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ANIMAL MODELS OF CANCER: A REVIEW

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ABSTRACT

Cancer is the second leading cause of death worldwide. In USA three persons out of five will develop some type of cancer. Beyond these statistics of mortality, the morbidity due to cancer presents a real scary picture. Last 50 years of research has rendered some types of cancer curable, but still the major fear factor associated with this disease is unchanged. Animal models are classified according to the method of induction of cancer in the animal. Spontaneous tumor models are the most primitive models. Although these models show good resemblance to the natural disease in humans, they were not capable of keeping pace with developing experimental therapeutics programs. It has therefore been necessary to take a further step towards artificiality, away from the clinical problem in the search for satisfactory testing method. From this step, the journey of artificially induced tumor models started. It is possible to induce cancer reproducibly in animals by exposing them to various agents and now, by transplanting tumor cells or tissue. The development of Genetically Engineered Animal models has provided a great help in knowing the disease. This article takes a review of present animal models used in anti-cancer drug discovery.

INTRODUCTION: Cancer research has been one of the fascinating areas of research and the most interesting aspect about it is its diversity. It includes research in origin and location of disease, the types of cells and drug targets that can be studied and approaches that can be pursued for diagnosis and treatment.

Whatever the area of study, the final goal remains same – Making the disease curable. This goal has been achieved to some extent, but still not completely. Or possibly the fruits of 30 years of research are about to ripe.

Cancer drug discovery continues to evolve at a phenomenal pace and enormous amounts of recourses are engaged for drug discovery and design. The evaluation of any such hopefully designed drug molecule is the critical stage of drug discovery program. Improper selection of an evaluation method may result in dropping of a potential agent from further development. Use of cell lines with high throughput screening is the primary screening method. But due to limitations like less relevance with clinical condition further screening using suitable *in vivo* model is mandatory. In such condition, the selection of animal model becomes crucial. The animal model should represent the human disease as closely as possible. At the same time its feasibility as well as economy -to be used in large drug screening programs- are also important factors.

Article Outline:

Animal models of cancer

1. Spontaneous tumor models

2. Virus induced tumor models
3. Radiation induced tumor models
4. Chemically induced tumor models
5. Transplantable tumor models

- Methods of transplantation
  - i. Heterotopic transplantation
  - ii. Orthotopic transplantation
- Depending on host used
  - i. Syngenic models
  - ii. Xenogenic models

6. Genetically Engineered Mice (GEMs)
   i. Transgenic animals
   ii. Knockout animals

1. **Spontaneous tumor models:** It includes selection and use of animals with natural incidence of cancer e.g. **Mice of some inbred strains** are particularly liable to develop distinct forms of cancer. Particularly, leukemia, mammary cancer, pulmonary adenomas and hepatomas [1]. In DA/Han rats more than 60% of female animals die from endometrial adenocarcinoma. In BDII/Han rats 87 to 90% animals die from endometrial adenocarcinoma [2]. These models mimic the clinical situation most closely. They resemble human cancers in kinetics and antigenicity.

   But, such systems have many limitations. It is impracticable to obtain at any one time sufficient numbers of such tumors of comparable size for screening purposes. Usually the tumors become measurable only late in their course and the metastatic pattern is not uniformity is difficult to establish accurate staging. These models are usually not reproducible and most of them are discovered to have viral origin. However, such tumors provide a stringent test of antitumor activity and are not normally used for primary screening. They play an important role for study of molecular aspects of cancer and carcinogenesis [1].

2. **Virus induced Tumor Models:** The two most commonly used virus-induced tumors are the Friend leukemia and Rous sarcoma. However, these types of models are not generally used for drug discovery [1].

   a. **Friend leukaemia:** This tumor was first discovered by Friend in adult Swiss mice. It can be transmitted to other mice by injection of cell-free filtrates of leukemic –spleen homogenates. Inhibition of splenic weight gain, decrease in titre of viable virus (assessed by bioassay) and prolongation of survival time are various evaluation parameters. The 2-4 month interval between inoculation of the virus and appearance of leukemia and laborious and time consuming evaluation parameters are the factors which hinder use of these models in anticancer screening research.

   b. **Rous sarcoma:** This tumor was first described by Rous in young chicks. It can be transmitted by implantation of tumor fragments or inoculation of cell free material from tumor homogenates. Inhibition of tumor growth and survival time, are the commonly employed evaluation parameters. This is a local tumor, so assessment of the tumor growth is easy. However, it is insensitive to many types of agents so many important compounds may be missed.

