ORIGINAL RESEARCH PAPER

Veterinary Science

KEY WORDS:
Adenocarcinoma, albino mouse, spontaneous tumor, Acinetobacter, animal tumor models.

A RARE CASE REPORT OF ADENOCARCINOMA IN THE SHOULDER REGION OF MOUSE WITHOUT THE EVIDENCE OF METASTASIS AND A REVIEW ABOUT THE EXPERIMENTAL MOUSE MODELS FOR CANCER RESEARCH

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ABSTRACT

An adenocarcinoma may develop in the shoulder area of mice, although it is not a spontaneous event. In the present case involving an experimental female Swiss albino mouse (aged 1year and 10 months), a spontaneous growth was observed and expanding continuously in the right shoulder region. Following surgical intervention and subsequent histopathological analysis, the tumor was identified as adenocarcinoma. Post-surgery, the mouse recovered to normal health; however, the tumor gradually recurred, leading to the animal's death at 2.5 months after the surgical removal. At necropsy no metastatic foci were detected in the lung sections. On culture examination lungs showed the growth of Acinetobacter baumanni, liver showed the growth of Citrobacter koserii and the right as well as left kidneys showed the growth of E.coli and Citrobacter koserii. Various literature demonstrated human patients with metastatic lung adenocarcinoma that present as a soft tissue mass or bone lesion in the shoulder region(8). However, there are no specific case reports detailing the development of adenocarcinoma in the shoulder area of mice. Therefore, this case represents a rare instance of adenocarcinoma that arose spontaneously, rather than as a result of metastasis from lung adenocarcinoma. Research has indicated that animal tumor models are essential for experimental studies, as tumors in animals can exhibit characteristics similar to those found in human tumors. At present, based on the causes of tumor formation; we divide the animal model into 4 categories: spontaneous; induced; transgenic; and transplant. Among them, the spontaneous, induced and transgenic model belong to the primary tumor of animals, and the transplantable tumor models include allogeneic and xenogeneic transplantation; the most used transplantation tumor model is human tumor xenografts in immunodeficient mice [26].

INTRODUCTION :-

An adenocarcinoma, a cancer originating from the glandular cells, can occur in the shoulder region in mice, but it is not a typical or spontaneous occurrence in the shoulder area of mice; it is usually a result of experimentally induced cancers, such as by genetically modified mice or injected cell lines, or can arise from the metastasis of a primary adenocarcinoma from another site, like the lungs, to the shoulder tissues[17,19]. In the present case of a female Swiss albino mouse of approximately 1year 10 months old, there was a continuously growing tumor on the right shoulder region which was operated for saving the life of animal and the tumorous tissue was sent for histopathological examination. It was diagnosed as adenocarcinoma. This mouse was the experimental animal and was maintained in the Animal House of UP University of Medical sciences (UPUMS), Saifai. After taking approval of Institutional Animal Ethics Committee (IAEC) of UPUMS, Saifai, this mouse was used to demonstrate the muscle relaxant property of diazepam using rota rod apparatus for postgraduate students training purpose in accordance to their course curriculum **(IAEC approval no.-IAEC 03/AH/2024-25 dated 14/06/24)**. In the past, after taking approval of IAEC, many mice were used for the same experiment i.e. to demonstrate the muscle relaxant property of diazepam, but there was not a single case of adenocarcinoma.

Fig.A&B Animal with tumor **Fig.C&D-**Microscopic slides of tumorous tissue at **100× H&E;** before operation **Fig.E&F-**Microscopic slides of tumorous tissue **400× H&E**

Materials & Methods:- By using aseptic technique, surgical removal of tumor was done. The slides of tumorous tissues were prepared for histopathological examination and examined microscopically at 100X and 400X by using Hematoxylin & Eosin stain. Sections examined from multiple tissue bits showed solid acinic pattern. Individual cells were highly pleomorphic with round to oval in shape having high N:C ratio, hyperchromatic nuclei, scant to moderate amount of eosinophilic cytoplasm with prominent nucleoli at few places. Features are suggestive of poorly differentiated for malignancy (i.e. adenocarcinoma). After surgical removal of the tumor, the animal got healthy and was feeding normally but the tumor recurred slowly and slowly and the animal survived till completion of 2.5 months of the operation.

