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Animal models for Cancer research and treatment

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Abstract

Use of laboratory animals is unavoidable in cancer research throughout the world. It would have been impossible to reconstruct the events that occur in human patients with cancers without use of appropriate animal models. Based on the type of cancer research, various models ranging from normal mice/ rats, GEMM, specialised and super specialised animal models are a choice. Use of more specialised and humanized mice models is advantageous due to higher percentage of tumor acceptance and metastasis rate. However, maintenance of such animals need improved housing and environmental conditions in addition to high quality food/water and trained manpower to maintain these animals. If all these facilities are available, it is not difficult to breed/maintain quality animals for the cancer research with reproducible and meaningful results

Introduction

Animal use is warranted for development of new and more effective methods of diagnosis and treating diseases that affect both humans and animals. Since use of humans is unethical for experimentation, animal usage is inevitable for any research targeted for their wellbeing. Literature survey indicates that rodents, canines, bovines, equines as well as reptiles and birds have been used in biomedical research. Rodents have been extensively used in biomedical research [1]. The laboratory mouse and rat is an excellent experimental model, in part because of well-defined strain genealogies, standardized mapping tools, well published background literature and the availability of sophisticated genetic monitoring tools.

Experimental models of human cancers are important in reconstructing the events that occur in human patients with cancers. Various factors decides the choice of tumor and animals model to be used for the research. These animal models are broadly divided into two types, first, graft of tumor material (syngenic or xenogenic) into normal or immuno-compromised animals and second, genetically engineered mice model (GEMM) that are used for a specific cancer genotype [2, 3].

Animal models have played crucial role in almost all medical related advances through animal research. Knowledge gained through use of animals for research is still improving the life of not only humans but domestic, pet as well as laboratory, zoo and wild animals. Similarly, various animal models have played important role in understanding the cancer biology as well as development of treatment modalities for cancer patients. Basic cancer research along with clinical trials on cancer patients is essential part of the cancer drug discovery procedure. For more than a half century the laboratory rodents has been the primary species in which experimental cancer chemotherapy have been tested [4]. The cancer drug screening historically relates to the murine ascitic tumors such as L1210 and P388. In the mid 1950's, the National Cancer Institute (NCI) of the USA started the cancer drug screening program with L1210 and P388 and in 1970's they added the solid tumors in the program using carcinoma 755 cell lines. This was made possible with use of conventional mouse models like C57BL/6, Swiss, BALB/c and DBA/2 strains. Subsequently, the discovery of the Nude and SCID mice allowed the widespread use of human tumor transplantation for anticancer research and testing [5, 6].

Spontaneous benign and malignant cancers are often reported in mice and rats. The incidence may vary from strain to strain. These spontaneous tumors do not always metastasize to different organs or if at all have mild local tissue invasion and therefore the incidence is very rare. However, it is noteworthy to mention that majority of the data generated on the metastatic behavior of cancer is reported to be derived from studies in rodent models [7, 8].

IMMUNOCOMPOROMISED MOUSE MODELS

Specialized animals

Nude mice were first discovered by Griest in 1962 but were studied and reported as lacking the T-cells in 1966 by Flanagan [9]. They lack the hairs (fig. 1) and thymus due to the mutation of *Nude* gene located on chromosome no. 11. Severe combined immunodeficiency (SCID) mutation was first discovered by Bosma and Carroll in 1983 [10]. The *scid* mutation in the *Prkdc* gene is responsible for in repairing double-stranded DNA breaks and in recombining the variable (V), diversity (D), and joining (J) segments of immunoglobulin and T-cell receptor genes. This gene has been identified on chromosome no. 16. Homozygous mutants have no mature T- and B- cells and therefore are important models for cancer research [11]. These mice are phenotypically similar to the Swiss mice (fig. 2). Since 2009, hairless SCID mice are made available commercially for the cancer researchers by the Charles River Laboratories. These mice are hairless on albino background and lack T- and B-lymphocytes and therefore like the nude mice they are convenient to transplant the tumor tissues and also monitor through imaging. These represent the 'specialized' group of animals for the cancer research without which it would have not been possible to discover the therapeutic drugs for cancer [12].

Housing and food

Appropriate housing, food, water, environmental conditions and trained personnel to take care of them are of prime importance for maintaining the colony of immune-compromised animals. These immune-compromised mice lack T-, B- as well as innate immunity and therefore housing of these mice under sterile condition is one of the prime requirements. Separate air handling unit or Heating Ventilation and Air Conditioning (HVAC) system is a necessity for survival of these mice. Restricted entry and that too of the authorized personnel must be practised for these rooms. Regular environmental monitoring and sterility check of the rooms is a key to maintain the animals under healthy and infection free conditions. Under Indian conditions, housing of these mice can best be done in the positive pressure isolators. Individually ventilated caging (IVC) system can also be used.