3. **Radiation induced tumors:** UV radiation is an established carcinogen. This fact is exploited for inducing cancer in experimental animals by exposing them to predetermined doses of radiation. These models mainly are skin tumor models. Sometimes radiation is used in combination with other chemical agent, like TPA or DMBA. UV induced skin tumorigenesis in SKH-1 hairless mouse. Two stage models for skin tumorigenesis etc are the examples of this type of models. Merits of these models include that tumors appear on the skin, so they are easily assessable. Use of radiation may pose a radiation hazard to the researcher.

Moreover, long tumor induction time and tedious evaluation parameters are the demerits of this type of models. However, this type of models can used to predict a general antitumor activity, depending on the evaluation parameters used. These models are not used in routine screening programs.

a. **UV induced skin tumorigenesis in SKH-1 hairless mouse** [3]: Inbred hairless (SKH-1) mice are irradiated for 5 day/wk at a total dose of 74.85 J/cm^2 UVA and 2.44J/cm2 UVB for 22 weeks. Irradiated mice develop an average of 16 tumors/mice by week 23 with average number of carcinomas per mouse being 2.1. Drug is applied topically twice a week at a dose of 8mg/cm2 immediately after UV radiation. Parameters like, skin tumor incidence (number of mice with tumors)**,** Tumor multiplicity (average number of tumors per mouse), Reduction in occurrence of skin papillomas, Onset of appearance of first tumor and Histopathological examination of the tumor including Western Blotting or immunohistochemistry for cell cycle regulatory proteins are studied.

b. **Two stage models for skin tumorigenesis** [4]: In this model the tumor induction is accomplished in two stages: Initiation and promotion. For example, Initiation by single topical application of DMBA (50 nmol) followed by a promotion with 2 weekly treatments with UVB light (250 mJ/cm2) for 25 weeks. Studies using these models have provided an insight into the process of carcinogenesis. The mode of action of drug can also be studied for example, whether the drug can reverse or prevent initiation or promotion process.

4. **Chemically induced tumors:** Tumors induced by means of chemical carcinogens arise from the host's own cells and therefore resemble human clinical cancer more closely than do transplantable neoplasms. **Limitations** with chemically induced tumors are the possible effects of carcinogen upon the behavior of the tumor and the hazards to other animals and to personnel which may arise from the excreted carcinogen and its metabolites in feces and urine of the animal [5].

Chemical carcinogens can be divided into two categories:

i. Direct acting agents- require no chemical transformation to induce carcinogenicity

ii. Indirect acting agents- become active only after metabolic conversion. Also known as procarcinogens and their active end products are called ultimate carcinogens.

Both direct acting and ultimate carcinogens are highly reactive electrophiles (i.e. have e^- deficient atoms) that react with the e^- rich atoms in RNA, DNA and cellular proteins.

Although any gene may be the target of chemical carcinogens, RAS gene mutations and TP53 genes are the important targets.

e.g. DMBA induced mammary tumors in rats, DMAB induced colon tumors, 3,4,9,10 dibenzopyrene induced fibrosarcoma in mice, 3,4 benzopyrene induced spindle cell sarcomas, 20-methylcholanthrene induced leukaemia and sarcomas

a. **DMBA induced mammary tumors** [6]: Female rats of 50 days of age are given a single dose of DMBA (9,10-Dimethyl-1,2-benzanthracene). The first malignant tumor can be detected 20 to 30 days later. The tumor weight is determined by palpation, comparing the volume of each tumor to that of preformed plasticine models. The tumor weight is calculated by multiplication of the model weight by a factor which takes into account the specific weights of plasticine and tumor tissue. The drug treatment (s.c. administration) is started after the total tumor mass has reached about 1 gm in the animal. Change in tumor volume can be recorded. Histopathological examination of tumor including mitotic index may throw some light on mechanism of action of drug.