Fig. G to L - Photographs of animal during surgical operation:-

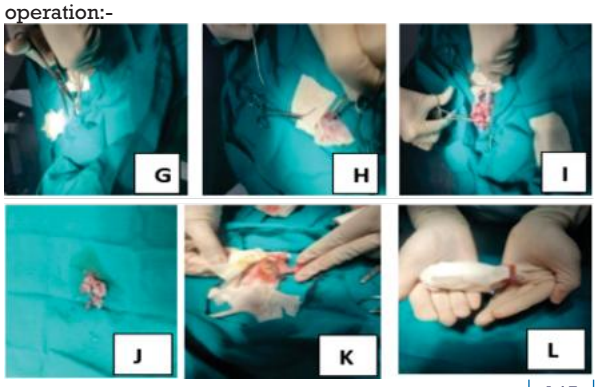
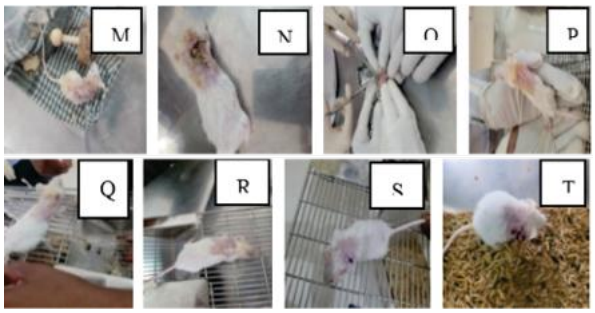


Fig.M to T- Photographs of animal taken on different dates during post operative care:-



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On post mortem examination, congestion and white necrotic patches at the edge of the lungs lobes were present and necrotic creamish coloured patches on the liver were found. Histopathological examination of the lungs and liver tissues showed mild mixed inflammation and no metastatic foci were seen in the sections examined. On culture examination, lungs showed the growth of Acinetobacter *baumanni,* liver showed the growth of Citrobacter koserii and the right as well a left kidneys showed the growth of E.coli and Citrobacter koserii.

RESULTS & DISCUSSION:- The studies have shown that in the context of the adenocarcinoma, the immune dysfunction associated with cancer can make the animal more susceptible to opportunistic infections[24] like in the present case Acinetobacter baumannii, Citrobacter koserii and E.coli infections occured. Diazepam alone does not typically cause adenocarcinoma in mice, but it can act as a tumor promoter by enhancing the development of liver tumors when given to mice already exposed to a known liver carcinogen like N-nitrosodiethylamine. Studies have shown that diazepam can promote the development of hepatocellular neoplasms (adenomas and carcinomas) in male mice after initiation with this carcinogen, with the effect being proportionate to the dose administered [5]. There are case reports found in human patients with metastatic lung adenocarcinoma presenting as a soft tissue mass or bone lesion in the shoulder area [13]. But there are no specific case reports of adenocarcinoma developing in the shoulder region of mice. So it can be said that this is a rare case of adenocarcinoma in the shoulder region of mouse which occurred spontaneously and not as a result of metastasis (from lung adenocarcinoma).

EXPERIMENTAL MOUSE MODELS FOR CANCER RESEARCH - A REVIEW

Introduction:- Animal tumor models are valuable for experimental analysis as the animal tumors can have similar characteristics to human tumors. We can study the occurrence and development of tumors by observing animal tumor models, and study the effect of genes on tumorigenesis and development by knocking out or knocking in a certain gene, and we can also establish PDX models for drug screening and preclinical trials. Furthermore, this experimental strategy is also advantageous as these experiments are low cost, the animals have short life cycles, and it can help to avoid human experiments. Thus the use of animal models is extremely valuable for tumor research. At present, based on the causes of tumor formation; we divide the animal model into 4 categories: spontaneous; induced; transgenic; and transplant. Among them, the spontaneous tumor model, the induced tumor model, and the transgenic model belong to the primary tumor of animals, and the transplantable tumor models include allogeneic transplantation and xenogeneic transplantation; the most used transplantation tumor model is human tumor xenografts in immunodeficient mice.

[I] Animal models for spontaneous tumors:- Spontaneous animal tumor models are utilized to investigate tumors that occur naturally in experimental animals without any conscious artificial intervention, and this occurrence type and incidence vary greatly with different species and strains of experimental animal. The advantage of the spontaneous animal tumor model is that the process of tumor formation is similar to that of human tumors, and the long period of tumor occurrence and development can be treated compre hensively with the intervention of many factors, and the role of genetic factors in tumorigenesis can be observed. However, the disadvantage of the spontaneous animal tumor model is that the experimental cycle is long, many experimental animals are required, and the cost is high. In addition, the spontaneous tumors of animals are often heterogeneous and there are individual growth differences, making it difficult to obtain a large number of tumor bearing animals with uniform growth at the same time. Following are the different types of spontaneous tumors:-

(1) Breast tumors- The incidence and mortality of breast cancer worldwide are increasing and have surpassed lung cancer in 2020 to become the most predominant cancer in the world. Mice have a high incidence of breast cancer. The occurrence of breast tumors in mice is affected by many factors, including factors such as viruses, hormones, heredity, and feed. Mouse mammary tumor virus (MMTV) is the most common cause of breast cancer, which causes breast cancer and lymphoma in mice mainly by insertion mutations and clone amplification in the genome; therefore mice carrying MMTV have a higher incidence of breast tumors. Wistar, SD, F344, and other rat strains also had a high incidence of breast cancer, Wistar rats were bred by the Wistar Institute of the United States in 1907, and SD rats were bred by Wistar rats in 1925. The incidences of breast fibroadenoma, breast adenoma, and breast cancer in female SD rats were 21.3%, 16.9%, and 3.4%, respectively. F344 rats were inbred and established by Dunning in subline 344 of the Fischer strain, and the incidence of breast fibroadenoma in the F344 rats was 41.2% [7].

