Establishment of tsA58 Transgenic Rats as a Source of Conditionally Immortalized Cell Lines with Differentiated Functions

Ri-ichi TAKAHASHI

YS New Technology Institute Inc., 519 Shimoishibashi, Ishibashi-machi, Shimotsuga-gun, Tochigi 329-0512, Japan

Abstract: To isolate a variety of rat cell lines with differentiated functions, we developed transgenic rat lines that ubiquitously express the temperature-sensitive large T-antigen gene of the simian virus 40 (SV40) tsA58 mutant under the control of the SV40 large T-antigen promoter. These rats might be advantageous for simultaneously establishing cell lines from different tissues of rats with the same genetic origin. The transgenic rat lines transmit a functional copy of the transgene and were bred with sib mating to generate the homozygous transgene. The established cell lines from this transgenic rat had temperature dependent growth and retained some of the differentiated functions of each particular tissue, and were useful as a ready source of novel conditionally immortalized cell lines. The possible use and perspectives of these transgenic cell lines are discussed.

Key words: temperature-sensitive large T-antigen, transgenic rat

Introduction

All organs and tissues in the body are derived from a single cell, the fertilized egg, by the developmental processes of proliferation and differentiation. These developmental processes generate cellular diversity and order within each developmental stage, and the differentiated cells are organized into tissues and organs. Further, the differentiation order within each generation ensures the continuity of life from one generation to the next. To understand the function of differentiation, how tissues or organs composed of various types of distinct cells are generated during development and maintain their functions during adult life. And what kinds of proteins or genes are required to express a specialized function of tissues or cells? The establishment of various types of cell lines with their original function would be a valuable tool for studying development, morphogenesis, tissue maintenance, and aging.

Appropriate cell lines are very important for studying the biological functions of the body at the cellular and molecular levels. Progress in regenerative medical treatment also depends on a variety of cell lines for the study of cell transplantation to repair damaged tissues. In research and development of pharmaceuticals, cell-based testing is very cost- and time-effective compared

(Received 5 September 2001 / Accepted 26 November 2001)
with *in vivo* testing in experimental animals, and is advantageous for mass screening of candidate pharmaceuticals. In addition, there is high demand for a reduction in the use of experimental animals for toxicology testing and for the development of a method to replace conventional animal testing in the development of pharmaceuticals. Many attempts have been made to use cell lines or reconstructed tissue-like systems with combinations of some cell lines for the substitution of *in vivo* testing, and cell lines that maintain their original functions would be a valuable alternative to *in vivo* testing.

Thus, there is high demand for a variety of cell lines with differentiated functions in medical and pharmaceutical sciences. This review discusses the development of transgenic rat lines that ubiquitously express the large T-antigen gene of a temperature-sensitive mutant strain of simian virus 40 (tsA58), which is a useful tool for establishing conditionally immortal cell lines from a variety of tissues.

---

### Establishment of cell lines from tissues

It is not easy to establish cell lines with differentiated functions from tissues. Generally, the cells can be prepared by simply dissociating the tissue of interest and culturing in appropriate conditions. Initially, the cells proliferate actively. Growth of the cells, however, gradually slows, and almost all cells stop proliferating due to senescence. If cell lines are obtained from the rare cells that carry mutations in growth-restraining genes, most of them have already lost their original differentiated functions. To overcome the difficulty in establishing cell lines with differentiated functions from various tissues, cells are often transfected with various oncogenes. Introduction of the large T-antigen gene of a temperature-sensitive (ts) mutant strain of simian virus 40 (tsA58) into cells is advantageous for the establishment of immortalized cell lines [13], and transgenic mice harboring the temperature-sensitive large T-antigen gene have been successfully established [14, 20]. Many cell lines with differentiated functions have been established from different tissues of these mice [15, 16] and these transgenic mice are very effective for establishing various cell lines from different tissues.

