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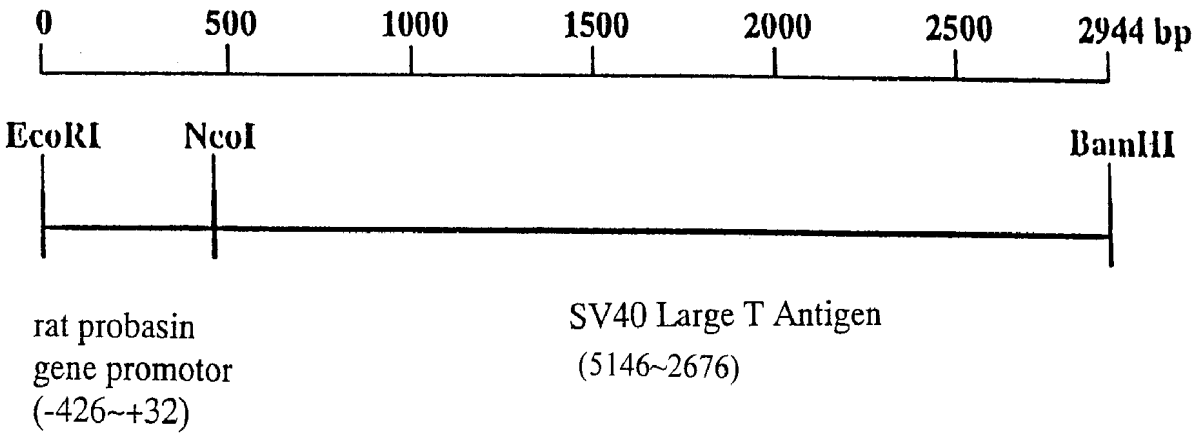

(57) Abrégé/Abstract:
To provide a rat model with the onset of prostate cancer in which the prostate cancer including an invasive cancer can be developed and can be bred for generations stably. A rat model with the onset of prostate cancer which can develop prostate cancer including invasive cancer and can be bred for generations stably is established by: ligating an SV40 large T antigen gene onto the downstream of rat probasin gene promoter; the resulting PBSVT transgene is introduced into a fertilized egg of a Sprague-Dawley rat; after the introduction, the fertilized egg is transplanted into a recipient rat; a transgenic rat obtained from the recipient rat is then mated with a wild-type Sprague-Dawley rat; the transgenic offspring rats thus obtained are similarly can be bred for generations thereafter; and transgenic rats developing prostate cancer are selected through histopathological observation of the prostate.

ABSTRACT

To provide a rat model with the onset of prostate cancer in which the prostate cancer including an invasive cancer can be developed and can be bred for generations stably. A rat model with the onset of prostate cancer which can develop prostate cancer including invasive cancer and can be bred for generations stably is established by: ligating an SV40 large T antigen gene onto the downstream of rat probasin gene promoter; the resulting PBSVT transgene is introduced into a fertilized egg of a Sprague-Dawley rat; after the introduction, the fertilized egg is transplanted into a recipient rat; a transgenic rat obtained from the recipient rat is then mated with a wild-type Sprague-Dawley rat; the transgenic offspring rats thus obtained are similarly can be bred for generations thereafter; and transgenic rats developing prostate cancer are selected through histopathological observation of the prostate.

SPECIFICATION

RAT MODEL WITH THE ONSET OF PROSTATE CANCER

TECHNICAL FIELD

The present invention relates to rat models with the onset of prostate cancer which can develop prostate cancer including invasive cancer and can be bred for generations stably, and to methods of establishing the same. More specifically, the invention relates to rat models with the onset of prostate cancer introduced with an SV (simian virus) 40 large T antigen gene which is under the control of probasin gene promoter, wherein the rat model can develop prostate cancer including invasive cancer in almost all the cases by the age of 15 weeks and can be bred for generations stably, and is particularly related to the PBSVT transgenic rat 2971 lines and to methods of establishing the rat. The present invention also relates to screening methods or the like for a substance promoting the onset and/or progress of, or suppressing the onset and/or progress of prostate cancer, where the rat model with the onset of prostate cancer is used.

RELATED ARTS

Prostate cancer is the second most common cause among cancer deaths in Western countries. In Japan, prostate cancer currently remains the eleventh cause of cancer deaths. However, with the prolonged average life expectancy, the number of prostate cancers is expected to increase rapidly. While the etiology of prostate cancer is largely unknown, it is androgen-dependent in its early stages, and then acquires independency, which will acquire drug-resistance. In order to elucidate the developmental mechanism of prostate cancer that undergoes such multiple stages, animal models of prostate cancer generated from various species and from various lines are needed.

Several types of animal models for prostate cancer have been generated and have been utilized to elucidate the developmental mechanism of prostate cancer and its modifiers. A previous report shows that a long latent period of approximately 20 months is needed to generate animal models that develop high incidences of

spontaneous malignant tumors with ACI/Seg and Lobund-Wistar rats. It is known that lateral prostate cancer in ACI/Seg rats is most moderately differentiated non-metastatic adenocarcinomas with a cribriform pattern, while prostate cancer in Lobund-Wistar rats is poorly differentiated adenocarcinomas with occasional metastasis.