(2) Lung tumors:- Lung cancer is one of the most common cancers and the leading cause of cancer-related death worldwide, with an estimated 2 million new cases and 1.76 million deaths annually. The histopathological subtypes of lung cancer include non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), which can be further divided into adenocarcinoma and squamous cell carcinoma (SCC). The incidence of spontaneous lung tumors in mice is high, and the pathological types are mainly adenoma and adenocarcinoma, depending on the mouse strain. 'A' mice and 'SWR' mice have a higher incidence of lung tumors, followed by BALB/C mice, CBA mice, and C3H mice, which have a lower incidence of lung tumors, and the lowest incidence of lung tumors was observed in DBA and C57BL/6 mice[22].

(3) Liver tumors:- Primary liver cancer is the fourth leading cause of cancer death worldwide, and its incidence is increasing every year. Histologically it can be divided into hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma. Spontaneous liver tumors in mice often originate from hepatocytes, cholangiocarcinoma is rare, and sarcomas are even rarer. The incidence of liver tumors is different in different strains of mice, but the incidence in male mice is usually higher than that in female mice. Male C3H/He mice are a highly spontaneous liver tumor strain, and as per literature, the incidence of liver cancer in 14 –month-old male C3H/He mice could reach 85% [9].

(4) Other tumors:- The incidence of renal adenocarcinoma in C+ mice was between 10% and 40%, and the incidence of tumors increased with age. AKR mice (new strain established by further inbreeding of AK mice by the Rockefeller Institute in 1936) are born with carcinogenic RNA virus. The incidence of leukemia in AKR mice after 8 months is approximately 60%-

90%, and that of 18 month old mice can be as high as 90 %. The incidence of reticulocytic sarcoma in the SJL mice was high, and the incidence of reticulocytic sarcoma in 13.5 month old female and 12.5 month old male mice was 88 % and 91% respectively [16].

[II] Animal models of induced tumor- The induced tumor model refers to the use of exogenous carcinogens to cause changes in the cellular genetic characteristics, resulting in the abnormal growth of active cells and the formation of tumors. Induction methods include physical, chemical and biological methods, of which the chemical methods are the most extensive and effective for the induction. Chemical methods have the advantages of easy application, short experimental time, and high reproducibility. The disadvantages of induction methods high animal mortality rate, and the time, location, and number of lesions are not uniform among individuals. Tumors can be induced chemically by using following substances [26]:-

(I) Urethane:- It was used as a herbicide and was later found to inhibit cell division and, thus, utilized as a chemotherapeutic drug for leukemia. Urethane is structurally one of the simplest carcinogens; it is soluble in both water and lipids and was the first water soluble carcinogen to be discovered. The biological effects of urethane depend on the direct inhibition of nucleic acid synthesis, especially of the pyrimidine bases, and an apparent antagonism between urea and pyrimidine. Mice are highly sensitive to urethane, especially newborn mice, and studies have shown that the rate of clearance of the urethane in newborn mice is 1/10 of that in adult mice, and 0.5 mg/g urethane can be metabolized in adult mice within 8 hours, while the same dose takes 72 hours in newborn mice.

(ii) NNK :- NNK also known as (methylnitrosamine-1-(3-pyridine)-1 butanone, is one of the main chemical carcinogens in cigarette smoke, and it can effectively induce lung cancer in mice, rats, and hamsters. The - hydroxylation of NNK catalyzed by a cytochrome 450 scan easily binds to DNA and forms DNA adducts causing gene mutations that lead to tumor development. There are various studies on different strains of mice that showed that the mice developed lung adenomas / lung cancer after administration of certain doses of NNK.

(iii) DMN/DEN:- Dimethylnitrosamines (DMN) and diethylnitrosamines (DEN) are nitrosamines, which can induce various tumors in mice and rats, but predominantly liver and lung tumors. Nitrosamines are activated by CYP 450 enzymes in vivo and converted into a strong alkylating agent, forming adducts in the DNA (active chemicals and cellular macromolecules from stable complexes through covalent bonds), resulting in carcinogenicity.

(iv) ENU:- Acetylnitrosourea (ENU) is a strong mutagenic agent that can cause rapid oncogenic genetic mutations in mice.

(v) NTCU:- N-nitroso-tris-chloroethylurea (NTCU) is a nitrosoalkylurea compound, which has been shown to induce lung squamous cell carcinoma (SCC) in mice.

(**vi) DMBA:-** Dimethylbenzanthracine (DMBA) is frequently used as a model for polycyclic aromatic hydrocarbon (PAH) induced mammary tumorigenesis because of its potent carcinogenic and immunosuppressive activities. Can be

(vii) NMBA:- Methyl benzylnitrosamine (NMBA) is also an important carcinogenic compound i.e. classified as a nitrosamine. NMBA is currently the most effective inducer of rat esophageal tumors, as they can be induced in 15 weeks or less.