b. **DMAB (3, 2-dimethyl-4-aminobiphenyl) induced colon tumors:** Tumor is induced in male F344 rats by s.c. injection of DMAB 50mg/kg once a week for 20 weeks. It can induce multiple colon tumors in

about 26-30% of animals fed a low fat diet, and 74% of animals fed a high fat diet. This produces both adenomas (benign tumors) and adenocarcinomas (malignant tumors) in large bowel. Various parameters like, tumor incidence, Size of tumor and Histopathology of tumor tissue can be studied. Limitations of model include requirement of multiple injections of DMAB to induce colon tumors. Moreover, there is induction of neoplasms at sites other than colon such as adenocarcinomas of mammary glands in female rats, sarcomas in salivary glands, squamous cell carcinomas of the ear duct and skin, gastric papillomas, sarcomas and lymphomas, carcinomas of urinary bladder. This complicates the comparison of drug response.

c. **3, 4, 9, 10 Dibenzopyrene Induced Fibrosarcoma In Mice:** Tumor is induced by single subcutaneous injection of 500µg in peanut oil in C57BL/6 mice. This causes uniform subcutaneous fibrosarcomas in all injected animals at the site of injection within 4 to 5 weeks of treatment. Evaluation parameters include tumor weight, histopathological examination of tumor including mitotic index etc. Advantage of model is that it can induce tumor with single dose of carcinogen. Moreover, carcinogen is not excreted in feces or urine and remains in the induced tumors, so animals are safer to handle.

5. **Transplantable tumors:** These models are based on the use of cancer cell lines or tissues that can be grown in mice or rats. There can be two methods of transplantation.

   A. Heterotopic transplantation

   B. Orthotopic transplantation

   A. **Heterotopic transplantation:** It involves transplantation of tumor cells or tissue at the site other than its site of origin. e.g. carcinoma transplanted intraperitoneally or subcutaneously. This method generally involves i.p. or s.c transplantation, where the tumor proliferates in the form of ascites or solid tumor, respectively. This inoculation procedure is simple and less time consuming. So, it becomes possible to inoculate a large number of animals at a time. In addition to that it requires less skill.

   B. **Orthotopic transplantation:** It refers to the transplantation of cancer cells to the anatomic location or tissue from which a tumor was derived. For example, lung tumor is transplanted in lungs. The use of this method has resulted in tumor models that may more closely resemble human cancers, including tumor histology, vascularity, gene expression, responsiveness to chemotherapy and metastatic biology. As more has been known about host-microenvironment interaction, it is clear why orthotopic tumors are preferred over conventional flank (s.c. transplant) models.

Orthotopic transplantation of cancer cells may be accomplished by (i) direct injection of tumor cells or (ii) the surgical orthotopic implantation (SOI) i.e. implantation of the intact fragments of tumor orthotopically by surgery. Use of SOI improves the reproducibility and metastatic outcome of the model [7].

In addition to this, transplantable models can be divided into two broad groups depending upon the origin of the tumor and the host used:

5.1 Syngenic models

5.2 Xenogenic (xenograft) models

5.1 **Syngenic models:** These models include the use of mouse or rat **(murine) cancer cell** line or tissues that are transplanted in inbred animals of the same genetic background as the derived cell line or tissue. e.g. L1210 leukemic cell line obtained from DBA/2 mouse grown in animals of same species. These are originally carcinogen induced or spontaneous tumors in animals, which are maintained as cell lines. Such cell lines or fresh tumor samples can be inoculated in mice of the same genetic background as those from which the tumor was originally obtained. The advantage of syngeneic models is that the transplanted tissues, the tumor microenvironment, and the host are from the same species.

This is particularly important when considering the close interaction between tumor and host. However, these model systems **lack** many of the important features of human tumors. For example, they usually are derived from homozygously inbred mice and therefore lack the genetic complexity of human tumors.

In addition, due to species-specific differences in oncogenesis, (for example differences in carcinogenic xenobiotic metabolism) they may not bear the same constellation of mutations observed in human patients[8].

5.1.1 Leukemia 1210 (L1210): The host used for implantation is DBA/2 mouse. The tumor arose originally in a female DBA/2 mouse following application of 0.2% 20-methylcholanthrene to the skin.

Subsequently transplanted subcutaneously or intramuscularly by Law and colleagues [9] and eventually obtained in an ascitic form by Connors and coworkers.

Methods of transplantation:

i. i.p. injection of 10^5 leukemic cells [10]

ii. s.c. transplantation of fragment of the solid tumor into the flank region

There is a period of rapid growth within peritoneum following i.p. inoculation. Dissemination occurs in both solid and ascetic forms killing animals in 9-12 days. Mean Survival Time (MST) of ascitic form depends on the size of inoculum.

