesis[20]. These results suggest that volatile anesthetics with different potencies could rapidly interfere with physiological patterns of synaptogenesis and thus might impair appropriate circuit assembly in the developing cerebral cortex. Increasing age might alter the response of dendritic spines to anesthetics from vulnerable to resistant. It is well established that NMDA receptor signaling plays a crucial role in synaptic development and neuronal survival[21]. During the critical period of synaptogenesis, inhibition of NMDA receptor signaling is detrimental to brain development[22]. NMDA receptor-interacting protein PSD-95 is also an important regulator of synaptic structure and plasticity. By using a combined electron microscopic, genetic and pharmacological approach, Nikonenko *et al*[13] have uncovered a new mechanism through which PSD-95 regulates synaptogenesis. They found that PSD-95 overexpression affected spine morphology and promoted the formation of multi-innervated spines contacted by up to seven presynaptic terminals. The formation of multiple contacts was specifically prevented by deletion of the PDZ2 domain of PSD-95[13], which interacts with NOS. Similarly, PSD-95 overexpression combined with small interfering RNA-mediated down-regulation or the pharmacological blockade of nNOS prevented axon differentiation into varicosities and multisynapse formation[13]. Conversely, treatment of hippocampal slices with a nitric oxide donor or cyclic guanosine monophosphate analogue induced multiinnervated spines and NOS blockade also reduced spine and synapse density in developing hippocampal cultures[13].

Given that PSD-95 promotes synaptogenesis and multiinnervated spine formation is specifically prevented by deletion of the PSD-95 PDZ2 domain[13], it is presumable that PSD-95 PDZ domain-mediated protein-protein interactions may be involved in hippocampal synaptogenesis and then contribute to synaptic plasticity and early anesthetic exposure-produced long-term cognitive impairment.

CONCLUSION

Approximately 1.5 million fetuses or newborns are exposed to anesthetic agents each year[23]. A recent clinical cohort study has shown that early exposure to anesthetics is a significant risk factor for later development of LD[1]. In animal studies, newborn rats exposed to commonly used anesthetic agents (e.g. isoflurane) develop neurodegenerative changes in multiple areas of the brain that are associated with long-term deficits in learning and memory[2,4,24]. These provocative findings bring into question whether anesthetic drugs can be used safely in pediatric anesthesia and have prompted many to investigate the neurotoxic effect of anesthetic drugs on the developing brain. Although recent studies have indicated that hippocampal neurogenesis and synaptogenesis may be involved in the mechanisms by which early anesthetic exposure produces long-term cognitive impairment[4,5,19], the underlying molecular mechanism remains to be elucidated.

Synaptic PDZ interactions might be potential targets in pathogenesis of neonatal anesthetic neurotoxicity. In the future, we may compare the effects of early anesthetic exposure and synaptic PDZ interaction disruption on hippocampal neurogenesis, hippocampal synaptogenesis, synaptic plasticity and long-term neurocognitive function; we may also define the relationship among PDZ interaction disruption, neurogenesis suppression, synaptogenesis interference and early anesthetic exposure-produced long-term cognitive impairment. By using PDZ interaction inhibitors, we could determine whether disruption of PDZ interactions can mimic the effect of inhaled anesthetics on hippocampal neurogenesis, synaptogenesis and long-term neurocognitive function; by using overexpression of anesthetic-resistant PDZ mutants, we could determine whether synaptic PDZ interactions are involved in the molecular mechanisms that underlie early anesthetic exposure-regulated neurogenesis, synaptogenesis and synaptic plasticity in the hippocampus. Together, evaluating both structural and functional synaptic plasticity will help us demonstrate the role of synaptic PDZ interactions in early anesthetic exposure-produced long-term cognitive impairment.

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OBSERVATION

Neuroendocrine differentiation and the ubiquitin-proteasome system in cancer: Partners or enemies?

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Abstract

Neuroendocrine (NE) differentiation of cancer and deregulation of the ubiquitin-proteasome system (UPS) are two processes that have been independently linked to the development of aggressive and treatment-resistant tumors. Striking data suggest a plausible interconnection between these two mechanisms, based on indirect evidence of neuropeptide-induced effects on UPS, reversed by proteasome inhibition and deubiquitinase-like properties of NE markers. Deciphering the model of their exact interactions is one of the keys to targeting the NE malignant phenotype more effectively.