---

### Why we use the rat?

Rats are widely used in the study of pharmacology and toxicology and there is a substantial amount of background data on the toxicologic parameters of rats. As rats are generally 10 times larger than mice, consecutive blood collections, biopsies from organs such as liver, large sample collections, and surgical treatments are more easily performed. Using the rat simplifies studies of biochemistry, pathology, and toxicokinetics. In addition, numerous experimental rat models have been developed to study hypertension, autoimmune diabetes, rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, encephalomyelitis, spondyloarthritis, and other diseases [2, 3]. Therefore, rats are actively used in the research and development of new pharmaceuticals and a vast number of various experiments have been performed using rats. What is the most important factor for choosing experimental animals? In basic biology, mice are genetically well-characterized and are essentially the only animal that is widely used for genetic engineering, such as transgenic mice for "gain of gene function" and knock-out mice for "loss of gene function". Mice, however, cannot be used for all purposes. Charrau et al. (1996) [4] reviewed the cases in which rats expressed conditions resembling those in humans and compared them to cases in mice. For example a transgenic rat carrying transgenes for HLA-B27 and human beta 2-microglobulin (spondyloarthritis) exhibits a spontaneous autoimmune condition similar to that in human disease [6], however, this disease condition does not appear in transgenic mice with the same transgene. The success of an experiment with rat cell lines can be extrapolated to *in vivo* experiments with rats. With the proper conditions for *in vitro* testing, research and development of pharmaceuticals can be performed more cost- and time-effectively. Cell lines from various tissues, especially from very small tissues, for example, the blood-cerebrospinal barrier, blood-retinal barrier, and urinary tubule, can be established more effectively in rats than in mice.

---

### Strategy for the use of transgenic rats to establish immortalized cell lines

Transgenic rats harboring the tsA58 large T-antigen gene that ubiquitously express the gene among differ-
ent tissues, are advantageous for establishing immortalized cell lines from various functional tissues from embryo to adult. These transgenic rat lines can be bred through many generations and are a ready source of novel conditionally immortalized cell lines (Fig. 1). Therefore, we introduced the gene into rats using a pronucleus DNA microinjection method, and established the transgenic rat lines. Further, we attempted to establish homozygous transgenic rat lines and to fix the chromosome number of the integrated gene using the fluorescence in situ hybridization (FISH) method [18]. To supply the animals, an embryo-bank was developed.
by mating homozygous transgenic male rats with wild type female rats.

In addition to the advantages for toxicology testing and biomedical science, stem cells, such as undifferentiated progenitor cells in various developmental stages, can be established from fetuses of these transgenic lines.

**Establishment of transgenic rats**

Twenty-three candidates of the transgenic line were obtained from 564 microinjected eggs. The integration efficiency (23/564) was consistent with that of a previous report [9]. It was most remarkable that over 10% (14/113) of the pups died before weaning and the majority of them (10/14) were transgenics. Over half (12/23) of the pups carrying the transgene died within 4 weeks after birth. Others lived and sexually matured. Most (15/18) of mice introduced the large T-antigen gene of SV40 died before sexual maturation [17]. Therefore, the large T-antigen gene might be lethal in the early postnatal period. Finally, 5 lines were established from 23 candidate lines and survived for at least 6 months. In particular, four of the five lines were bred with normal reproductive efficiency. The is large T-
antigen gene was expressed in the fetal fibroblast cells prepared from the established transgenic lines and temperature dependent cell growth was evident in the cells from lines #1507-5 and #1519-8. Both transgenic rat lines were successfully generated as homozygous and can breed with normal reproductive efficiency equal to that of their heterozygotes (Fig. 2). These results indicate that these two lines are very advantageous for practical use, and differ from that of a previous report on transgenic mice harbouring the same gene [20].

Selection of appropriate transgenic lines from many candidate lines is necessary for establishing a transgenic line expressing the ts large T-antigen gene.