Fig. 1. Nude mouse.

Fig. 2. SCID mice with well grown human tumor. A and C- subcutaneous tumor growth. B and D- tumor growth as seen after cutting open the skin flap

Providing high protein content diet with irradiation or atleast autoclaving is practiced worldwide for maintaining these mice under optimal conditions. Unfortunately, commercially available food of Indian make are not good enough for feeding these mice which supports growth and breeding. The only option left is to procure the feed from reputed imported sources.

Source of clean water is another requirement for maintaining these mice. Regular municipal water put through the UV and charcoal based water purifier is good enough. The water collected through these purifiers may also be autoclaved before offering to the animals. Alternatively, RO water can best be used where water supply is with very high total-dissolved solutes (TDS).

Need for Specialised animal models

Cancer research necessitates better preclinical models that more accurately predict the response of human cancer to chemotherapy. Carcinogen induced tumors in murine models provide the solution to some extent. However, high engraftment rates of human tumor with short period of acceptance are the prerequisites to make the *in vivo* model feasible for cancer research [2, 3]. Triple mutation for Nude, Bg and Xid mutation is also available as NIH-III mice. This is developed in the National Institute of Health, USA. They lack T-, B- as well as NK cells. NOD-SCID, SCID beige, NSG, NOG and Rag-1 and 2 mutation mouse model also lack T-, B- as well as NK cell activity and are the next level of '*super specialized*' models which supports not only hetero-transplantation but also metastasis studies.

The conventional mouse models have made important contribution to our knowledge of cancer mechanism and would continue to do so. Further the discovery of Nude and SCID mice model have made significant improvement in these models and have made it possible to identify clinically efficacious agents through anticancer research and testing and is said to be a 'W*orkhorse*' of the pharmaceutical industry [13, 2]. Before the discovery of the Nude/ SCID mice, whole body irradiation coupled with thymectomy of the neonate rodents was the choice for studying the human tumors *in vivo*. Hetero-transplantation of human tumor biopsies or tumor cells into Nude/ SCID mice has opened new path for understanding the tumor processes/ preclinical screening for development of newer cancer drugs. Because of the absence of T-cells, immuno-compromised models often supports growth of solid tumors and therefore are routinely used models for transplantation of human surgical specimens and established human tumor cell lines. These models have not only helped in characterization of cancer cell lines but also helped in understanding metastatic processes and efficacy of the newly developed anticancer drugs [14]. There are numerous reports wherein scientists have reported metastases in different parts of the body of the experimental animals. This information is not always possible in human patient unless otherwise operated or detailed post mortem is conducted after their deaths.

Tissue microenvironment is inherent in the mouse model system. Consequently, the mouse tumor response must closely mimic the human tumor response to therapy [15]. Tumor growth induction under the subcutaneous tissue has been quoted as a model by several authors. However, these tumors have lower acceptance rate, are often encapsulated, rarely infiltrate/ metastasize and do not always reflect the clinical situation even when highly aggressive tumors have served as a source of xenografts [16, 17]. In this context, implantation of tumor under renal capsule/ orthotropic organs has been shown to produce higher uptake and metastasis to different organs [18, 19, 20, 21].

There has been significant improvement in diagnosis, surgical techniques, general patient care, and local and systemic adjuvant therapy for cancer. However, most deaths from cancer are due to metastasis that are resistant to conventional therapies [22, 23, 24, 25]. Prevention of metastasis has always been of more interest to academic scientists as well as to the practicing oncologists [26]. Therefore, appropriate model for cancer development and metastasis is of utmost importance for the researchers.