Key words: Neuroendocrine differentiation; Ubiquitin; Proteasome; Cancer

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The study of neuroendocrine (NE) cancers and NE differentiation of solid tumors has been hampered by a number of circumstances, including our limited understanding of the cellular and molecular biology of NE cells and the mechanisms of tumorigenesis, a shortage of *in vitro* and animal models to study disease pathogenesis and treatment, a paucity of critical targets for new therapies, and a lack of uniform pathological classification and staging systems. The carcinoid tumor group is heterogeneous and comprises a mixture of foregut-, midgut- and hindgut-derived tumors. Furthermore, clinical studies are commonly performed on patients with tumors displaying varying degrees of differentiation, proliferation rates and disease stage, thus limiting the validity of the conclusions drawn. Experimental data from well-characterized *in vitro* and animal models are therefore needed to properly evaluate novel treatment principles[1].

The ubiquitin-proteasome system (UPS) constitutes a large multiprotein complex present in all cells, which degrades damaged, oxidized or misfolded proteins which are targeted for ubiquitination. Furthermore, it orchestrates the orderly degradation of regulatory proteins that govern cell cycle, transcription factor activation, apoptosis and cell trafficking[2]. A classic example is regulation of nuclear factor κB (NFκB), a key transcription factor promoting cell survival, angiogenesis and metastasis, related to cancer progression and resistance to chemotherapy in various solid tumors. NFκB activation is dependent on proteasome-mediated degradation of the NFκB inhibitory protein IκB[3].

Coincident with progression from prostate cancer *in situ* to metastatic disease is an increase in the number of tumor cells exhibiting NE differentiation. NE cells express a variety of hormone peptides, including endo-

thelin-1 (ET-1), the bombesin (BBS)-like peptide, gastrin-releasing peptide, and their receptors[4]. Although there is a strong positive correlation between the degree of NE differentiation and the metastatic potential of prostate cancers, a mechanistic link between increased expression of markers of NE differentiation such as neuropeptides and the UPS had not been established before the study of Levine *et al*[4] who demonstrated that BBS treatment induced IκB degradation, NFκB translocation to the cell nucleus, increased NFκB DNA binding and upregulation of expression of pro-angiogenic factors IL-8 and VEGF, whereas proteasome inhibition by MG132 blocked these effects. These were the first data suggesting a positive modulatory role of neuropeptides in UPS regulation. Later preclinical studies reported a remarkable activity of the proteasome inhibitor bortezomib against several NE tumor cell lines of pancreatic and bronchial origin, with IC_{50} values of the drug < 1 μmol/L[5]. The mechanism of action of bortezomib in these cell lines was supported to be induction of apoptosis *via* activation of the extrinsic apoptotic pathway at longer exposure times (> 24 h), while at shorter drug exposure times (24 h) the intrinsic apoptotic pathway, resulting in nuclear condensation and fragmentation[6].

Treatment of human pulmonary and gastrointestinal carcinoid cells with MG132 resulted in growth inhibition and apoptosis which was in parallel with a dose dependent inhibition of NE markers chromogranin A and Achaete-Scute complex-like 1. These effects also coincided with an increase in the level of phosphorylated Glycogen Synthase Kinase-3β (GSK-3β), which is a highly active serine/threonine protein kinase in carcinoid cells. Phosphorylation of GSK-3β has been shown to inhibit NE tumor growth and the carcinoid phenotype[7].