(viii) MCA :- Methylcholanthracene is a potent carcinogen i.e. often used to induce the transformation of cultured cells, and it was found that repeated intratracheal injection of MCA into BC3F1 and DBA/2 mice could induce respiratory tract SCC, and the induced SCC, had obvious infilteration and metastasis.

(ix) AOM:- Azomethane is a chemical reagent that can promote base mismatch and cause cancer through the alkylation of DNA, which is often used in colonic carcinogenicity.

(x) Fattening diet:- Asgharpour et al. successfully established a model by following a fattening diet (high fructose-glucose solution and high fat, high carbohydrate diet- 42% calorie fat and 0.1% cholesterol) in 8-12 week-old mice. 89 % of the mice developed HCC (Hepatocellular Carcinoma) at 32-52 weeks, and each mouse had three or more tumor foci.

(xi) Ionizing radiation:- Moderate to high doses of radiation are well established causes of cancer, and ionizing radiation such as X-rays, -rays, -rays, -rays can break through genetic material and cause DNA fracture damage and gene mutations. Some studies have shown that irradiated mice may develop a series of malignant tumors, including sarcomas, and single high dose radiation significantly increases the incidence of tumors when compared with fractionated radiation[26].

[III] Transgenic models:- Gene mutation is an important cause of tumorigenesis, and targeted therapies for driving genes have achieved good results in tumor patients with a specific genetic background, but the problem of drug resistance still restricts the further benefits. Due to high similarity between mouse and human protein coding genes, transgenic mice have been used to study the effects of gene mutations on tumorigenesis and development since the mid-1980s. Transgenic animal tumor models have great advantages in studying tumorigenesis mechanisms and tumor immune escape. The establishment process of transgenic animal models is long, the feeding costs are high, and it is difficult to obtain a large number of experimental animals, which hinders rapid and high throughput research.[26]

(i) KRAS- Approximately 30% of human tumors carry 'ras' gene mutations, and the ras gene family includes Kras, Nras, and Hras. Kras is the most commonly mutated gene in the lung, colon, and pancreatic tumors, with a mutation rate of approximately 70%–90% in pancreatic cancer, 50% in colon cancer, 25%–50% in lung cancer, and 15%–25% in lung adenocarcinoma. Point mutations (including G12D, G12V, G12C, G13D, AMP, and G12R) are the most common Kras mutations. Most mutations in lung cancer models are Kras dependent; Kras mutations are found in 90% of spontaneous and chemically induced lung tumors in mice, and genetically engineered Kras mice have been widely used in lung cancer research, which is very similar to the genetic and pathophysiological characteristics of human lung cancer. In pancreatic cancer, the Kras mutation occurs in the early stages of tumorigenesis and accounts for approximately 90% of pancreatic ductal adenocarcinomas; it is often combined with other classical mutations (PTEN, etc.) to induce pancreatic cancer.

(ii) TP53 -Somatic mutations in the tumor protein P53 (TP53) gene are the most common changes in human cancers. The incidence of TP53 mutations in ovarian, esophageal, colorectal, head, neck, laryngeal, and lung cancers is 38%–50%, and the mutation rate is approximately 5% in primary leukemia, sarcoma, testicular cancer, malignant melanoma, and cervical cancer, and it is more common in advanced or invasive cancer subtypes. Normally, TP53 functions by activating or inhibiting the transcription of

numerous critical genes in diverse bioactivities, including cell cycle arrest, DNA repair, metabolism, senescence, and apoptosis. Trp53 (TP53 is called Trp53 in mice) knockout mice are common transgenic animal models that formulate spontaneous tumors at the age of 6 months (including breast cancer, sarcoma, brain tumor, and adrenocortical carcinoma). Compared with homozygous mice, mice with the Trp53 allele heterozygotes had later spontaneous tumors; the most common tumor type in homozygotes was malignant lymphoma, and the main heterozygotes were osteosarcoma and soft tissue sarcoma.

(iii) PTEN- Pten (phosphatase and tensin homolog deleted on chromosome ten) is a classical tumor suppressor gene with a mutation rate of approximately 12% in breast cancer, 1% in thyroid cancer, 2.6% in endometrial cancer, 1.6% in renal cell cancer, 5% in colon cancer, and 2% in malignant melanoma. The PTEN deletion was also reported in 15% of poorly differentiated serous ovarian cancers, and Pten mutations were also found in 20% of endometrioid ovarian cancers; approximately 53% of patients with primary bladder cancer showed a decrease or deletion of the Pten protein in the cytoplasm or nucleus of their tumor cells. The tumor inhibitory activity of PTEN depends to a large extent on its phosphatase activity, which regulates the activity of many important cellular pathways, such as PI3K/AKT; thus, it can regulate many cell processes, including proliferation, survival, energy metabolism, cell structure, and movement. The current transgenic model of Pten is widely used in the study of tumorigenesis mechanisms; however, half of the Pten knockout mice died within 1 year after birth, and the rest developed a variety of tumors, including lung, breast, thyroid, endometrial, and prostate cancer, and T-cell lymphoma.