The present inventors have established rat models of prostate cancer using the followings as carcinogens: N-methyl-N-nitrosourea (MNU); N-nitrosobis (2-oxopropyl) amine (BOP); 3,2'-dimethyl-4-aminobiphenyl (DMAB); and 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP). The inventors have successfully generated rats developing prostate cancer with high incidence by combining carcinogen administration with the induction of cell proliferation (Jpn. J. Cancer Res., 76: 803-808, 1985). These lesions of prostate cancer were originated in the ventral lobes, and most of them showed a cribriform pattern with no invasion. Their sizes were generally small with no sign of metastasis. Prostate cancers induced by DMAB demonstrated different specificity among the animal species. F344 and ACI rats were the most susceptible, and Wistar and Sprague-Dawley rats showed resistance, yet their histopathological patterns displayed no obvious species specificity.

As in the cases of MNU and BOP, the development of prostate cancer by DMAB can be modified by the long-term and high-dose administration of testosterone propionate (TP). The present inventors subcutaneously implanted TP, which was incorporated into silicon tubes, to raise the serum testosterone concentration to 10-fold and found that the cancer spectrum of the sexual organs of rats that were given DMAB shifted from the ventral lobe to the dorso-lateral and anterior lobes as well as to the testis (Cancer Res., 51:1264-1269, 1991). In contrast to the ventral lobe prostate cancer, the prostate cancer developed in the latter was invasively growing adenocarcinomas with metastatic lesions in the abdominal cavity, liver, lung, and the like. It is also known that the shifting of cancer spectrum from the ventral lobe to the dorso-lateral and anterior lobes and to the testis depends on the dose and duration of TP, and that the degree of their invasiveness depends on the same (Cancer Lett., 83: 11-116, 1994, Jpn. J. Cancer Res.,86:645-648, 1995).

Molecular studies of cancer induced by carcinogen administration have suggested

that Ki-*ras* gene is involved in the prostate and testis cancers induced by DMAB. Three out of nine adenocarcinomas in the ventral lobe, five out of 13 adenocarcinomas in the dorso-lateral lobe and one out of 11 adenocarcinomas in the testis, demonstrated point mutations in the Ki-*ras* genes. In contrast, no mutation was found in Ha-*ras* or p53 genes (Mol. Carcinog., 13:21-26, 1995). There was no difference in the occurrence of *ras* gene mutation between invasive and non-invasive cancers. Therefore, it is thought that in experimental rat prostate cancers, the activation of the *ras* protooncogene by point mutation is not associated with the cancer progression and that deactivation of the p53 gene does not correlate with the progression either.

Atypical hyperplasias in the whole regions and the ventral lobe carcinomas are immunohisitochemically androgen receptor-positive, but invasive cancers in the dorso-lateral and anterior lobes are not. Three cell lines established from DMAB plus TP-induced invasive prostate cancer in the dorso-lateral lobes are reported to grow in an androgen-independent manner in vivo (Jpn. J. Cancer Res., 87:1218-1226, 1996, J. Toxicol. Pathol., 11:27-32, 1998). Castration after the treatment with DMAB alone or DMAB plus TP completely arrested the progress of cancers in the ventral lobes that were androgen receptor-positive but it did not terminate the progress of invasive cancers in the dorso-lateral and anterior lobes of the prostate, or in the testes that were androgen receptor-negative (Jpn. J. Cancer Res., 90:23-30, 1999). The recent analysis of the androgen receptor-gene with the use of adenocarcinoma cell lines demonstrated hypermethylation in the promoter region of the receptor, which is thought to be the reason for the androgen-independency of invasive cancers.

Although the induction of prostate cancer through administering carcinogens as described above allows various types of prostate cancers to develop, the animals have to be kept for a long period of one year or more before the development of cancer. Therefore, animal models introduced with cancer-causing genes have been actively generated in recent years. To date, several transgenic animals for prostate cancer have also been reported. Mice, generated by Greenberg in 1995 with the introduction of the probasin promoter of 0.5 kb and the SV40 large T antigen gene, developed prostate cancers that were metastatic. Mice, generated by Barrios in 1996 with the

introduction of the probasin promoter of 0.5 kb and *ras* T24 gene, showed hyperplasias in their prostates but did not develop cancers. Mice, generated by Yan in 1997 with the introduction of the probasin promoter of 11.5 kb and the SV40 large T antigen gene, developed prostate cancers. Mice, generated by Maroulakoou in 1994 with the introduction of the C3 (1) promoter and the SV40 large T antigen gene, developed prostate cancers. Mice, generated by Tehranian in 1996 with the introduction of the C3 (1) promoter and the polyoma middle T gene, developed prostate cancers. Mice, generated by Zhang in 1997 with the introduction of the C3 (1) promoter and the human bcl-2 gene, had hyperplasias in the prostates but did not develop cancers. Mice, generated by Perez-Stable in 1996 with the introduction of the fetal G γ -globin promoter and the SV40 large T antigen gene, developed prostate cancers. Mice, generated by Kitsberg in 1996, with the introduction of the MMTV promoter and the KGF gene, showed hyperplasias in the prostates but did not develop cancers.