Furthermore, expression of the neuron cytoplasmic protein gene product 9.5 (PGP9.5)/ubiquitin C-terminal hydrolase 1 (UCHL-1) was found elevated in multiple myeloma cells and primary specimens and was suggested as a potentially useful marker for the screening of proteasome inhibitor sensitivity, given its involvement in deubiquination as a thiol protease that recognizes and hydrolyzes a peptide bond at the C-terminal of ubiquitin[8]. However, PGP9.5 is also known as a specific tissue marker for the NE system and here lie implications for possible associations between NE phenotype and UPS. It was the same protein that was found consistently upregulated both at the gene and protein level in airway epithelial samples of smokers compared with non-smokers. UCHL1 expression was evident only in NE cells of the airway epithelium in non-smokers but was also expressed in ciliated epithelial cells in smokers. It was therefore suggested that the overexpression of UCHL1 in chronic smokers, combined with previous data of UCHL1 overexpression in > 50% of lung cancers, may represent an early event during lung carcinogenesis[9]. The already established role of UCHL1 in the degradation of unwanted, misfolded or damaged proteins within the cell might imply that an increased deubiquitination hallmarks NE differentiation and transition to a NE phenotype. This scenario may further be enriched by our recent *in vitro* results in androgen-independent prostate cancer, consistent with an increased proteasomal activity, pronounced NFκB activation and increased levels of secreted ET-1. The exact mirror image was observed in androgen-dependent prostate cancer cells displaying low secreted ET-1 levels and a low-level activation of the NFκB pathway associated with low 20S proteasome activity[10]. Thus, it seems that during transition to a NE differentiated, androgen-independent state, neuropeptide abundance coincides with upregulation of proteasome activity.

The induction of both ubiquitination-mediated protein degradation and deubiquitination processes in the context of a NE phenotype may seem difficult to explain at first. However, numerous proteins with divergent roles within the cell are targeted by the proteasome, including proteins related with NE differentiation. In this case, an elevated deubiquitinase activity would be needed to protect such proteins from being degraded and thus maintain a NE phenotype. BCCIP is a proteasome-substrate protein, whose overexpression in prostate tumor cells induces an apparent NE differentiation. BCCIP degradation is promoted by overexpression of its associated protein, LYRIC/AEG-1, and blocked by proteasome inhibition[11].

It is therefore evident that preclinical data support a bidirectional association between UPS and NE phenotype, possibly enabling us to consider them as partners, although this remains to be further elucidated. In the era of targeted anticancer therapies, this association provides a rational for testing the use of proteasome inhibitors in these tumor types at the clinical level. A first attempt was made in a small phase 2 study of bortezomib at a dose of 1.5 mg/m^2 on days 1, 4, 8 and 11 every 21 d in patients with metastatic NE (carcinoid and islet cell) tumors. As a result, disease stabilization was seen, although no partial or complete remissions were observed. This might at least partially be due to an inadequate dosing schedule as the mean percentage of 20S proteasome inhibition achieved in whole blood at 1 and 24 h after bortezomib administration was 68% and 30%, respectively, thus reducing therapeutic efficacy[12].

More importantly, an in-depth analysis of the molecular part of NE differentiation which involves UPS components (ubiquitin ligases, ubiquitin-like modifiers, deubiquitinating enzymes, proteasome subunits) and how these components interact with neuroendocine proteins modulating their stability, is anticipated to offer a better understanding of the UPS - NE phenotype association, thus resulting in better clinically-oriented research. Hopefully, research directed to identifying the particular subpopulation of patients with a particular cancer prior to intervention would allow for a selective process and potentially a more favorable clinical outcome.

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REVIEW

A short perspective on gene therapy: Clinical experience on gene therapy of gliomablastoma multiforme

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Abstract

More than two decades have passed since the first gene therapy clinical trial was conducted. During this time, we have gained much knowledge regarding gene therapy in general, but also learned to understand the fear that persists in society. We have experienced drawbacks and successes. More than 1700 clinical trials have been conducted where gene therapy is used as a means for therapy. In the very first trial, patients with advanced melanoma were treated with tumor infiltrating lymphocytes genetically modified *ex-vivo* to express tumor necrosis factor. Around the same time the first gene therapy trial was conducted, the ethical aspects of performing gene therapy on humans was intensively discussed. What are the risks involved with gene therapy? Can we control the technology? What is ethically acceptable and what are the indications gene therapy can be used for? Initially, gene therapy was thought to be implemented mainly for the treatment of monogenetic diseases, such as adenosine deaminase deficiency. However, other therapeutic areas have become of interest and currently cancer is the most studied therapeutic area for gene therapy based medicines. In this review I will be giving a short introduction into gene therapy and will direct the discussion to where we should go from here. Furthermore, I will focus on the use of the Herpes simplex virus-thymidine kinase for gene therapy of malignant gliomas and highlight the efficacy of gene therapy for the treatment of malignant gliomas, but other strategies will also be mentioned.

