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Maintenance of the rat transgenic model of familial amyotrophic lateral sclerosis expressing human SOD1^{G93A} mutation

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Abstract

A colony of transgenic rats expressing the human mutant Cu,Zn superoxide dismutase gene (hSOD1^{G93A}) that is associated with some cases of familial form of amyotrophic lateral sclerosis (ALS) has been maintained in the Animal House of the Polish Academy of Sciences Medical Research Centre since 2003. This transgenic model, generated by Howland et al. (Proc Natl Acad Sci USA 2002; 99: 1604-1609), has been obtained under the material transfer agreement from Wyeth Corporation. The transgenic SOD1^{G93A} (or 'Howland') rats develop neurological and neuropathological symptoms reminiscent of human ALS, i.e. progressive loss of motoneurons leading to paralysis and death. This paper describes maintenance of the transgenic rat colony, and general procedures used in experiments with these animals (i.e. genotyping, neurological observations, anaesthesia, etc.). At the beginning of the colony, up to the 3rd generation of the rats, symptoms of the model disease appeared at 95-125 days of age, and the animals survived till 120-145 days of age. Thereafter a gradual change in the disease phenotype occurred, and in the 8th generation approximately 1/3 of the rats displayed much slowed disease progression.

Key words: *motor neuron disease, neurodegeneration, transgene, Cu,Zn superoxide dismutase, phenotype, variability.*

Introduction

Amyotrophic lateral sclerosis (ALS) is an incurable fatal human neurodegenerative disease that predominantly affects motoneurons and causes death from respiratory paralysis, usually within 3-5 years from onset [2]. About 10% of ALS cases are familial (fALS); the inheritance is usually due to an autosomal dominant trait [13]. In approximately 1/5 of fALS cases the disease is caused by mutations in the gene that codes for Cu,Zn superoxide dismutase (SOD1) [4,8,19]; more than 100 such mutations have been identified so far.

A transgenic mouse carrying the mutation in the human *SOD1* transgene that results in glycine93→alanine substitution in a hSOD1 molecule *(hSOD1^{G93A})* was the first published animal model of fALS [8]. It remains highly popular because it imitates major clinical (i.e. progressive paralysis) and histopathological (i.e. selective motoneuron death) features of ALS. More recently, a few transgenic rat models of fALS have also been created; the rats are endowed with multiple copies of and highly overexpress either the same [10,14] or another variant of mutant human SOD1 [14]. While usually showing a faster progression of the model disease than the

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corresponding transgenic mice, these rat lines also recapitulate a number of characteristics of the respective mutant SOD1-associated fALS variants [10,14]. The transgenic rats are expected to facilitate experimental studies on ALS due to larger animal size that allows sufficient tissue or CSF for biochemical studies to be obtained, implanting infusion pumps for chronic intrathecal drug delivery at the desired level of the spinal cord, and performing and monitoring therapeutic delivery of neural stem cells.

The aforementioned transgenic rats carrying the *hSOD1*^{G93A} gene develop, beginning usually during the 4th month of life, symptoms of an incurable illness that imitates ALS in most respects, and are an established model of fALS with early onset and a short symptomatic phase. This provides a unique opportunity to evaluate potential treatments. Transgenic animals carrying the mutant human gene are subject to active treatment beginning usually at some time point before the emergence of disease symptoms, whereas the respective transgenic controls are given the vehicle. The absolute end-point of the study is disease-related death, with an intermediate check-point delineated by the emergence of neurological deficits; the therapeutic effect is measured by the increase in time needed for the events to occur.

This paper describes a colony of transgenic rats expressing the mutant human *SOD1*^{G93A} gene and developing the model paralytic disease, which is maintained in the Animal House of the Polish Academy of Sciences Medical Research Centre in Warsaw. These rats were used in research aimed at characterizing neuropathologic and ultrastructural phenotype of the disease which have been the subject of successive reports [5,15,17], and in studies of some pharmacologically active compounds, the results of which will be published elsewhere.

Materials and methods

Transgenic Sprague-Dawley rats carrying the mutant *hSOD1*^{G93A} gene [SD-Tg(hSOD1^{G93A})], which are used in the Polish Academy of Sciences Medical Research Centre in Warsaw, were generated and described by Howland et al. [10]. A colony of the rats was established in the local animal facility in 2003. The original breeding material of hemizygotic males carrying the mutant transgene was obtained from Taconic Farms under the material transfer agreement with Wyeth Co. (USA). Simultaneously, the stock of outbred Sprague-Dawley (SD) rats was purchased from Taconic Farms; this stock is propagated in parallel with the transgenic rats as a source of healthy females for reproduction with the hemizygotic males.

The colony was founded using 6 SD-Tg(SOD1^{G93A}) males mated each with two SD females (Tac:SD); this mating ratio has been continued until the present time. A pregnant female is always kept separately from the others for the delivery and weaning period. The rats are given free access to standard pelleted laboratory rodent chow and filtered tap water, are bred and kept behind a sanitary barrier in a controlled environment, at 22±2°C, 50-60% relative humidity, 13h/11 h light/dark cycle (lights on at 07:00 a.m.), and have specific pathogen-free (SPF) health status.