Animal models developing cancers are indispensable both for elucidating the developmental mechanism of cancer and for developing drugs for the treatment. Animal models of various cancers have been generated up to now, but substances and methods for induction of the cancer differ and experimental menu varies depending on animal species used. Therefore, the establishment of model animals of as many species as possible is needed. In this regard, rats are excellent models for acquiring sufficient materials for the analysis because, like mice, the periods for their gestation and sexual maturation are short, they deliver many, something like 10 neonatal rats at one time and because their weight is about 10 times as heavy as mice. In addition to these, rats have many other useful features such as their piled up data concerning chemically caused cancers. However, rat models with the onset of prostate cancer that can surely develop prostate cancer and can be bred for generations stably have not been established. The subject of the present invention is to provide a rat model with the onset of prostate cancer that can develop prostate cancer including invasive cancer and can be bred for generations stably.

DISCLOSURE OF THE INVENTION

The present inventors have made a keen study to solve the subject mentioned above and have found that rat models with the onset of prostate cancer, which can surely develop prostate cancer and can be bred for generations stably, can be established through the following steps: introducing SV40 large T antigen genes that are under the control of probasin gene promoter into rats; breeding the resulting transgenic rats; observing their prostate histopathologically; and selecting the transgenic rat lines in which all the rats develop prostate cancer. Here the inventors have completed the invention.

The present invention relates to: a rat model which exhibits an onset of prostate cancer, develops prostate cancer including invasive cancer and can be bred for generations stably. The rat model has an SV40 large T antigen gene under the control of probasin gene promoter that is 5'-flanking region of 458bp from -426 to +32, introduced therein. The rat model may be obtained by the steps of: excising an SV40 large T antigen gene (5146-2676) with NcoI and BamHI; then binding it on a site downstream of 5'-flanking region (458bp: from -426 to +32) of a rat probasin gene promoter excised with EcoRI and NcoI; introducing the resulting probasin-simian virus 40 large T antigen (PBSVT) transgene of 2944bp into a fertilized egg of a Sprague-Dawley rat; transplanting the introduced fertilized egg to a recipient rat; and mating a transgenic rat born from the recipient rat with a wild-type Sprague-Dawley rat. The rat model may belong to PBSVT transgenic rat 2971 line.

The present invention also relates to: a method of establishing a rat model which exhibits an onset of prostate

cancer, develops prostate cancer and can be bred stably for generations, which method comprises the steps of:

(A) excising simian virus (SV) 40 large T antigen gene having a nucleic acid sequence from 5164 to 2676 with NcoI and BamHI;

(B) binding the SV40 large T antigen gene to a site downstream of 5'-flanking region of 458bp from -426 to +32 of a rat probasin gene promoter excised with EcoRI and NcoI to form a probasin-simian virus 40 large T antigen (PBSVT) transgene of 2944bp;

(C) introducing the resulting PBSVT transgene of 2944bp into a fertilized egg of a Sprague-Dawley rat;

(D) transplanting the fertilized egg into which the PBSVT transgene has been introduced, to a recipient rat;

(E) mating a transgenic rat born from the recipient rat with a wild-type Sprague-Dawley rat to obtain first transgenic offspring rats;

(F) breeding the transgenic offspring rat by mating the transgenic offspring rat with a wild-type Sprague-Dawley rat to obtain first filial generation offspring rats;

(G) histopathologically observing prostate of the first filial generation offspring rats; and

(H) selecting transgenic rats which exhibit an onset of prostate cancer among the first filial generation offspring rats.

The present invention further relates to a method for screening a substance that promotes the onset and/or

progress of, or suppresses the onset and/or progress of prostate cancer, wherein a test substance is administered to the rat model with the onset of prostate cancer, before or after the onset of prostate cancer, and the onset and/or progression severity of prostate cancer in the rat model is determined and assessed. The determination and assessment of the onset and/or progression severity of prostate cancer may be made by analyzing and assessing histopathological figures of the prostate cancer obtained from the rat model with the onset of prostate cancer. The determination and assessment of the onset and/or progression severity of prostate cancer may be determination and assessment of prostanoic acid phosphatase (PAP) and/or a prostate-specific antigen (PSA) produced in prostate cancer cells. The determination level of a wild-type rat of the same breeding line as the rat model with the onset of prostate cancer may be used for comparison and assessment, when determining and assessing the onset and/or progression severity of prostate cancer.

The present invention still further relates to a substance promoting the onset and/or progress of prostate cancer obtained by the above-mentioned screening method; a substance suppressing the onset and/or progress of prostate cancer obtained by the above-mentioned screening method; a drug to prevent, to suppress or to treat the prostate cancer; and a pharmaceutical composition used to treat a patient in need of suppressing the onset and/or progress of prostate cancer, wherein the pharmaceutical composition comprises, as an active ingredient, the substance suppressing the onset and/or progress of prostate cancer.

BRIEF DESCRIPTION OF DRAWING

Fig. 1 shows a fragment of PBSVT gene used to generate a transgenic rat model of prostate cancer of the present invention.