(iv) EGFR- Epidermal growth factor receptor (EGFR) is a member of the HER family, which includes HER1 (erbB1, EGFR), HER2 (erbB2, NEU), HER3 (erbB3), and HER4 (erbB4). Studies have shown that there is high or abnormal expression of EGFR in many solid tumors, which is related to tumor cell proliferation, angiogenesis, invasion, metastasis, and inhibition of apoptosis. In a study using EGFR knockout mice, the types of cells most affected by the EGFR deletion were epithelial cells and glial cells, while the types of cells overexpressing EGFR in the human tumors were epithelial cells and glial cells. A large number of EGFR mRNA deletions have been observed in a number of neoplasms, first in glioblastoma, but recently in non-small-cell lung cancer, breast cancer, pediatric gliomas, medulloblastomas, and ovarian cancer.

(v) Combined mutations- Tumors are often caused by multiple gene mutations, the most common of which is the activation of oncogenes and inactivation of tumor suppressor genes. Kras and TP53 are common combined gene mutations in human cancer, and KP mice with both Kras and Trp53 mutations are the most classic transgenic model [26].

[IV] Transplantable animal tumor models - Human tumor generated mouse models are established by transplanting human tumor cells and/or tissues of research interest into recipient animals (almost always possessing immune function deficiencies). The advantage is that most types of human tumors can establish transplantable tumor models in immunodeficient animals, and under the same inoculation conditions, the growth rate of animals is the same, the difference in tumor formation rate is small, and the inoculation tumor formation rate is high. The disadvantage is that the recipient host animal needs to be in an immunodeficient state requiring special housing in an aseptic environment, which is expensive to maintain. Furthermore, not all cell types of human tumors can be successfully established in rodent models and the stroma of the human tumor tissue obtained may contain the components of the recipient animals. It is worth noting that the key to developing an optimal transplanted tumor model lies in the immune status of the host (immunodeficiency state) and the composition of the graft (containing important or all components of the tumor) [26].

(i) Immunodeficient mice:- The immune status of the host has undergone many improvements, which is the key to the development of a successful transplant tumor model. Transplantable tumors are divided into allogeneic transplantation and xenogeneic transplantation, and the most used is the xenotransplantation of human tumors. Due to the existence of immune rejection, wild mice cannot be used for xenotransplantation of human tumor. However, human tumors can be implanted in immunodeficient mice, and the tumors can maintain the histological, immunological, and biological characteristics of original tumors.

- Nude mice lack thymus and T lymphocytes, and thus, they lacked T cell-mediated immune responses and could be used as recipients for human tumor xenotransplantation. However, because nude mice still have B cells and NK, they are not suitable for use as hosts for lymphoma and leukemia, which limits the extensive application of nude mice in relation to transplanted tumors.

- SCID mice are more severely immunodeficient than nude mice, and the mutant genes of the SCID mice were identified in 1996. The maturation defects of B lymphocytes and T lymphocytes in SCID mice were caused by a point mutation in the Prkdc (protein kinase, DNA-activated, catalytic subunit) gene, which causes the affected lymphocytes to die prior to maturation or be cleared by the macrophages, granulocytes, and NK cells in the body. However, it is usually difficult to establish a successful model for lymphoma and metastatic tumors in SCID mice because of leakage (some SCID mice will restore the function of some T and B lymphocytes with age). In addition, NK cells and other innate immunoreactive substances in SCID mice were present at high levels, which limits the success rate of transplantation, such as human hematopoietic stem cell (HSC) transplantation [15].

- Subsequently, the establishment of NOD/SCID mice ushered in a new breakthrough in transplanted animal tumors, which are a type of spontaneous type 1 diabetic mice, caused by T lymphocytes infiltrating and destroying islets, as well as complement loss and impaired function of NK, macrophages, and dendritic cells. NOD/SCID mice were established through the hybridization of NOD and SCID mice, and NOD/SCID mice will not develop type 1 diabetes, as they lack an adaptive immune system and the loss of effector T cells; moreover, due to extensive defects in innate and adaptive immunity (low activity of NK cells and loss of T- and B-cell function), NOD/SCID mice have become a stable and excellent animal model for human HSCs and human solid tumor transplantation. At the beginning of the 21st century, scientists introduced IL-2Ry mutations on the basis of NOD/SCID mice, resulting in NSG and NOG mice, which, in addition to being the mice with the highest degree of immunodeficiency at present, could also be used to construct humanized immune system mice to study the antitumor effects of chimeric antigen receptor T-cell immunotherapy (CAR-T) and immune checkpoint inhibitors (ICIs)[20].