This model enjoyed its status as 1st stage prescreen in the NCI Drug Screening Program from 1955-1985 [10]. This model has low cost and allows for a relatively high throughput of compounds. However, afterwards it was realized that screening against rapidly growing leukemic cells could bias selection toward compounds that are preferentially active against rapidly growing tumors.

The development of drugs active against the solid tumors would presumably require a different approach.

5.1.2 Lewis Lung Carcinoma model: It was first isolated by Dr. Margaret R. Lewis in 1951 from a spontaneous epidermoid carcinoma of the lung in C57BL/6 mouse.

Methods of transplantation:

1. S.C. transplantation of tissue fragment (MST – 27 days).

2. Tumor cells in suspension are inoculated through the right main stem bronchus into the right lung in a lightly anaesthetized animal.

Both these methods show lower rates of metastasis. Metastatic potential of the tumor is highly increased by surgical orthotopic transplantation of tumor fragments [11] or by injection of tumor cell suspension in tail vein [5] (*Experimental metastasis approach*). It has been important tumor model for metastatic and angiogenesis studies [12].

To make the distant metastasis easily detectable, a gene (FP gene) coding for fluorescent protein is inserted into the cells. For this a retroviral vector is used which in turn transfects the L.L. carcinoma cells. The use of such modified cells provides for easy, sensitive and reproducible detection [11].

5.1.3 Ehrlich Ascites carcinoma: This tumor originated spontaneously in a female albino mouse at the base of ear. Tumor in experimental animal can be generated by intraperitoneal injection of $2x10^5$ tumor cells per animal on day 0. After 24 hrs of tumor inoculation drug treatment is started. Drugs are administered by i.p. route. On 5th, 7th and 9th days, animals are sacrificed and peritoneal fluid is collected. Tumor cells from peritoneal cavity are collected by repeated wash with saline. Additional groups of animals can be used for survival time assay [13].

Parameters like volume of peritoneal fluid, viability of tumor cells in peritoneal fluid, packed cell volume (PCV) in peritoneal fluid and % Increase in survival time of drug treated animals are used for evaluation. It is a non metastatic tumor.

Cancer cells grow in the peritoneal fluid and may attain a very high cell density of 25 to 100 million per ml of ascitic fluid. This is widely used model for primary screening procedure as it can predict a general antitumor activity. A modified Solid tumor model is developed by injection of $4x10^6$ tumor cells s.c. in flank of the animal. The tumor may grow to a diameter of 12 mm in 14 days. Tumor volume and histopathological examination of tumor are the evaluation methods used [14].

5.2 **Xenograft models:** For tumor models that more closely resemble the clinical disease, transplantable tumors of human origin should be used. But transplantation of such human tumors in mice may result in severe immune rejection. For this purpose *athymic (nude)* mice or *severe combined immunodeficiency* (scid) mice are used. These animals lack immune response to such foreign transplanted material. Before the availability of athymic mice, mice immunocompromised by irradiation, thymectomy or steroid were used for transplantation [10].

The first **nude mice** arose spontaneously in a closed colony of albino mice in a laboratory in Ruchill Hospital, Glasgow, Scotland and were described by Isaacson and Cattanach as lacking fur [15]. On chromosome 11, a mutant gene (*nu*, for nude) is present as an autosomal recessive gene, responsible for the absence of hair, retarded growth, short lifespan and low fertility. Mice with homozygous mutation *nu/nu* lack a thymus, while heterozygous *nu/1* mice have a thymus. Immunologically, the *nu/nu* athymic mice have a small number of T cells received from heterozygous mother. However, B cell function is normal and activity of natural killer cells is higher.

The success of human tumor xenografting into nude mice and the ability to maintain the histologic and biologic identity of tumors through successive passages *in vivo* revolutionized many aspects of cancer research.

Transplantation of tumor cell lines into nude mice can be accomplished via multiple routes: subcutaneous, intraperitoneal, intravenous, intracranial, intrasplenic, renal subcapsular, or through a new orthotopic model by site-specific organ inoculation. Each site has specific advantages and limitations.