BEST MODE TO CARRY OUT THE INVENTION

Any rat line can be a rat model with the onset of prostate cancer of the present invention as long as it can develop prostate cancer including an invasive cancer in almost all the rats and can be bred for generations stably. A rat model with the onset of prostate cancer, including probasin-simian virus 40 large T antigen (PBSVT) transgenic rat 2971 line, exemplifies a rat model developing prostate cancer which is established from a transgenic rat introduced with a transgene (PBSVT transgene) to which SV40 large T antigen gene is bound. The SV40 large T antigen gene is expressed specifically in the prostate, and induces cancer by inactivating p53 and Pb proteins by directly binding to them under the control of probasin gene encoding androgen- or zinc-regulated proteins.

The method of establishing a rat model with the onset of prostate cancer of the present invention can be exemplified with previously known methods of generating transgenic animals (for instance, Proc. Natl. Acad. Sci. USA77: 7380-7384, 1980). The method of the invention will be described below with an example of the case wherein a gene fragment, in which the SV40 large T antigen gene is positioned under the foregoing probasin promoter control, is used as a transgene. A PBSVT transgene prepared according to the previously known method is amplified in an appropriate

expression vector and gene fragments (PBSVT transgenes) are excised after the amplification. These PBSVT transgenes are introduced into rat fertilized eggs in the pronuclear stage by microinjection and the post-introduction fertilized eggs are transplanted to recipient rats, and neonatal rats are obtained. DNA is extracted from part of the bodies (for example, tips of the tails) for southern blotting analysis or PCR assay and so on, to confirm whether the neonatal rats are introduced with PBSVT transgenes. Individuals confirmed to have the PBSVT transgene are the founders, which are then mated with normal wild type rats. 50% of the obtained neonates (F1) inherit the PBSVT transgene. These F1 rats that are confirmed the introduction of the PBSVT transgene are mated with normal rats, and the procedure goes on likewise, while observing the histopathology of the prostates and selecting transgenic rats that develop prostate cancer. A rat model line for developing prostate cancer can thus be established.

A promoter derived from a prostate specific antigen (PSA) or a prostate specific membrane antigen (PSMA) which is specifically expressed in the prostate can be also used for the foregoing transgene instead of the probasin gene promoter. SV40 large T antigen gene, which induces cancer, can be substituted with the large T antigen gene of SV40 thermo-sensitive mutant line tsA58, EIA gene of adenovirus, HPV16 gene of human Papilloma virus and the like. Further, Electric Pulse, Liposome, Calcium phosphate methods and so on can be conducted instead of the above-mentioned microinjection for an introducing method of fertilized eggs.

The above-mentioned PBSVT transgenic rat 2971 line, the rat model of the present invention for developing prostate cancer, which is created as a rat model with the onset of prostate cancer capable of developing prostate cancer including an invasive cancer and capable of breeding for generations stably, is established by the following steps: excising the SV40 large T antigen gene (5146-2676) with NcoI and BamHI, then binding it on the downstream of 5'-flanking region (458bp: from -426 to +32) of the rat probasin gene promoter excised with EcoRI and NcoI; introducing the resulting PBSVT transgene of 2944bp into a fertilized egg of a Sprague-Dawley rat; after the introduction, transplanting the fertilized egg to a recipient rat; mating a transgenic rat born from the

recipient rat with a wild-type Sprague-Dawley rat; breeding transgenic offspring rats obtained in a similar manner thereafter; observing the prostates histopathologically; and selecting transgenic rats that developed prostate cancers. The PBSVT transgenic rat 2971 line is maintained at the 1st Department of Pathology, Nagoya City University Medical School, where the rats of this line are available under certain conditions for those concerned.

The rat model of the present invention with the onset of prostate cancer as described above can be used not only for elucidating the developmental mechanism of prostate cancer but for screening any substance to promote or suppress the prostate cancer development and progression, and so forth. These screening methods, for example, include a method of determining and assessing the onset and/or the progression severity of prostate cancer in the rat models with the onset of prostate cancer of the present invention, wherein a test substance is administered to the rat models before or after their cancer development. A determining and assessing method of the onset and/or the progression severity of prostate cancer is exemplified by a method wherein the histopathological figures of prostate cancer obtained from the rat models administered with a test substance is analyzed and assessed. When determining and assessing the onset and/or the progression severity of prostate cancer, it is preferable to use simultaneously wild type rats of the same line as a rat model with the onset of prostate cancer as this will make comparative experiments accurate on individual levels.

A substance suppressing the onset and/or the progress of prostate cancer obtained by the screening method of the present invention provides possibility for its use as a preventive drug, a suppressing drug, a treatment, or the like for prostate cancer. For instance, when performing the screening method mentioned above, administration of a test substance before the development of the prostate cancer is suitable for the screening of a preventive drug for the prostate cancer. On the contrary, administration of a test substance after the development of the prostate cancer is suitable for the screening of a drug for the treatment and for amelioration of the symptoms of the prostate cancer. On the other hand, a substance promoting the onset and/or the progress of prostate cancer is thought to be useful to give rise to the prostate cancer to the sufficient level in an animal

model with the poor development of prostate cancer. In addition, a pharmaceutical composition of the present invention for the treatment of a patient in need of suppressing the onset and/or the progress of prostate cancer contains a substance for suppressing the onset and/or the progress of prostate cancer, as an active ingredient, together with publicly known substances necessary for the drug preparation.