Key words: Gene therapy; Glioblastoma multiforme; Herpes simplex virus - thymidine kinase

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GENE THERAPY

Gene therapy has experienced several ups and downs during the last few decades. The last one was particularly troublesome as many promising therapies have failed in clinical trials. However, with the increasing knowledge of epigenetics and their impact in many diseases, such as cancer and atherosclerosis, the potential of gene therapy has regained attention. Also, with the introduction of safer and more specific gene transfer vectors, the fear of gene therapy has been released, at least to some extent. According to the European Medicines Agency (EMA), a gene therapy medicinal product means "a biological medicinal product that contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view of regulating, replacing, adding or deleting a genetic sequence,

as well as if its therapeutic, prophylactic or diagnostic effect relates to the recombinant nucleic acid sequence it contains, or to the products of genetic expression of this sequence". Gene therapy can be categorized into (1) germ line gene therapy and (2) somatic gene therapy. In somatic gene therapy, the genetic material is not passed along to the next generation, whereas in germ line gene therapy it is. This difference is of importance as to date, gene therapy is only allowed on somatic cells. Gene therapy to somatic cells is prohibited.

There are several strategies how foreign genetic material (i.e. a transgene) can be introduced to the patient. Firstly, the transgene can be introduced directly to patients (i.e. *in vivo* gene therapy) (Figure 1)[1-3] or alternatively, the genetic material can be introduced *ex vivo*[4-6]. In this case, either autologous or heterologous cells are transduced outside the patient (i.e. *ex vivo*) and then administered to the patient (Figure 1). When heterologous cells are used, the cells need to be protected from the recipient's immune system. This might be achieved by cell encapsulation of transduced cells into microparticles[7-9].

Different gene delivery methods have been used for introducing the genetic material into the cells. These methods can be categorized into physical, viral and non-viral methods. Examples of physical methods are electroporation, ultrasound and gene gun methods. In case of viral or non-viral gene delivery, a biological (a virus) or a synthetic (liposomes or nanoparticles) are used as gene carriers to deliver the genetic material into the cells. Of the viral vectors, adenoviruses are currently the most dominant gene delivery vectors used in gene therapy, followed by retroviral vectors (including lentiviral vectors) and plasmid DNA. Adeno associated viral vectors have also gained momentum recently and represent an interesting alternative to retroviral vectors. Other gene transfer vectors that are more or less commonly used are vaccinia viruses and poxviruses (Figure 2A). The use of bacteria as gene transfer vectors is not new but only recently has gained more attention.

CURRENT STATUS

So far, neither the Food and Drug Administration nor the EMA has approved any human gene therapy product for commercial use. Initially, the main targets for gene therapy were monogenic disorders, such as adenosine deaminase deficiency and adrenoleukodystrophy. Even though drawbacks have been reported, which is exemplified by the work of Hacei-Bey-Abina for example, there have also been successes[5,10-13]. Eventually gene therapy has shifted from these monogenetic diseases into other disease areas such as cancer and cardiovascular diseases[1,14-19]. Currently, cancer is the most common disease area that gene therapy is applied to. More than 60% of all ongoing gene therapy clinical trials are developed for the treatment of cancer (Figure 2B). Many cancer types have been targeted with gene therapy including tumors of the brain, lung, breast, pancreatic, liver, colorectal, prostate, bladder, head and neck, ovarian and renal cancer. The first clinical trial on cancer started in 1990, where patients with advanced melanoma were treated with tumor infiltrating lymphocytes genetically modified *ex-vivo* to express tumor necrosis factor[20].

In October 2003, China became the first country to approve the commercial production of a gene therapy product[21-23]. Shenzhen SiBiono GenTech (Shenzhen, China) obtained a drug license from the State Food and Drug Administration of China for its recombinant adenovirus-p53 gene therapy (Gendicine). Gendicine was approved for the treatment of head and neck squamous cell carcinoma (HNSCC). Two years later, in 2005, the conditionally replicating adenovirus H-101 (i.e. an adenovirus wherein the E1B-55 kDa gene has been deleted, allowing the virus to selectively replicate in and lyse p53-deficient cancer cells) gained marketing approval for HNSCC[24]. More recently, Rexin-G, a pathotropic targeted retroviral vector designed to interfere with cyclin *GI* gene by integrating into the host DNA, has recently been approved in the Philippines for the treatment of all solid tumors that are refractory to standard chemotherapy[25]. In addition, it is currently in clinical trials in the U.S. and has been granted Orphan Drug Status by the FDA for three cancer indications: (1) pancreatic cancer; (2) soft tissue sarcoma; and (3) osteosarcoma[25-30].