When human tumor cells are transplanted in nude mice they undergo kinetic changes. Most frequently, doubling time becomes shorter than that of the original tumor, which further decrease during subsequent passages. Despite this, many xenografted human tumors maintain original morphologic and biochemical characteristics. Therefore, human tumor xenografts are mainstay of cancer drug discovery programs [16]. **Table 1** Shows the Human tumor xenografts panel used in NCI drug screen.

TABLE 1: HUMAN TUMOR XENOGRAFTS PANEL USED IN NCI DRUG SCREEN [16]

Site of Tumor	Host of Origin	Tumor of Origin	Historical Description	Site
CX-1 colon	Isolated in tissue culture, subsequently maintained in nude mice	Human colon. Untreated primary tumor from 44-year-old Caucasian female.	Adenocarcinoma of the colon	Subrenal capsule
LX-1 lung	Isolated and maintained in nude mice	Metastatic lesion from arm of 48-year-old	Carcinoma	Subrenal capsule
MX-1 mammary	Isolated and maintained in nude mice	Human breast. Primary tumor from 29-year-old female with no previous chemotherapy. CL-1 line	Carcinoma	Subrenal capsule

5.2.1 Subcutaneous implantation: It is the important site for transplantation of human tumor into the nude mouse because it is simple and gives easy access to tumor. NCI has included this method as primary in vivo test for its drug discovery and screening program. A tumor cell suspension (approx. 10^6 to 10^7 cells per animal) is usually injected into the flank region of animal. Tumors usually require between a few days to a few months to grow, depending on the growth rate of the cell line used. Invasion in adjacent tissues and metastasis is rare with subcutaneous xenografts.

Growth delay and clonogenic assay are the evaluation methods suitable for this model.

5.2.2 Renal Subcapsular (RSC) Assay: This method was first described by Bogden and colleagues in 1978. Cells are inoculated in nude mouse as tumor fragment, approximately 1 mm in size, under the capsule of kidney. Advantage of these tumors is that, they maintain true morphologic, functional, and growth characteristics of the original tumor such as, cell-cell contact and spatial relationship of the tumor. Therefore, they better represent the metastatic characteristics of human tumors. Growth assay, clonogenic assay and animal survival assay are the suitable evaluation methods.

Unlike the subcutaneous xenograft assay, the renal subcapsular assay has a relatively short and constant period between tumor inoculation and the appearance of a grossly palpable mass. Tumors can usually be assessed in a period of 6 days. Therefore, this model is particularly appropriate when a short term in vivo assay is required. Despite many advantages it is not the ideal model, because the subcapsular area of the kidney is not a totally immunoprivileged site. Tumor in this area has shown invasion with variable amounts of lymphocytes, which may be a factor different from the original tumor. However, it might be an ideal orthotopic model for renal cell carcinoma.

5.2.3 Intraperitoneal, Microencapsulated Tumor Assay: Because of the limitations of the RSC and its poor adaptability to slow growing tumors, alternative short-term in vivo assays have been developed. Microencapsulated tumor assay is one of this, which employs microencapsulation technology.

Tumor cells are encapsulated in semipermeable gels that can be formed into microcapsule of from 0.05 to 1 mm. These microcapsules can be inoculated into the peritoneal space of experimental animals. Under typical assay conditions using mice, approximately 600 microcapsules are injected into the peritoneum. The semipermeability of the capsule protects the tumor cells from host cell–mediated immune cytotoxicity, so that athymic (nude) mice need not be used. At the same time, it allows nutrient and systemic cytotoxic agents to diffuse and reach the tumor cells.

Anticancer effect is assessed by recovering microcapsules and counting viable tumor cells in treated versus control animals. The microencapsulation assay is simple, rapid, and relatively inexpensive. It requires fewer mice when compared to the subcutaneous transplanted tumor assay. Tumor cells are evaluated after exposure to drug concentrations that are obtainable *in vivo*.

In addition, the system is adaptable to most solid tumors and, unlike the subcutaneous transplanted tumor assay, use immunocompetent mice. More than one tumor can be evaluated at the same time, in the same mouse. For these reasons, the microencapsulated tumor assay is being evaluated by the NCI screening program as an In vivo second-line screen to follow initial drug leads that pass the In vitro screening system.

5.2.4 Orthotopic Xenograft Model: Transgenic tumor models and subcutaneously-growing human tumors in immunodeficient mice, do not sufficiently represent human clinical cancer, because when they are implanted heterotopically, they lose metastatic potential and change drug sensitivity.