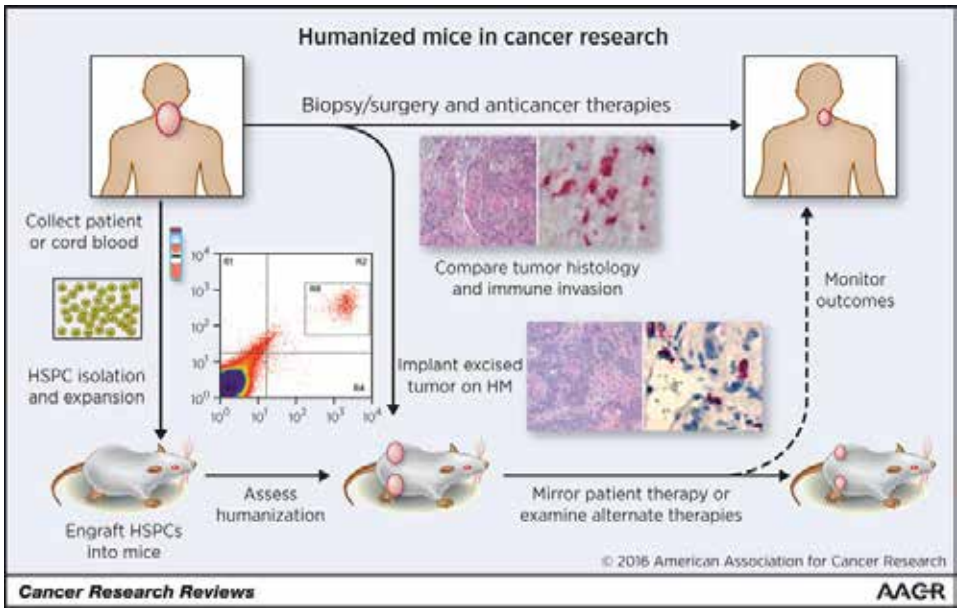
PATIENT DERIVED XENOGRAFT TUMOUR MODELS

Progress in oncology drug development has been sluggish due to lack of preclinical models that reliably predict clinical activity of novel compounds in cancer patients. In an effort to address these shortcomings, there has been a recent increase in the use of patient-derived tumour xenografts (PDTX) engrafted into immuno-compromised rodents such as NOD/ SCID or NSG mice for preclinical modelling. Numerous tumour-specific established PDTX models are biologically stable when passaged in mice in terms of global gene-expression patterns, mutational status, metastatic potential, drug responsiveness and tumour architecture [27, 28]. Well characterized PDXT models are now available worldwide commercially or through collaborative network research [29]. However, it is suggested to develop the PDXT models seen in our region/ country locally. This is for the reasons that the tumor incidence in other countries may be due to the reason not valid for our country and secondly they may not truly represent the tumor profile seen in our country.

Humanized mouse models

Use of humanized mouse (HM) model is one of the latest trend for not only cancer biology but also for human haematopoiesis, innate & adaptive immunity, autoimmunity, infectious diseases, regenerative medicine and allergy research. Humanized mouse model refers to normal immunocompetent mice expressing human genes via transgenesis or to immunodeficient mice engrafted with human hematopoietic/ lymphoid cells or tissues. Schematic diagram of creation of HM from cord or patient blood, implantation of patient tumors, and comparative analysis of therapy is shown in fig. 3. Several types of humanized mice can be produced for cancer research where they express specific human protein considered relevant for the cancer growth of our relevance. Humanized mice can also be produced by direct injection of human peripheral blood [30]. Examples of some of the humanized mouse models are given in table 1 [31].

Fig. 3. Schema showing the creation of HM from cord or patient blood, implantation of patient tumors, and comparative analysis of therapy.



Adopted from Morton et al., 2016.

Table 1. Models of humanized mice.

Model	Approach	Characteristics	Use
Hu-PBL-SCID (Human Peripheral Blood Lymphocyte)	Engraftment of mature PBMC obtained from blood, spleen or lymph nodes	Predominately engrafts human CD3 T cells. Study of mature T cell function	Study of mature immune cell function
Hu-SRC-SCID (Human Scid Repopulating Cell)	Engraftment of hematopoietic stem cells	Develops human hematopoietic and naïve immune systems	Study of complete hematopoietic system and naïve immune system
SCID-Hu	Engraftment of human fetal liver and thymus	Develops human hematopoietic and immune systems	Study of complete hematopoietic system and naïve immune system
Hu-Tg Mice	Transgenic expression of human genes	Expresses specific human genes in vivo	In vivo study of human gene function

Adopted from Brehm et al., 2010.

References

1. Isselbacher Kurt J, Barger A Clifford, Cuatrecasas Pedro, Loew Franklin M, Purpura Dominick P, Thompson Richard F (1991). Why Are Animals Used in Research? In Frank Press and Samuel O. Their (eds). Science, Medicine, and Animals, National Academic Press, Washington, pp 4-5.
2. Morton CL and Houghton PJ (2007). Establishment of human tumor xenografts in immunodeficient mice. *Nature Protocols;* 2: 247-250.
3. Richmond A and Su Y (2008). Mouse xenograft models vs GEM models for human cancer therapeutics. *Dis Model Mech;* 1 (2-3): 78-82.
4. Kerbel RS (2003). Human tumor xenograft as predictive preclinical model for anticancer drug activity in humans. *Cancer Biol Ther;* 2 (4-1): S 134-139.
5. Johnson JI, Decker S, Zaharevitz D et al (2003). Relationship between drug activity in NCI preclinical in vitro and in vivo model and early clinical trials. *Br. J. Cancer;* 9: 1424-1431.

6. Sausville EA and Burger AM (2006). Contributions of human tumor xenograft to anticancer drug development. *Cancer Res;* 66 (7): 3351-3354.

7. Giavazzi R, Campbell DE, Jessup JM et al (1986). Metastatic behaviour of tumor cells isolated form primary and metastatic humnan colorectal carcinomas implanted into different sites in Nude mice. *Cancer Res;* 46: 1929-1933.