GENE THERAPY FOR MALIGNANT GLIOMAS

Brain tumors bear several features that make them amenable to gene therapy, particularly for suicide gene therapy. First of all, brain tumors are, in most cases, single, localized lesions of dividing cells in a background of non-dividing cells. Secondly, primary brain tumors rarely metastasize outside the central nervous system and if recurrence occurs, it typically happens in the close vicinity of the original lesion[31-33]. Different approaches have been utilized for the treatment of malignant gliomas using different gene transfer vectors. Among these, prodrug activation/suicide gene therapy, anti-angiogenic gene therapy, oncolytic virotherapy and immune modulation are the most commonly used strategies[1,2,34-44]. The very first clinical gene therapy trial against brain cancer was registered in 1992[45]. In that trial, autologous tumor cells were modified *ex vivo* with retrovirus to express *interleukin-2* gene in neuroblastoma. In the following year, brain cancer patients were treated with herpes simplex virus thymidine kinase suicide gene therapy using retrovirus vectors producing cells and concomitant administration of ganciclovir. However, transduction efficiency was a major problem in these trials, resulting in poor therapeutic efficacy. In 1996, Eck *et al*[46] published the first phase I clinical trial where adenovirus Herpes simplex virus-thymidine kinase was used with intention in patients with recurrent gliomas. In 2000, Sandmair *et al*[34] published a study wherein 21 patients were enrolled to compare the efficacy of retrovirus-packaging cells to adenovirus mediated gene transfer. In this study,