The present invention will be explained below based on the examples but the invention will not be limited to these examples.

(Construction of the PBSVT transgene)

SV40 large T antigen gene used was the gene incorporated as the NcoI-BamHI fragment into the multi-cloning site of pBluescriptIIKS(-) which was provided by Human Science Research Resources Bank. The above SV40 large T antigen gene (5146-2676) excised with NcoI and BamHI from the pBluescriptIIKS(-) was ligated on the downstream of 5'-flanking region (458 bp: from -426 to +32) of the rat probasin gene promoter excised with EcoRI and NcoI, thus the PBSVT transgene was constructed (Fig.1). The PBSVT transgene that can express both SV40 large T antigen of 2944 bp and the small t antigen was digested with restriction enzymes, EcoRI and BamHI, after having been amplified with plasmid pBluescriptII (Transgene). The transgenes then underwent 1% agarose gel electrophoresis to have the plasmid parts removed and were separated for linearized DNA fragments. Then these linearized DNA fragments were collected using GENE CLEANE*II (BIO 101 INC) and the DNA obtained after this purification was dissolved in the injection buffer (10mM Tris-HCl including 0.1mM EDTA, pH7.6) to 5 μg/ml and kept at -20℃ until manipulated for injection.

(Generation of the transgenic rat)

The PBSVT transgene solution described above was microinjected into rat fertilized eggs in the pronuclear stage according to the following protocol. 8-week-old Sprague-Dawley (SD) female rats were fed under 12-hour cycles of light and dark (light hours 4:00-16:00), temperature at about 23℃ and moisture of about 55%. Then vaginal smear test was conducted to observe sexual cycles of the female rats and to select the

*Trade-mark

day for the hormone treatment. 150 IU/kg of pregnant mare's serum gonadotropin (NIPPON ZENYAKU; "PMS ZENYAKU*") was intraperitoneally administered to the female rats for superovulation, and 48 hours thereafter, 75 IU/kg of human chorionic gonadotropin (SANKYO ZOKI; "Puberogen*") was administered. The female rats were then co-resided with males for mating. After 32 hours of human chorionic gonadotropin administration, fertilized eggs in the pronuclear stage were collected through oviduct perfusion. The mKRB solution (Y. Yoshida and M.C. Chang, J. Reprod. Fertil., Vol.36, pp9-22(1974)) was used for the oviduct perfusion and egg cultivation. The collected fertilized eggs were enzymically treated for 5 minutes at 37℃ in the mKRB solution supplemented with 0.1% hyaluronidase (Sigma) to remove the cumulus oophorus cells. The fertilized eggs were then washed three times in the mKRB solution to remove the enzyme and were kept in a CO_2 incubator (CO_2 5%, 37℃, saturated moisture) until manipulated for DNA injection. Male pronulceus of Wistar rat fertilized eggs prepared as described above were introduced with the foregoing PBSVT transgene solution to 10 molecules per one fertilized egg. 200 fertilized eggs introduced with the solution were transplanted to 10 recipient rats and 40 neonates were given birth. DNA prepared from the tails just after weaning were analyzed by PCR method with primers according to Seq. ID Nos.1 and 2 on the sequence listing. The result proved that four founder (F0) male transgenic rats of SD line were generated.

These founder male transgenic rats were mated with normal wild-type female rats of SD line to create the first filial generation (F1) and the time-course pathological changes occurring in the systemic organs were studied. Two rats of 2944 and 2971 lines among four transgenic rats were found capable for establishing their lines. Rats of 2971 line can be bred for generations stably and are thereby successfully established as PBSVT transgenic rats. On the contrary, rats of 2944 line, as breeding continue, suffered frequent developments of hydrocephalus and the line became extinct.

Paralysis in both hind limbs appeared in F0 rats of 2944 and 2971 lines at around 12 weeks of age and they died at weeks14 and 25. Marked atypical hyperplasias were diffusively found in the prostate ventral lobes in both lines but no cancer lesions were

*Trade-mark

observed. SV40 T antigen was expressed in all prostate lobes and was expressed frequently in the ventral lobe in particular. Bone tumors of the spine were observed in both the lines and further hypoglossal tumors were also found in 2971 line. All of the tumors were malignant small round cell tumors and positive for the SV40 T antigen.

General observation with regard to those dead and killed in the process of breeding of 2971 and 2944 lines are shown in Table 1, "General observation of PBSVT transgenic rats of 2971 line (cases for dead or killed)" and in Table 2, "General observation of PBSVT transgenic rats of 2944 line (cases for dead or killed)". In addition to these, the histopathological findings in their prostates are shown in Table 3, "Histopathological findings in the PBSVT transgenic rat prostates". As shown in Table 1, 2971 line rats are confirmed to be capable for breeding. Parvocellular undifferentiated malignant tumors were found to develop in tongues and the proximal of spinal cords other than in their prostates. In addition, many individuals of 2944 line rats died of hydrocephalus as indicated in Table 2. Other than in prostates, parvocellular undifferentiated malignant tumors were developed in tongues, the proximal of spinal cords, and the like.