8. Morikawa K, Walker SM, Jessup JM et al (1988). In vivo selction of highly metastatic cells from surgical specimens of different primary human colon carcinomas implanted into Nude mice. *Cancer Res;* 48: 1943-1948.

9. Anderson Graham and McCarthy Nicholas I, (2015). Laying Bare the Nude Mouse Gene. *J Immunol;* 194:847-848.

10. Bosma MJ, Custer RP and Caroll AM, (1983). A Severe combined immunodeficiency mutation in the mouse. *Nature;* 301 (5900): 527-30.

11. Fulop GM and Phillips RA, (1990). The *scid* mutation in mice causes a general defect in DNA repair. *Nature;* 347: 479- 82.

12. Dore J.F., Bailley M. and Bertrand S. Metastases of human tumors in experimental animals. *Anticancer Res;* 1987, 7 (5B): 997-1003.

13. Teicher BA (2006). Tumor model for efficacy determination. *Mol. Cancer Ther;* 5 (10): 2435-2443.

14. Tzukerman Maty, Rosenberg Tzur, Ravel Yael et al (2003). An experimental platform for studying growth and invasiveness of tumor cells within teratomas derived from human embryonic stem cells. *PNAS;* 100 (23): 13507-13512.

15. Pompili, L, Porru, M, Caruso, C et al (2016). Patient derived xenograft: a relevant preclinical model for drug development. *J Exp Clin Cancer Res;* 35,189.

16. Ingle AD and Hosetti BB (2010). Use of immuno-compromised mouse model for establishment and study of human/ animal tumors. *Ind J Vet Pathol;* 34, 2: 156- 61.

17. Ingle AD and Hosetti BB (2012). Metastatic behavior of human tumour xenografts in immuno-compromised mouse model. *Ind J Vet Pathol;* 36,2: 192-197.

18. Fu X.Y, Besterman JM, Monosov A et al (1991). Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically intact patient specimens. *Proc Natl Acad Sci;* 88: 9345-9349.

19. Fei Xi Feng, Zhang Quan Bin, Dong Jun et al (2010). Development of clinically relevant orthotopic xenograft mouse model of metastatic lung cancer and glioblastoma through surgical tumor tissues injection with trocar. *J. Expl. Clin. Cancer Res;* 29: 84-91.

20. Priolo C, Agostini M, Vena N et al (2010). Establishment and genomic characterization of mouse xenografts of human primary prostate tumors. *Am J Pathol;* 176, 4: 1901-1913.

21. Jung Joohee, (2014). Human Tumor Xenograft Models for Preclinical Assessment of Anticancer Drug Development. *Toxicol Res;* 30,1: 1-5.

22. Lee YT (1985). Patterns of metastasis and natural course of breast carcinoma. *Cancer Met Rev;* 4: 153-172.

23. Fidler IJ (2003). The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nat. Rev. Cancer;* 3: 453-458.

24. Minn AJ, Kang Y, Seganova Y (2005). Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. *J Clin Invest;* 115, 1: 44-55.

25. Nemati F, Sastre-Garau X, Laurent C et al (2010). Establishment and Characterization of a Panel of Human Uveal Melanoma Xenografts Derived from Primary and/ or Metastatic Tumors. *Clin. Cancer Res*; 16 (8): 2352-2363.

26. Wang X, Fu X, Brown PD et al (1994). Matrix mettaloproteinase inhibitor BB-94 (Batimastat) inhibits human colon tumor growth and spread in a patient-like orthotopic model in nude mice. *Cancer Res;* 54: 4726-4728.

27. Tentler JJ, Tan AC, Weekes CD et al (2012). Patient-derived human tumor xenografts model for cancer drug development. *Nat Rev Clin Oncol;* 9: 338-50.

28. Ingle AD and Hosetti BB (2013). Therapeutic response of Human Brain, Breast and Oral Cavity Tumors to Paclitaxel in NOD SCID mice. *Int. J Bio-Pharma Res;* 2,1: 39-44.

29. Hidalgo M, Amant F, Biankin A et al (2014). Budinská, E., Byrne, A. T., Caldas, C.,Villanueva, A., Patient Derived Xenograft Models: An Emerging Platform for Translational Cancer Research. *Cancer Discovery;* 4(9): 998-1013.

30. Morton J Jason, Bird Gregory, Refaeli Yosef et al (2016). Humanized Mouse Xenograft Models: Narrowing the Tumor–Microenvironment Gap. *Cancer Res;* 76,21: 6153-58.

31. Brehm Michael A, Shultz Leonard D, and Greiner Dale L (2010). Humanized Mouse Models to Study Human Diseases. *Curr Opin Endocrinol Diabetes Obes;* 17(2): 120–125.