(ii) PDX- Transplant components have also been the focus of transplantable tumor model research in recent years, and in the past, we transplanted tumor cell lines into animals, which are simpler and easier to use, but there are some defects:

(1) This tumor model cannot fully represent the unique characteristics of each cancer patient.

(2) This model cannot reconstruct the remaining non-tumor

cell components in the tumor tissues, which plays an important role in tumorigenesis and development. Consequently, the patient-derived xenotransplantation (PDX) model has been actively generated and applied, and the PDX model can directly implant cancerous tissues from the patient's tumor into immunodeficient mice, thus preserving both the cell–cell interaction and the tumor microenvironment [11].

- The PDX model retains the characteristics of the primary patient tumor, including the gene expression profile and drug response, and offers great advantages for drug screening, biomarker development, and the evaluation of therapeutic effects. The PDX model usually takes approximately 6 months to 2 years to establish, and the success rate varies (10%–90%), depending on the tumor source and disease characteristics. Specifically, invasive, recurrent, and the transplantation rates tend to be higher for highly metastatic tumors. Gastrointestinal tumors (such as colon and pancreatic cancers) have higher transplantation success rates than other cancers, and the transplantation rate in breast cancer is low.

Animal models also play an important role in drug development and screening. In the past, the average success rate of translating animal research into human clinical trials was approximately 8%, and <5% of antineoplastic drugs were approved to enter the market. Although the accuracy of the PDX model on drug efficacy and drug resistance was up to 90%, the traditional PDX model takes a long time to construct; owing to this, it cannot quickly reflect the drug sensitivity of patients and cannot meet clinical needs [26].

(iii) Humanized mice model- Although PDX, which depends on immunodeficient mice, has been widely used in the research of tumor immunity and the development of new therapies, the lack of models for the human immune system and tumor immune microenvironment limits the study of immune mechanisms and transformation of immunotherapy to a great extent. The humanized mouse model is a mouse model that reconstructs the human immune system by implanting human hematopoietic cells, lymphocytes, or tissues into immunodeficient mice, which can effectively reconstruct the human immune system and better simulate the characteristics of human immunity [21]. From the earliest nude mice to the later SCID and NOD/SCID mice, the success rate of human cell implantation has been low either due to the existence of innate immunity or because of high sensitivity to radiation and the limited life cycle. Because of this, all these models limited the application of immune system-humanized mice in practical research to some extent. In NOG mice, mature T/B cells and NK cells are lacking, complement activity is decreased, and the function of macrophages and dendritic cells is impaired; therefore, NOG mice are an ideal model for human immune cell transplantation. Subsequently, the IL-2 receptor mutation was further developed. NSG mice had a complete IL-2 receptor chain-invalid allele, similar to NOG mice, with a loss of T/B and NK cells, lack of complement activity, and defects in macrophages and dendritic cells. In addition, other highly immunodeficient mice, such as BRG and BRGS mice, have been established as humanized mouse models that lack T/B cells and NK cells [12]. BRGS mice provides superior human cell engraftment due to additional *Sirpa* mutation often serving as a model for atopic dermatitis and advanced immune system.

- Although NOG and NSG mice are very useful humanized models, they still lack the ability to reconstruct myeloid and NK cells, and a new generation of super immunodeficiency models HuNOG-EXL and HuNSG-SGM3 have been developed, which can express some growth factors to promote myeloid regeneration. According to the method of human immune system reconstruction, the humanized mouse model can be divided into three categories[4]:

[I-]Hu-PBMCs (humanized - peripheral blood mononuclear cells)	[II-] Hu-HSCs (humanized - hematopoietic stem cells)	[III-] Hu-BLTs (humanized - bone marrow, liver, thymus)
The Hu-PBMC model is a simple and economical humanized mouse model in which mature lymphocytes from peripheral blood mononuclear cells (PBMCs) are injected into immunodeficient host mice through intraperitoneal (i.p.) or intravenous (i.v.) injection. This method was first described in CB17-SCID mice in 1988 and has been widely used to study the human immune response in autoimmunity and infectious diseases. However, graft-versus-host disease (GvHD) occurs in the Hu-PBMC model in 2–3 weeks, and the survival time is short. At present, this model is often used to study the activation of human effector T cells and to evaluate immunosuppressive drugs	In this model, human CD34+ HSCs from human umbilical cord blood, adult bone marrow, or fetal liver were injected into adult mice (intravenous or intrafemoral) or newborn mice (intracardiac or intrahepatic), and sublethal irradiation of host mice is needed to eliminate HSC and promote the transfer of human HSC. Fetal liver and umbilical cord blood are the most commonly used sources of human CD34+ HSCs, which are easier to colonize in immunodeficient mice than that in adult HSCs. Although this method can produce a variety of HSCs in adult mice, the number of T cells produced is small and the model does not possess functional immune cells. In newborn mice (less than 4 weeks old), good human cell transplantation can be obtained, and T cells, B cells, macrophages, NK cells, and DCs can be produced.	This model is established by transplanting human fetal liver and thymus tissue into the renal capsule of adult immunodeficient recipient mice after sublethal irradiation, and at the same time, fetal liver or bone marrow-derived CD34+ HSCs from the same individual is injected intravenously (i.v.) into recipient mice. This model is often used to study adaptive immune responses, such as HIV infection. The incidence of GvHD in the Hu-BLT model is higher than that in other CD34+ HSC transplantation models, and complex and precise surgical procedures are required, thus limiting the application of the Hu-BLT model in the research and development of tumor immune drugs.