To avoid drift in the disease onset time, *hSOD1*--positive males used for breeding were checked daily for the emergence of disease symptoms starting on the 90th day of life. If the onset of symptoms was delayed past the 115th day of life, male offspring of such a "slow performer" were not used for further breeding.

The offspring are genotyped for the presence of the transgene by tail DNA blot as described by Howland et al. [10]. In short, at the age of 3-4 weeks all litters are tagged with ear clips (National Band and Tag, Newport, KY, USA) and their tail tips are taken for genotyping. DNA from the tips is isolated by incubating the tissue with proteinase K, and subjected to PCR using *hSOD1*-specific primers SOD-i3f (5'-GTGGCATCAGCCCTAATCCA-3') and SOD-E4r (5'-CACCAGTGTGCGGCCAATGA-3') [10]. The presence of the transgene in the PCR product is verified electrophoretically (Fig. 1). The white bands of approximately 200 bp size (for comparison see the GeneRuler™ 100 bp DNA Ladder on the right size of the picture) reveal the presence of the *hSOD-1* gene in approximately half of the rats in each litter.

All animal use procedures were in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and were accepted by the local animal experimentation ethics committee. Central nervous tissue (i.e. brain and spinal cord) sampling was performed after the animals were given an intraperitoneal injection of an aqueous solution of chloral hydrate (3% w/v, 11 ml/kg body weight) and decapitated while deeply anaesthetized. More details on tissue processing are given in the reports that follow.

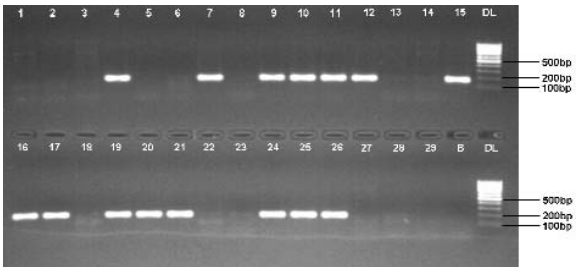

Fig. 1. A typical result of PCR genotyping showing the difference between transgene-positive (lanes 4, 7, 9-12, 15-17, 19-21, 24-26) and transgene-negative (lanes 1-3, 5-6, 8, 13-14, 18, 22-23, 27-29) rats. B – reagent blank lane; DL – lane with 100 bp DNA ladder

Fig. 2. A transgenic Sprague-Dawley rat bearing the mutant *hSOD1*^{G93A} gene; terminal stage of the model disease

For experiments with pharmacological treatments (results of which will be reported elsewhere) paired groups of rats were formed by randomizing transgenic littermates to the group that was to be treated actively and the control group that was to be treated with vehicle. Treatments started on day 61 of age, when all animals were still asymptomatic. The rats were tested every day for the presence of disease symptoms. The date of the start of symptomatic motor neuron disease was taken as the day on which an abnormal gait or evidence of hindlimb weakness was noted. Disease-related death was scored on the day on which full paralysis of at least 3 limbs occurred; this was preceded by substantial weight loss, poor grooming and porphyrin staining around the eyes (Fig. 2). The data concerning asymptomatic survival and total survival were used to plot Kaplan-Meier curves.

Results

From August 2003 to May 2006, a total of 151 litters were delivered in the colony. The average number of live newborns per litter was 9.9±3.4 (mean±SD), median: 10 rats/litter, mode value: 12 rats/litter. The total number of rats born was 1515, including 802 females (52.9%) and 713 males (47.1%). Of these, 764 (50.4%) rats were genotyped as transgenic *(hSOD1+)* and 751 (49.6%) as non-transgenic *(hSOD1-)*. The male/female ratio was 1.08 for the transgenic and 1.17 for the non-transgenic rats. All pups survived to weaning.

In the first two generations of the locally bred transgenic hSOD1 rats, 'clinical' symptoms of the model disease appeared between day 95 and day 125 of life and death was scored on day 118 to day 146 in untreated rats (Fig. 3). Starting with the 3rd generation, a small subset of transgenic rats developed disease symptoms later than on day 125 of life and survived for more than 146 days. The relative size of this subset grew from generation to generation and amounted to about 1/3 of untreated transgenic rats in the 8th generation. The survival curve for the remaining (short-surviving) subgroup in this generation remained similar to that for the 2nd generation (Fig. 4). There was no statistically significant gender-related difference in the duration of symptom-free survival and total survival of the transgenic rats (data not shown).

Discussion

Ectopic expression of a transgene may be achieved by microinjections of a cloned DNA construct into the pronuclei of fertilized eggs [9,16]. The injected eggs are then introduced into pseudopregnant females and allowed to develop. If integration into the genomic DNA occurs at the single cell stage, the integrated DNA will be contained in all cells of the body including germline cells and will become a heritable addition to the genome. Usually the DNA construct used for transgenic production is designed to contain a transgene of interest expressed under the control of

a regulatory promoter element which designates when (i.e. at what developmental stage) and where (i.e. in what cell types) transgene expression will take place in the transgenic animal.