The orthotopic xenograft model is a system in which tumor cells are implanted at the site of the organ of origin. Comparisons of the SOI (Surgical orthtotopic implantation) models with transgenic mouse models of cancer indicated that the SOI models have more relevance with clinical metastatic cancer. This organ-specific site presumably provides the tumor cells with an optimal environment for growth and progression. Because of its relevant expense and novelty, this model has as yet not been used widely by the NCI drug-screening program.

However, it is being used extensively to explore its role as an in vivo evaluation model for cytotoxic agents specific for organ sites such as the lungs in lung cancer. **Table 2** shows orthotopic models for study of human cancers grown in athymic nude mice.

Multiple tumor xenografts, including renal cell carcinoma [17,] pancreatic carcinoma [15], certain brain tumors [18] and prostate, colon, and (to a larger extent) lung cancer, have already been developed using nude mice [19]. All of these models are potentially amenable to orthotopic development.

TABLE 2: ORTHOTOPIC MODELS FOR STUDY OF HUMAN CANCERS GROWN IN ATHYMIC NUDE MICE [16]

Human Cancer Organ Site of Origin	Implantation Site in Nude Mice	Nomenclature
Central nervous system	Percutanous intracranial implantation into cerebral cortex	Intracranial model
Colon	Wall of cecum	Intracolonic model
Lung	Intrabronchially into right mainstem bronchus	Intrapulmonary model
	Percutaneously into right pleural space	Percutaneous intrathroacic model
Pancreas	Pancreas parenchyma	Intrapancreatic model
Renal	Subrenal capsule	Subrenal capsule model
	Kidney parenchyma	Intrarenal model

The **lung tumor model** is the predominant orthotopic model that has been explored by the NCI, and application of other models is currently under way. In the case of lung cancer, tumor cells in suspension are inoculated through the right main stem bronchus into the right lung in a lightly anesthetized animal. Tumor response can be evaluated by sacrificing the animal and histologically quantifying tumor growth, or, noninvasive chest x-rays may be sufficient to provide interim evaluation of tumor response.

Another method described by Hollingshead is **Hollow fiber Assay**[20]**.** In which the tumor cells are sealed in hollow fibers, which are than implanted into the mouse. Drug treatment is given to the animal. Cells recollected after sacrificing the animal and cell viability is determined. Polyvinylidene fluoride (PVDF) hollow fibers (500k Da M.wt. exclusion, 1mm i.d.) containing target cells are heat sealed and cut at 2 cm intervals and implanted into rodents. 3 or more tumor cell lines can be grown concurrently, in 2 physiologic sites, i.p. and s.c. within each mouse.

The mice are treated with experimental compounds once daily for four days. Fibers are collected 24 hr following the last dose of compound. After collection the viable cell mass is determined using an MTT dye conversion assay. The cytostatic/cytocidal effect of a compound is determined from differences in the viable cell mass in fibers from compound treated Vs diluent treated mice. Limitations of this method include absence of host stroma and its interaction with the tumor cells, absence of an immune system component, and limited number of compound doses that can be given in a short term assay. Moreover, ability to administer higher doses of compound than would be tolerated in a long term assay may result in poor prediction of the toxicity. It is a short term assay, which may be preferable in initial screening stage.

As it is a minimum challenge model, it is used by NCI to prioritize compound for testing in classical tumor models. Activity against more than one tumor cell line grown concurrently in two physiologic sites can be assessed in a single mouse. It allows successful maintenance of allogeneic as well as xenogeneic cells in immunocompetent hosts, thus decreasing the cost. Other advantages include minimum compound consumption and low false negative rate.

The pure tumor cell sample isolated from fibers can be used in various assays (e.g. western blotting). Protein or nucleic acids can be isolated or the intact fiber can be fixed, sectioned and subjected to immunohistochemical stains to determine target expression in the treated cells. With this technique, hollow fiber assay can be used to test compounds against specific targets *in vivo* while overcoming limitations of in vivo system.

Use of luciferase transfected cell lines allows HF cell mass to be evaluated without need for removal from host. This provides for real time evaluation of effect of compound.