At present, it is believed that the Hu-HSC model can establish the human innate immune system and lymphocytes, with little or no occurrence of GvHD, which can be used for long-term research; however, owing to the species differences between humans and mice, there is a lack of human cytokines in mice, and the development of human stem cells in mice is limited [23].

Prospects:- Since the establishment of the earliest spontaneous animal tumor model, we have obtained a great deal of information about tumor generation and progression. But we have gradually eliminated this method because of it's randomness, high cost, and long cycle of tumorigenesis. Then, chemical drugs were used to induce tumors in mice to establish tumor models and study the carcinogenic effect of a certain factor. Induced tumor is the most widely used method for establishing animal tumor models at present, due to the low technical requirements and cost, most of the laboratories can perform this technique. With the development of the transgenic animal model and the PDX model, we have made

great progress in understanding specific gene function, building animal models of human diseases, and evaluating the safety and effectiveness of new drugs, and it is hopeful that it will be a bright light for the study of animal tumor models in the future [26].

Genetically Engineered Mouse Tumor Models (GEMMs):-

Genetically engineered mouse models (GEMMs) of human cancer were first created nearly 45 years ago. These early transgenic models demonstrated that mouse cells could be transformed in vivo by expression of an oncogene. A new field emerged, dedicated to generating and using mouse models of human cancer to address a wide variety of questions in cancer biology. The GEMMs that revealed oncogene dependence were generated by using sophisticated mouse modeling techniques developed throughout the years. Ideal GEMMs of human cancer feature tumors that are initiated in a small subset of cells within an organ by a genetic lesion found in the human counterpart and for which steps in tumor progression (eg, angiogenesis, acquisition of secondary mutations, ability to metastasize) also resemble the human disease. Moreover, to additionally mimic most human sporadic cancers, the genetic lesion being studied should be induced in adult tissues rather than during embryonic development. As a result of extensive refinement of techniques for generating mouse models, GEMMs available today have many of these features. The first mouse models of cancer were created with the advent of technologies to generate transgenic mice by pronuclear injection of DNA. These techniques have allowed nearly every cancer type to be modelled in the mouse, including common adult cancers like breast, prostate, and lung cancers and rarer tumors like pancreatic cancer. Moreover, childhood tumors like medulloblastomas and rhabdomyosarcomas have also been modeled in GEMMs. For detailed descriptions of several of these models, the reader is referred to recent comprehensive reviews [18].

GEMMs Versus Xenografts

Historically, xenografts have been the most extensively used mouse models in preclinical drug testing. In xenografts, human cancer–derived cells or tumor fragments are implanted into immunodeficient mice subcutaneously or orthotopically (i.e, into the organ of interest) to propagate tumors that are monitored for response to drug treatment. Xenografts are useful for these studies, because many different drugs and dosing schedules can be tested against the same set of cells (in different mice).

Advantages of tumor xenografts:- (i) The starting material is usually derived from advanced cancers or metastases;

(ii) The cells presumably represent real human tumors replete with genetic complexity.

Disadvantages of xenografts:- (i) Tumor-derived cell lines may not completely recapitulate intratumor heterogeneity, because the cells that grow may represent only a subpopulation of tumor cells.

(ii)Tumor growth occurs in a host with an impaired immune system and usually (in subcutaneous models) in an artificial site where the original human tumor did not develop.

- GEMMs circumvent these issues, because tumors arise in situ where immune function, angiogenesis, and inflammatory processes can all interact normally with the developing tumor. Thus, GEMMs allow for the analysis of tumors as they develop through defined stages of tumorigenesis, facilitating studies of the biology of the tumors early and late in the process[18].

Mouse models of drug resistance and tumor relapse:-

A major clinical problem is the development of acquired drug resistance. Elucidation of mechanisms of acquired resistance could lead to new treatment strategies. Ideally, mechanisms of drug resistance could be studied on human tumor samples that have acquired resistance to a particular drug. Although this approach has been successful for understanding acquired resistance to EGFR TKIs in the case of lung cancer and to imatinib in CML and gastrointestinal stromal tumors, obtaining adequate numbers of samples and sufficient material for thorough molecular studies is challenging. Tumor cell lines offer an attractive alternative approach to study drug resistance, because unlimited material is available. However, the findings in cell lines do not always parallel the in vivo situation; hence, the clinical relevance of results obtained using cell lines is not always clear [1].