Table 1

General observation of PBSVT transgenic rats of 2971 line (cases for dead or killed)

Animal No.		Sex	Weeks of age	Dead or killed	General observation
F0		M	25	dead	Tumors in spinal cord, tongue and lymph node
F1	27	F	26	dead	Tumors in spinal cord, tongue and lymph node
	28	M	3	killed	–
	31	F	26	dead	Tumors in spinal cord, tongue and lymph node
	34	F	26	killed	Tumors in spinal cord, tongue and lymph node
	55	M	17	dead	Tumor in spinal cord
	56	M	22	dead	Tumors in spinal cord, tongue and lymph node
	59	M	24	killed	Tumors in spinal cord, tongue and lymph node
	61	M	15	dead	Tumors in spinal cord, tongue and lymph node
	62	M	16	dead	Tumors in spinal cord, tongue and lymph node
	66	F	19	dead	Tumors in spinal cord, tongue and lymph node
	69	F	13	dead	Tumors in tongue and lymph node
	70	F	25	killed	Tumors in spinal cord, tongue and lymph node
	75	M	24	dead	Tumors in spinal cord, tongue and lymph node
	76	M	19	dead	Tumors in spinal cord, tongue and lymph node
	77	F	21	dead	Tumors in spinal cord, tongue and lymph node
	78	F	24	dead	Tumors in spinal cord, tongue and lymph node
	79	F	24	dead	Tumors in tongue and lymph node
	81	F	25	killed	Tumors in spinal cord, tongue and lymph node
	84	M	25	dead	Tumors in tongue and lymph node
	100	F	21	dead	Tumors in spinal cord, tongue and lymph node
	102	M	17	dead	Tumors in spinal cord, tongue and lymph node
	104	M	15	dead	Tumors in spinal cord, tongue and lymph node
	105	M	20	killed	–

Table 2

General observation of PBSVT transgenic rats of 2944 line (cases for dead or killed)

Animal No.		Sex	Weeks of age	Dead or killed	General observation
F0		M	14	dead	Tumor in spinal cord
F1	4	M	15	dead	Tumors in spinal cord, tongue and lymph node
	7	F	17	dead	Tumors in spinal cord, tongue, thyroid gland and lymph node
	8	F	18	dead	Tumors in spinal cord, tongue and lymph node
	9	M	4	killed	hydrocephalus
	17	F	4	killed	hydrocephalus
	18	F	19	dead	Tumors in spinal cord, tongue and lymph node
	19	F	19	dead	Tumors in spinal cord, tongue and lymph node
	20	F	16	dead	Tumors in spinal cord, tongue and lymph node
F2	120	M	3	killed	hydrocephalus
	121	M	3	killed	hydrocephalus
	122	M	3	killed	hydrocephalus
	125	F	14	dead	hydrocephalus,tumors in tongue, thyroid gland and lymph node
	126	F	14	dead	hydrocephalus,tumors in tongue and lymph node
	129	F	15	dead	hydrocephalus,tumors in spinal cord, tongue and lymph node
	130	F	7	dead	hydrocephalus
	133	M	9	dead	hydrocephalus
	139	F	18	dead	Tumors in spinal cord, tongue and lymph node
	142	F	4	dead	hydrocephalus
	145	M	15	killed	hydrocephalus,tumors in tongue, thyroid gland and lymph node
	146	M	15	killed	hydrocephalus,tumors in tongue, thyroid gland and lymph node

Table 3

Histopathological findings in the PBSVT transgenic rat prostates

Animal No.		Line	Weeks of age	Prostate		
				Compound lobe	Dorso-lateral lobe	Anterior lobe
F0		2944	14	AH	AH	n.p.
F1	4	2944	15	AH	AH	n.p.
	9	2944	4	n.p.	n.p.	n.p.
	145	2944	15	AH	–	–
	146	2944	15	AH	–	–
F0		2971	25	Atrophy	Atrophy	–
F1	28	2971	3	n.p.	n.p.	n.p.
	55	2971	17	Atrophy	Atrophy	CA
	56	2971	22	Atrophy	Atrophy	–
	59	2971	24	Atrophy	–	–
	62	2971	16	Atrophy	Atrophy	–
	104	2971	15	–	AH	CA

AH: atypical hyperplasias CA: cancer n.p.: no practice

Five 2971 line PBSVT transgenic rats, 15 weeks of age, were killed for histopathological examination of the prostate and testis. The examination revealed that each prostate lobe developed adenocarcinomas in almost all the cases that were atypical tumor cells arranged in a glandular structure accompanied by many mitotic figures. Particularly in the anterior lobe, invasive cancers were observed to develop. The results are shown in Table 4 "Histopathological findings in PBSVT transgenic rat prostates and testes".

Table 4

Histopathological findings of PBSVT transgenic rat prostates and testes

	Number of rats	Prostate								Testis	
		Compound lobe		Dorsal lobe		Lateral lobe		Anterior lobe			
		AH	Ca	AH	Ca	AH	Ca	AH	Ca	AH	Ca
15 week -old	5	5	4 (0)	5	2 (0)	5	4 (0)	5	5 (5)	0	0

AH: atypical hyperplasias CA: cancer ( ): invasive cancer

Immunohistochemical observation of PBSVT transgenic rats for the protein expression of SV40 large T antigen revealed that the expression was found in each lobe of the prostates, in the salivary gland ducts, and in the testis Sertoli cells. The results are shown in Table 5, "Immunohistochemical observation results for the protein expression of SV40 large T antigen in PBSVT transgenic rats".