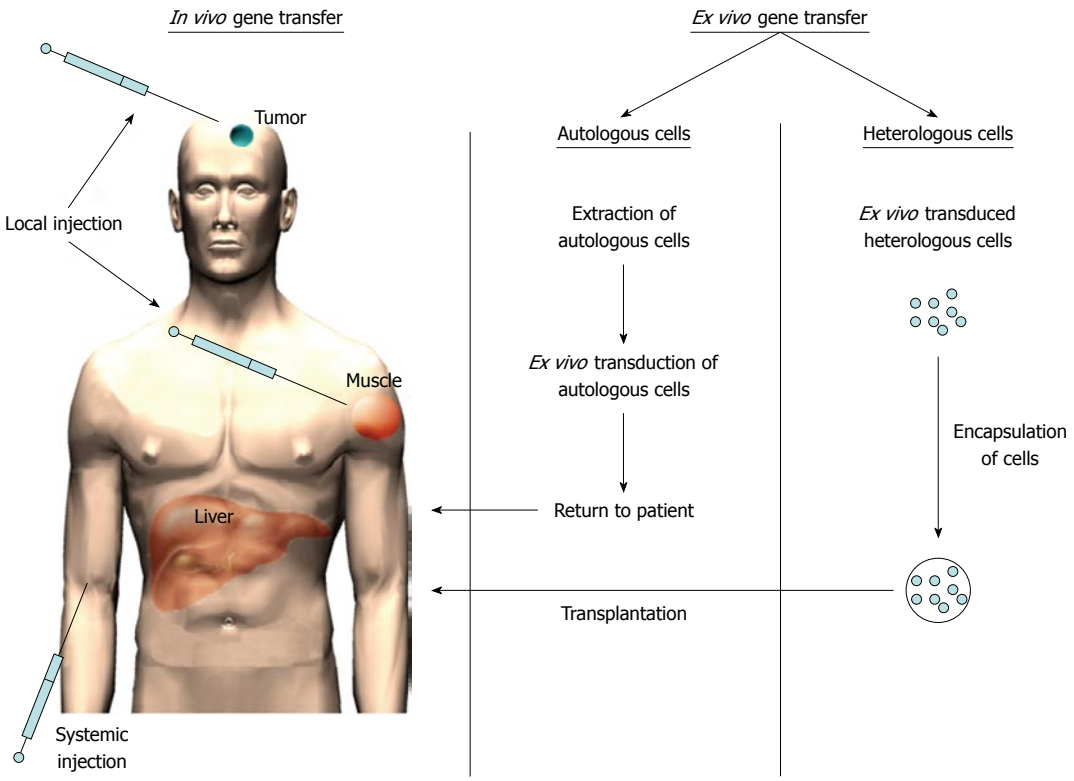

Figure 1 Gene transfer can be performed either *in vivo* or *ex vivo*. In *ex vivo* approaches, the cells which are transduced can be autologous or heterologous in origin. In the case of using heterologous cells, cells need to be protected from the recipient's immune system.

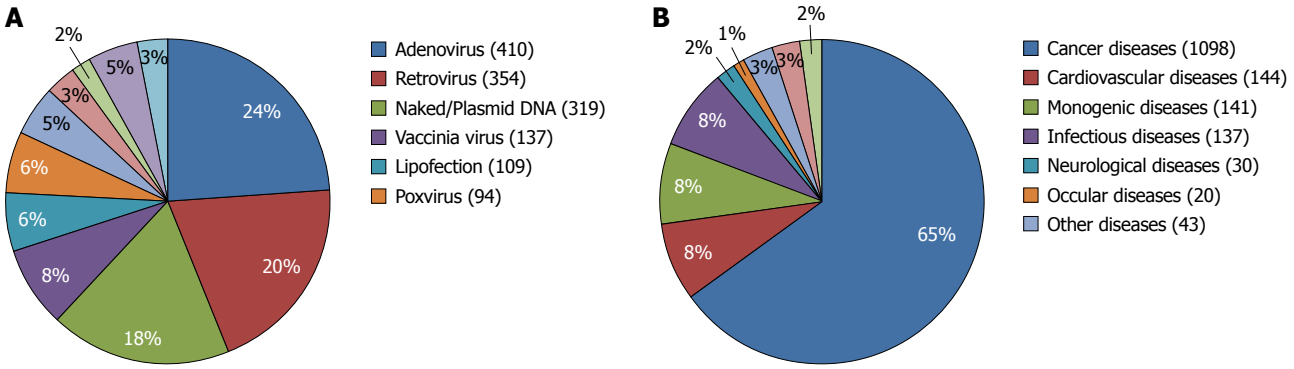

Figure 2 Different gene transfer vectors used in clinical settings (A) and different indications that have been addressed by gene therapy in clinical trials (B). A: By far, adenoviral, retroviral and naked plasmid/DNA have been the most commonly used gene transfer vectors; B: Even though initial studies have been conducted on monogenetic diseases, cancer soon became a major interest, with 65% of all clinical trials to date. The reasons for this are the highly unmet medical need in cancer therapy as well as its big market size. Also, the ethical acceptance of gene therapy as a therapeutic modality is a factor that has supported the shift from monogenetic diseases to cancer.

the therapeutic efficacy of the Herpes simplex virus-thymidine kinase, using these two approaches, was compared in context of the treatment of primary or recurrent gliomas. The mean survival time in the adenovirus Herpes simplex virus-thymidine kinase group was 15 mo and significantly longer, when compared to the survival time of the retrovirus-packaging-cells group, which was 7.4 mo. The control group, which received adenovirus LacZ had a mean survival time of 8.3 mo. Although the retrovirus-packaging-cells approaches were found safe, no efficacy was observed in malignant glioma patients. The low gene transfer efficacy with retrovirus and the lack of the treatment response indicated that retroviral Herpes simplex virus-thymidine kinase gene therapy may not be efficient enough in human clinical settings. This was further confirmed by the results from the first randomized, open-label, parallel group phase Ⅲ clinical trial of 248 patients, where Herpes simplex virus-thymidine kinase produced by retroviral producing cells did not result in an improvement of survival[2]. Some years later, in 2003, a phase I clinical trial described the use of an adenoviral vector encoding for the tumor suppressor gene TP53 for the treatment of patients with recurrent malignant gliomas[47]. In that study, 15 patients underwent