For these reasons, GEMMs are emerging as important vehicles to study tumor relapse and drug resistance. Mouse tumors with defined genetic mutations are monitored serially for tumor regression and recurrence by using small animal imaging techniques (magnetic resonance imaging, computed tomography, positron emission tomography [PET], ultrasound)[18].

Using GEMMs to identify biomarkers and imaging strategies for early detection and response prediction:-

For most cancers, outcomes are significantly better when tumors are diagnosed at early stages. However, many epithelial cancers are more frequently diagnosed at advanced stages of the disease because of a lack of useful biomarkers for the early detection of these tumors. New approaches to find blood biomarkers for cancer or to develop imaging techniques to screen high-risk individuals and the general population for these cancers are necessary. One such approach has been to use GEMMs, which allow for collection of blood from mice with genetically defined tumors at specific stages of tumorigenesis to identify plasma biomarkers of disease. Studies in GEMMs for which defined genetic mutations are engineered to cause cancer are likely to be useful for the identification of plasma protein changes that occur on treatment with specific drugs. Monitoring of plasma protein levels in patients who are undergoing treatment would be an easy and relatively inexpensive way to assess the effectiveness of therapy[18]. GEMMs also have the potential to be important assets for the development of molecular imaging techniques to monitor disease progression, response to therapy, and eventual disease relapse. Such techniques could also pinpoint the biologic characteristics of individual tumors (eg, proliferation rate, signaling pathway changes).As an example, in a GEMM of high-grade glioma, the thymidine analog 3 -deoxy-3 -^{18}F-fluorothymidine(^{18}F-FLT) was used for PET imaging to quantify tumor cell proliferation[2]. This imaging technique can now be used to assess the effectiveness of different therapies in this particular model and may be developed clinically to monitor how brain tumors respond to specific drugs. In another example, deletion of the tumor suppressor *Lkb1* (a serine/threonine kinase that regulates adenine monophosphate-activated protein kinase [AMPK]) in mice expressing oncogenic *Kras* in the lung accelerated tumor onset, and metastases were observed in two thirds of the mice examined[10]. Finally, metastatic disease was observed in a mouse model of melanoma only when expression of the $Braf^{V600E}$ mutant was combined with deletion of the tumor suppressor *Pten* [3]. Invasion of metastatic cells into the subcutis and lesions in the lymph nodes and lungs were found in almost all of the mice.

Technical advances to improve the cost effectiveness of GEMMs:-

Increasing knowledge of the multitude of genetic changes present in human tumors means that, to effectively understand the impact of these changes on tumorigenesis and on the response to specific drugs, we need to be able to

mimic in the mouse, rapidly and cost effectively, the compendium of mutations deemed to be important. This represents a challenge, because the generation of knock-out, knock-in, and transgenic alleles is time consuming, as is breeding the different alleles. Several laboratories have devised strategies to overcome some of these problems. For example, multiple transgenic constructs can be introduced sequentially into embryonic stem (ES) cells (potentially already null for a specific tumor suppressor gene). The ES cells are then used to generate chimeric mice, which can be directly used as experimental animals[25].A major advantage of this system is that modified ES cells carrying multiple combinations of genes can be frozen and stored until needed, greatly reducing the costs that would be associated with maintaining these complex strains. Somatic delivery of viral vectors bearing one or more cDNAs or shRNAs of interest directly to organs is also likely to be an effective and widely used strategy to test the role of multiple genes in tumorigenesis in vivo [6,14].

Multiple uses of genetically engineered mouse models (GEMMs) of human cancer [18] -

(i) GEMMs are useful for preclinical development of novel targeted therapies in genetically defined tumors (as particular gene is responsible for generation of tumor-oncogene).

(ii)GEMMs are emerging as important vehicles to study tumor relapse and drug resistance.

(iii) The use of GEMMs allows for collection of blood from mice with genetically defined tumors at specific stages of tumorigenesis to identify plasma biomarkers of disease.

(iv) These are useful in the identification of genetic determinants of metastatic progression and the development of strategies to interfere with metastasis formation.

(v) These are useful to test the effectiveness of agents to prevent cancer formation/ progression and to establish the effect of environmental changes on tumor burden in mice genetically modified to develop specific cancer types [18].

CONCLUSION:-

- GEMMs of human cancer have contributed significantly to our understanding of basic cancer biology.
- There are still major pitfalls for meaningful experimentation and inconsistencies between the models and human disease, so constant refinements and innovations are needed to generate more realistic mouse models of human disease.
- Since the ultimate goal of cancer research is to design effective therapeutic approaches to curb lethal metastatic disease, so the efforts need to be once more geared up and channelled toward the generation of the perfect surrogate mouse models of human cancer.
- The animal tumor models still have significant limitations in modelling human cancer, which is mainly reflected in the immune microenvironment and tumormicroen vironment, or the more subtle differences caused by species-specific differences.
- Although we have developed the PDX model and humanized model to reduce these differences, it is urgent that we develop and establish more effective animal tumor models to facilitate more detailed investigation of the tumor development process.

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