6. **Genetically Engineered Mouse Models (GEMs):** Cancer in the genetically engineered animals resembles human cancer better than the other models outlined because the tumor develops spontaneously in its natural organ, unlike the xenograft tumor, which is usually implanted in other than an orthotopic site. The tumors have a natural growth rate and metastatic characteristics that resemble the natural history in humans. These tumors are nonimmunogenic within the natural host; hence, they overcome the requirement for the immunosuppressed animal to grow.

GEMs can be devided into two categories:

6.1 Transgenic mice

6.2 Knockout mice

6.1 Transgenic Mice: The transgenic mouse is the resultant progeny of the pronucleus of a fertilized egg that is **injected with a foreign gene.** This progeny then carries and expresses this exogenous gene and passes it on to its descendents. Genes can be transferred to the pronucleus by microinjection, retroviral infection, or embryonal stem cell (ESC) transfer. Transgenic animals are excellent models for studying the oncogenic phenotype that results from the disregulation of a known gene.

Examples in transgenic mice, which provided invaluable information regarding the characteristics of oncogenes, include the NF1 gene in the case of neurofibromatosis, c-fos, N-myc, erb B2, and others. Oncogene-expressing transgenic animals that develop spontaneous tumors as a result of a known pathway defect are an excellent model for testing directed drugs targeted to a specific molecular pathway.

For example, it is known that *ras* inactivation plays a major role in the pathogenesis of many cancers, including breast cancer. Transgenic mice carrying *ras* mutations that develop mammary tumors have been used to screen for the efficacy of new chemotherapeutic agents specifically active in breast cancer.

6.1.1 The TRAMP transgenic Mice: This consists of a minimal probasin promoter that drives expression of SV40 tumor antigens. These mice develop prostate cancer within 12 weeks of age and ultimately develop metastasis by 30 weeks[21]. The TRAMP mice recapitulate many salient aspects of human prostate cancer.

6.1.2 *p53+/- Wnt-1* transgenic mice: *p53+/-* mice have been crossed with MMTV- Wnt-1 transgenic mice to develop a model of mammary tumorigenesis where MMTV is the mouse mammary tumor virus promoter [22].

6.1.3 *Apc* deficient mice: These mice spontaneously develop preneoplastic intestinal polyps due to a dominant mutation of a *Apc (*adenomatous polyposis coli) gene. Mutation of this gene is common to most human colon cancers [22].

6.2 Knockout Mice: A knockout is an animal model that is generated by **omitting both alleles of a specific gene.**

6.2.1 The *Nkx 3.1* knockout mice: *Nkx 3.1* is a prostate specific tumor suppressor gene. It is essential for prostate differentiation and function. Loss of function of this gene results in histopathological defects that resemble prostate cancer in humans. This model provides a model for studying mechanism of prostate cancer initiation as well as to explore the tissue specific features of the disease[21].

6.2.2 Homozygous *p53 knockout* mice: Mutation of *p53* tumor suppressor gene is the most frequently observed genetic lesion in human cancer. Over 50% of all human tumors have identifiable *p53* gene point mutation or deletions. These mice are highly susceptible to spontaneous tumorigenesis particularly lymphomas[22].

6.2.3 *Brca1* conditional knockout model: *Brca1* deletion is induced using Cre loxp system by expressing Cre under the control of MMTV-LTR or WAP. Animal develop mammary tumor by the age of 10 to 13 months [23].

CONCLUSION: Spontaneous tumors were the most primitive models of cancer. Though not much useful in study of drugs, these models have provided great insight in studying natural progression of disease. Use of **virus-induced tumor** is rare now. **Chemically induced and radiation induced tumors** are having their own place in drug screening and evaluation. However, due to some of their limitations (especially long induction period) it is impracticable to use them in large scale screening programs. In such set up, which requires short term, reproducible and cheaper techniques **transplantable tumor** provides the best option. Thus, transplantable tumors have got their main application in drug screening.

Another important aspect of cancer research is investigating the activity of a drug on specific type of cancer. (Disease Oriented Approach). Use of **cell lines** (in vitro or in transplantable tumor) provides a great flexibility for this type of screening.

Genetically Altered Mice models also have potential to be used for disease-oriented screening. (However, cell lines are preferred over them with obvious reasons) But, they are mainly used for study of carcinogenesis. Use of these models has resulted in a number of hopeful targeted drug molecules, which are showing exciting results in clinical studies.

Thus, every model has its own merits and demerits. No one is ideal. So the thorough understanding of the available models and its rationale use becomes the important thing.

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