Table 5

Immunohistochemical observation results for the protein expression of SV40 large T antigen in PBSVT transgenic rats

Prostate		Salivaly gland	
Compound lobe	++	Duct cell	±
Dorsal lobe	++	Acinous cell	−
Lateral lobe	++		
Anterior lobe	+		
		Colon	−
		Liver	−
Testis	−	Pancreas	−
Testis (Spermary)		Thymus	−
Sertoli cell	±	Lymph node	−
Epididymis	−	Cerebrum	−
Lung	−	Cerebellum	−

++: intensively positive +: moderately positive

±: poorly positive −: negative

The experiment as described below was conducted to study the effect of the testosterone administration on the development of cancer.

Male PBSVT transgenic rats were grouped into three at five weeks of age. The first group served as the control group, the second underwent castration, and into the rats of the third group, testosterone filled in 1.5cm Silicon tubes was subcutaneously transplanted. Transgenic rats of all three groups were killed at 15 weeks of age and their pathological changes in the prostate were histopathologically examined. All

controls of the first group displayed marked atypical hyperplasias diffusively in the anterior lobes of the prostate and some of them developed cancer. In the second group, prostates generally displayed atrophy with no sign of neoplastic lesions. In the third group, the entire prostates showed considerable swelling with the high incidence of invasive cancers. However, castration after the testosterone administration for a duration of 15 weeks resulted in involution of the prostate and all of the proliferative lesions disappeared. These findings revealed that the prostate tumor development in the PBSVT transgenic rats is androgen-dependent and testosterone administration will progress their prostate tumors in a short period and considerably. In addition, prostate cancers were proved to develop by inactivating p53 and Pb proteins.

INDUSTRIAL APPLICABILITY

These animal models allow prostate cancer to develop at early stages with high incidence. Therefore, they are quite useful prostate cancer models for analyzing the developmental process of prostate cancers in detail and for developing drugs for the treatment of prostate cancer. The characteristics of transgenic rats of the present invention show that they are useful in the field of medical research.

SEQUENCE LISTING

<110> JAPAN SCIENCE AND TECHNOLOGY CORPORATION

<120> Rat Prostate Carcinogenesis Models

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<140>
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<150> JP 2000/42491
<151> 2000-02-21

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<170> PatentIn Ver. 2.1

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CLAIMS:

1. A method of establishing a rat model which exhibits an onset of androgen-dependent prostate cancer, develops androgen-dependent prostate cancer and can be bred stably for generations, which method comprises the steps of:

(A) excising simian virus (SV) 40 large T antigen gene having a nucleic acid sequence from 5146 to 2676 with NcoI and BamHI;

(B) binding the SV40 large T antigen gene to a site downstream of 5'-flanking region of 458bp from -426 to +32 of a rat probasin gene promoter excised with EcoRI and NcoI to form a probasin-simian virus 40 large T antigen (PBSVT) transgene of 2944bp;

(C) introducing the resulting PBSVT transgene of 2944bp into a fertilized egg of a Sprague-Dawley rat;

(D) transplanting the fertilized egg into which the PBSVT transgene has been introduced, to a recipient rat;

(E) mating a transgenic rat born from the recipient rat with a wild-type Sprague-Dawley rat to obtain first transgenic offspring rats;

(F) breeding the transgenic offspring rat by mating the transgenic offspring rat with a wild-type Sprague-Dawley rat to obtain first filial generation offspring rats;

(G) histopathologically observing prostate of the first filial generation offspring rats; and

(H) selecting transgenic rats which exhibit an onset of prostate cancer among the first filial generation offspring rats.

2. The method according to claim 1, wherein in step (C):

the fertilized egg is in a pronuclear stage; and

the introduction of the PBSVT transgene into the fertilized egg is carried out by a microinjection, electric pulse, liposome or calcium phosphate method.

3. The method according to claim 1 or 2, which further comprises:

analyzing DNA extracted from a body of the transgenic rat born from the recipient rat by southern blotting analysis or PCR assay using primers having the DNA sequence of SEQ ID NOs. 1 and 2, prior to the mating step (E), to confirm the transgenic rat having the PBSVT transgene introduced therein.

4. The method according to any one of claims 1 to 3, wherein the step (E), the transgenic rat born from the recipient rat is male; and the wild-type Sprague-Dawley rat is female.

5. The method according to any one of claims 1 to 4, wherein in step (F), the transgenic offspring rat is male; and the wild-type Sprague-Dawley rat is female.

6. A method for determining whether a test substance promotes or suppresses an onset or progress of androgen-dependent prostate cancer, which method comprises:

(I) providing a rat model which (i) has an SV40 large T antigen gene under control of probasin gene promoter introduced therein, the probasin gene promoter being 5'-flanking region of 458bp from -426 to +32, (ii) exhibits an onset of androgen-dependent prostate cancer, (iii) develops androgen-dependent prostate cancer and (iv) can be stably bred for generations;

(II) administering the test substance to the rat model, before or after the onset of androgen-dependent prostate cancer; and

(III) assessing the onset or a progress severity of androgen-dependent prostate cancer in the rat model.