intratumoral stereotactic injection of the adenoviral vector *via* an implanted catheter, followed by *en bloc* resection of the tumor and treatment of the post-resection cavity. Due to the design of the study, tumor response could not be assessed but it proved to be safe, demonstrating minimal toxicity. No systemic viral dissemination was observed and a maximum tolerated dose was not reached in this study. Analysis of tumor specimens demonstrated restricted transgene expression close to the injection site. Chiocca *et al*[38] published a phase I dose-escalation trial of the oncolytic adenovirus ONYX-015, which preferentially replicates and thereby lyses p53-deficient cells (a common feature in tumor cells). In that trial, 24 patients with recurrent malignant glioma were injected with ONYX-015 with doses ranging from 10^7 to 10^{10} pfu (plaque forming units) in a total of 10 injections into 10 different sites of the cavity of resected tumors. None of the patients experienced serious adverse events related to the virus. However, in that trial, the maximum tolerated dose was not reached. All patients showed tumor progression with a median time of 46 d and a median survival time of 6.2 mo. One patient with anaplastic astrocytoma had stable disease and two patients who underwent a second resection had lymphocytic and plasmacytoid cell infiltration at the site of injection. Nevertheless, despite a good safety profile, the overall therapeutic efficacy was poor. In another study performed by Chiocca *et al*[48], 11 patients were injected with different doses of interferon-β-expressing adenoviruses ranging from 2×10^{10} to 2×10^{11} viral particles stereotactically into the tumor. This was followed by surgical removal of the tumor 4-8 d later with additional injections of the adenovirus into the tumor bed. Generally, the treatment was well tolerated with only one patient experiencing a dose-limiting side effect after post-operative injection with the highest dose. However, all patients had disease progression and/or recurrence within 4 mo after the treatment. The median time to tumor progression was 9.3 wk and the median overall survival was 17.9 wk.

The clinical efficacy of sitimagene ceradenovec was evaluated first in two separate phase Ⅱ clinical trials; a phase Ⅱa trial and a phase Ⅱb trial[1,34,49]. Sitimagene ceradenovec is an adenoviral vector encoding for the Herpes simplex virus-thymidine kinase, which is injected into the tumor cavity of resected gliomas, followed by the administration of the pro-drug ganciclovir (Figure 3). In the randomized and controlled phase Ⅱb trial published by Immonen, carried out in 36 patients, seventeen patients with operable or recurrent malignant gliomas receiving sitimagene ceradenovec implicated a survival advantage over control patients who did not receive gene therapy. The mean survival of the patients in the sitimagene ceradenovec group (70.6 wk) was significantly longer ($P < 0.0095$) when compared to the standard care group (39.0 wk) or a historical control group ($P < 0.0017$). This study was also historically the first randomized, controlled trial with sitimagene ceradenovec where increased survival of the patients was shown when compared to standard therapy. The results from the study were very

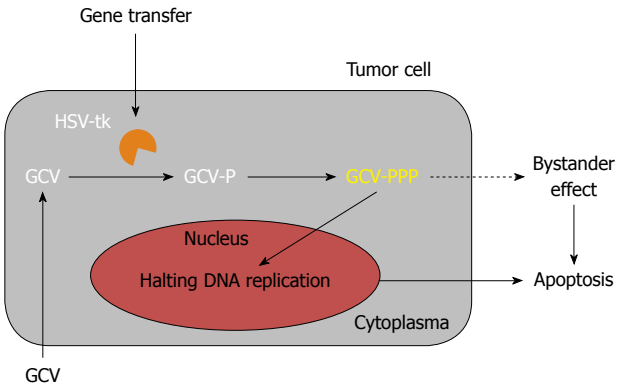

Figure 3 Sitimagene ceradenovec is an adenoviral vector based suicide gene therapy, which is injected into the wall of the tumor cavity of glioma patients, after the resection of the tumor. Mainly healthy cells will be transduced producing the HSV-tk and converting the pro-drug ganciclovir into ganciclovir monophosphate. The actual cytotoxic metabolite ganciclovir triphosphate is killing possible residual proliferating tumor cells.

encouraging and it was concluded that sitimagene ceradenovec could provide an effective adjuvant treatment for patients with operable primary or recurrent malignant glioma. Therefore a multicenter, standard care controlled, randomized clinical phase Ⅲ trial was commenced. However, the results from that trial were not as significant as those from the previous Ⅱb trial. As a result, suggestions by the EMA were given for further clinical evaluation as they concluded that the data did not provide sufficient evidence of significant clinical benefit compared to current standard treatment.