Many experts believe that transgenic rodent animal models of ALS provide a good opportunity to study multiple characteristics of the disease, and to evaluate preclinically potential therapeutic effects of new treatments [6,7,12]. For such an evaluation transgenic animals carrying the mutant human gene (in most cases these are transgenic mice bearing human $SOD1^{G93A}$ mutation) are subject to active treatment beginning usually before the predicted time point of the emergence of disease symptoms; their symptom-free and/or total survival is compared with those of the respective control group. Numerous reports have been published that show the efficacy of various substances and procedures in prolonging survival of these animals. To quote just a few of the most recent ones: prolongation of survival in murine transgenic models of ALS was noted after administration of tianeptine (a selective serotonin reuptake enhancer) and/or morphine [3], thalidomide as well as its derivative enalidomide (immunomodulating drugs interfering with TNFalpha and other proinflammatory cytokines) [11], or ketogenic diet [22]. Recently, of 113 compounds reviewed by Traynor et al. [20] 24 drugs were identified that have been tested in animal models of ALS and proved somewhat effective. After more detailed pharmacological evaluation, 20 of these drugs were found suitable for further clinical evaluation in ALS. Experiments performed in transgenic rodent models of ALS are, indeed, helpful in selecting compounds for clinical development. However, considering the estimated prevalence of ALS, which is about 1 in 100,000 [1], the number of drugs selected for development is, perhaps, still too high. A remedy might be in further refinement of transgenic models of ALS.

According to our experience with the local colony rats expressing the human mutant *$SOD1^{G93A}$*

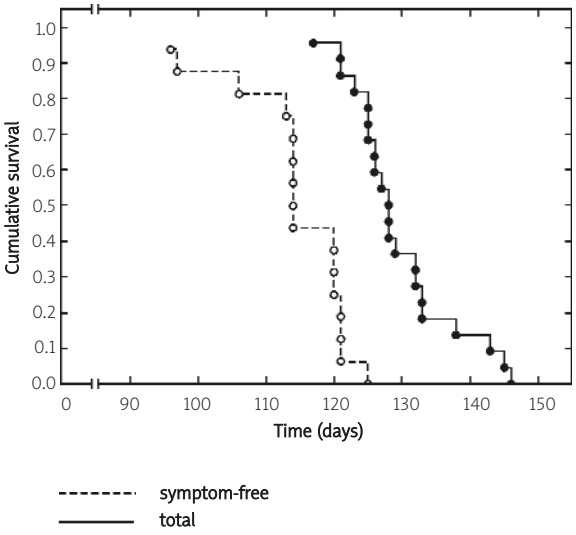

Fig. 3 Kaplan-Meier curves of symptom-free survival (open circles, dashed line) and total survival (closed circles, solid line) of locally bred 3rd generation untreated SD-Tg ($hSOD1^{G93A}$)

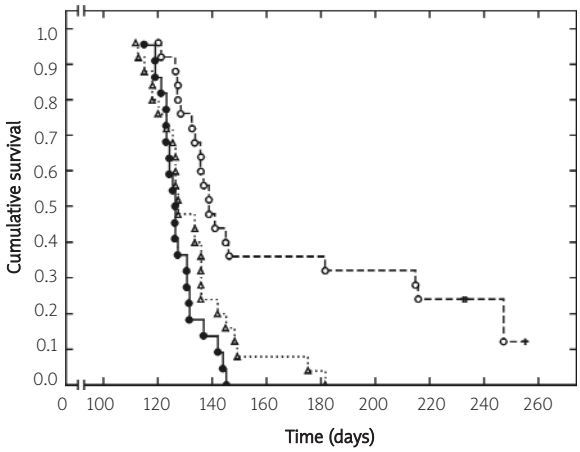

Fig. 4. Kaplan-Meier curves of total survival at the beginning of colony propagation (closed circles, solid line – rats born as the 2nd generation of the colony; triangles, dotted line – rats born as the 3rd generation of the colony) and 2.5 years later (open circles, dashed line – rats born as the 8th generation of the colony). Markedly prolonged survival in about 1/3 of the 8th generation rats is evident

transgene, the phenotype of the model disease undergoes a drift over several generations even when the males are carefully selected for reproduction based on their disease phenotype. The cause of this drift is unknown. It may result from phenomena resembling transgene elimination in transgenic plants (see [18] and the references cited therein), or from an RNA-based mechanism that directs genome-wide DNA rearrangements and serves to disable invading genetic agents, which has been described in *Tetrahymena* [21]. Whatever is the nature of the mechanism that underlies phenotypic change in the rat transgenic model of fALS, experiments with these rats should always be performed by randomizing transgenic littermates between study groups that are to be compared.

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