Established cell lines from tsA58 transgenic rat

Blood-organ barrier cell lines

The blood-organ barriers have a key role in maintaining a constant milieu and restricting the entry of substances from circulating blood to organs such as the brain and retina. Brain (TR-BBB) and retinal (TRiBBR) capillary endothelial cell lines have been established from the transgenic rat line #1507-5 [10, 11]. These cell lines have a spindle-fiber shape morphology, express a typical endothelial marker (i.e., von Willebrand factor), and exhibit uptake activity of acetylated-low density lipoprotein. Moreover, they express vascular endothelial growth factor (VEGF) receptor-2. These cell lines express a large T-antigen and grow well at 33°C but do not grow at 37°C or 39°C due to inactivation and decomposition of the large T-antigen. Consequently, temperature-dependent cell growth was observed. These cells expressed GLUT1, which is capable of 3-O-methyl-D-glucose transport activity. The Michaelis-Menten constant in the cells was similar to that in the capillary blood vessels of the brain and retina. In addition to the expression of P-glycoprotein with a molecular weight of approximately 180 kDa, expression of mdr 1a, mdr 1b and mdr 2 genes was detected using reverse transcriptase-polymerase chain reaction analysis. These cell lines would be a good in vitro model for drug transportation to the brain or retina and valuable for examining possible delivery of drugs to the brain and retina [19].

Bone marrow-derived endothelial cell lines

Post-neonatal neovascularization is thought to result exclusively from the proliferation, migration, and remodeling of fully differentiated endothelial cells. Hattori et al. (2001) [7] reported that bone marrow contains cells that can differentiate into endothelial cells and contribute to neoangiogenesis in adults. They established three independent cell lines from the transgenic rat line #1507-5 (TR-BME) incorporating 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate with a spindle shape. One of the lines strongly expressed VEGFR-2, and weakly expressed VEGFR-1 and the von Willebrand factor. In contrast, another of the lines strongly expressed Tie-1, 2, and the von Willebrand factor, and weakly expressed VEGFR-1, 2. All markers were expressed strongly in another tested cell line. These data confirm that the above three TR-BME cell lines are novel endothelial cells derived from bone marrow progenitors. These cell lines will be useful for examining the interactions between endothelial cells and organ/tissue cells. In the future, it will be possible to inhibit or activate angiogenesis in situ using these cells as vehicles for gene therapy.

Other organs

Research projects using this transgenic rat have become more widespread since 1998. The rats have been supplied to over 20 institutes. Cell lines from various organs/tissues will be continuously established.

Future studies

Availability of appropriate cell lines with original differentiated properties is very important in the study of tissue and organ functions. In vitro systems, such as culture, co-culture, and reconstructed tissue systems on micro-plates might allow high throughput screening of new pharmaceuticals, substituting for conventional procedures. A new in vitro model for the study of barrier function with conditionally immortalized cell lines [12, 19] has been reported and is very useful for examining the possible delivery of drugs into the brain or retina. On the other hand, stem cells with self-renewal and differentiation properties are useful tools to study cell transplantation for repairing damaged tissue. Allen et al. [1] reported that mouse hepatocytes established from H2kb-tsA58 transgenic mice can survive in vivo after
being transferred to the liver, and will be useful as a model for hepatic gene therapy. Cells with a marker such as green fluorescent protein are useful to study cell transplantation and cell migration. Recently, we established an enhanced green fluorescent protein (EGFP) transgenic rat [8], and confirmed the EGFP Tg rat is a useful tool for organ transplantation research [5]. The double transgenic rats (tsA58 x EGFP) will be more useful for studies of cell transplantation and cell migration studies as cellular therapeutic models.

The transgenic rat is a ready source of novel, conditionally immortalized cell lines and will be an effective tool in medical and pharmaceutical studies.

Acknowledgments

I wish to thank to Dr. M. Obinata (Institute of Development, Aging and Cancer, Tohoku University) and Dr. N. Yanai (Miyagi Gakuin Women’s College), Dr. M. Ueda (YS New Technology Inst., Inc.) and the staff of our lab. for their many suggestions and help.

References