IMPROVING GENE DELIVERY

One of the major hurdles has been how to get the relevant genetic material into a sufficient number of target cells and how to avoid the transduction of non-target cells (i.e. how to target the gene transfer vector to cells of interest). Obviously, as gene therapy has matured from clinical trials to the first commercial products, understanding of the mechanisms of gene delivery has increased notably. This is also reflected in the progress we have made in the development of viral vectors[50-53]. A number of improvements have been achieved in order to tackle issues of transduction efficiency, biodistribution and safety[51,52,54,55]. Ideally, a gene transfer vector should be able to efficiently and specifically transduce the target cells (dividing and non-dividing) and result in the expression of the transgene for a sufficient duration of time. The vector should not have any limitations in the transgene insertion capacity and it should be able to be manufactured easily and cost effectively in high concentrations. Furthermore, the vector should not induce any immune responses within the host, enabling repeated, safe vector administrations without adverse effects. In order to fulfill these criteria, different strategies have been exploited. However, none of the strategies are without limitations. For example, to improve gene transfer efficiency, specificity and thereby patient safety, target cells

may be removed from the patient, transduced with viral vectors and re-introduced back into the patient[5,56,57]. This approach has been shown to be effective, but is limited to the cells which are available (i.e. either by extraction or by growing from the stem cells *in vitro*)[58]. When the vector is delivered directly to the patient (*in vivo*), either locally (for example intratumoral) or systemically (i.e. into the blood circulation), a limiting factor can be the size and shape of the gene transfer vector, resulting in poor distribution within the tissue (when administered locally into the tissue) or the lack of specificity when administered systemically. To improve specificity and hence also safety of systemically administered gene delivery vectors, the surface of these vectors has been modified[52,59-62]. For example, retroviruses and lentiviruses have been frequently pseudotyped to widen their tropism, increase their yield in production and improve their safety, most often with the Vesicular Stomatitis virus G-protein[63-65].

CONCLUSION

Gene therapy is an intriguing therapeutic modality and sooner or later will be part of the standard care for a variety of different diseases. However, at the same time, when the first patients were treated utilizing gene therapy based technology, debates about the ethical aspects of gene therapy started[66-69]. It is important that we acknowledge and understand the differences in human beings and in what they believe in. Obviously, there are concerns when it comes to the use of gene therapy. We have to ask ourselves several questions before we can justify gene therapy in humans: What is our current understanding regarding gene therapy? For example, what are the technical details of the DNA and vector to be used? The technical aspects involved, risks endeavored by the patient, and the fear of human genetic engineering are some of the major reasons why human gene therapy trials have long been difficult to conduct. Are we able to control this technology? In which diseases is gene therapy is ethically acceptable and what are the costs for this type of therapy? Why is gene therapy more tolerated for life-threatening diseases (e.g. diseases like cancer or AIDS) than in the correction of learning disorders? Also, somatic gene therapy appears to be more tolerated than germline gene therapy. Where do we draw a line when dealing with genetic or chromosomal disorders? Would it be ethically acceptable to practice gene therapy on people with Dawn syndrome? What would be the justification of using gene therapy in the enhancement of some individual physical or mental properties? The use of viral vectors, such as lentiviruses and adeno-associated viruses, raises skepticism because of their ability to integrate into the genome[11,65,70,71]. Understandably, this raises concerns about the safety of these vectors. This is furthermore supported by the somewhat contradictory data available in the literature[72,73]. Non-viral vectors are not efficient enough yet but have gained better acceptance in the society. Despite the progress in gene therapy research, we are still at the beginning of the era of gene therapy based medicines. Emphasis should be put on the development of targeted and regulated gene transfer vectors. Especially in case of integrating vectors (such as retroviral vectors), emphasis should be laid on the development of vectors, where the integration of the transgene can be controlled, in order to avoid insertional mutagenesis. In either case, it is of utmost importance that the normal principles of good clinical research apply in the conduct of the ethical evaluation of gene therapy protocols. The safety of an individual must be the first concern of the treatment protocols and, last but not least, the integrity and free will of a patient should be respected.

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3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

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6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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