7. The method according to claim 6, wherein the assessment of the onset or progression severity of androgen-dependent prostate cancer is made by analyzing and assessing histopathological figures of the androgen-dependent prostate cancer obtained from the rat model.

8. The method according to claim 6, wherein the assessment of the onset or progression severity of androgen-dependent prostate cancer is carried out by measuring prostanoic acid phosphatase (PAP) or a prostate-specific antigen (PSA) produced in prostate cancer cells.

9. The method according to any one of claims 6 to 8, wherein the assessment of the onset or progression severity of androgen-dependent prostate cancer is carried out by comparing with a wild-type rat of the same breeding line as the rat model.

10. The method according to any one of claims 6 to 9, wherein the rat model belongs to a Sprague-Dawley rat.

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11. The method according to any one of claims 6 to 10, wherein the rat model is established by the method as defined in any one of claims 1 to 5.

12. A use of a rat model for screening a substance that promotes or suppresses an onset or progress of androgen-dependent prostate cancer, wherein the rat model (i) has an SV40 large T antigen gene under control of probasin gene promoter introduced therein, the probasin gene promoter being 5'-flanking region of 458bp from -426 to +32, (ii) exhibits an onset of androgen-dependent prostate cancer, (iii) develops androgen-dependent prostate cancer and (iv) can be stably bred for generations.

13. The use according to claim 12, wherein the rat model belongs to a Sprague-Dawley rat.

14. The use according to claim 12 or 13, wherein the rat model is established by the method as defined in any one of claims 1 to 5.

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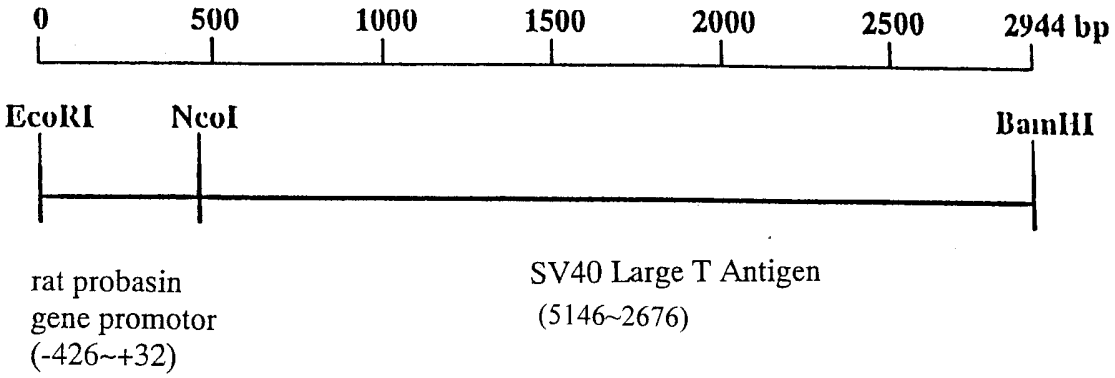
PATENT AGENTS

FIG. 1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1000
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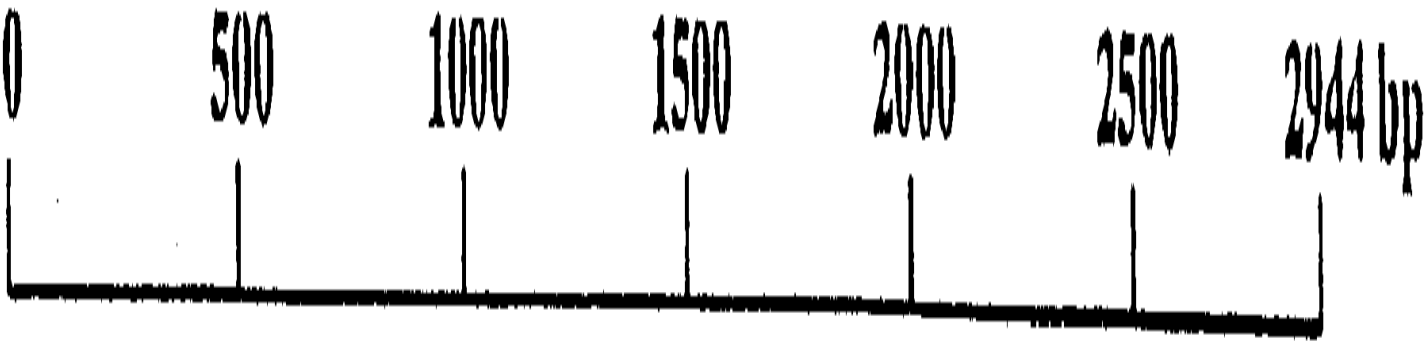
NcoI
BamHI
rat probasin
gene promotor
(-426~+32)
SV40 Large T Antigen
(5146~2676)



rat probasin
gene promotor
(-426~+32)

SV40 Large T Antigen
(5146~2676)