1. INTRODUCTION

Cancer is a generic term for a group of more than 100 diseases that can affect any part of the body. Other synonym terms used for cancer are malignant tumor and neoplasms. One defining feature of cancer is the rapid creation of abnormal cells which grow beyond usual boundaries of the organs. It also invades adjoining parts of the body and spread to other organs, a process refereed to as ‘metastasis’. In short it is a devastating disease for all vertebrate animals. In spite of advancement in diagnostic technology as well as treatment modalities, diagnosis and management of cancers in human being as well as animals is always a challenge. Cancer of tongue, mouth, hypopharynx, esophagus, stomach, gall bladder, lung, breast, cervix, uteri and thyroid are major cancers in India. The most common cancers of men in India are head and neck, lung and esophagus; whereas those of women are breast and cervical. Brain cancer is the major cancer observed in children. As per the World Health Organization, 9 million people may die by the end of 2015 due to cancer and 11.4 million in 2030 (Pinchuk, 2007). Due to marked resistance to radiation and chemotherapy, the prognosis for patients with glioblastomas is reported to be very poor. Majority of patients die within 9-12 months and less than 3% survive more than 3 years. Majority of tumors of the central nervous system are glioblastoma which are most malignant and frequent (Yeole, 2008).

Being diverse population and diet in India, cancer biology and pathogenesis present more challenging job as compared to other parts of the world. National Cancer Registry Program of the Indian Council of Medical Research (ICMR) has made it possible to have a systematic program of cancer data collection so as to have reliable incidence and mortality rates. However, the registry cover only selected urban centers. Rest of the centers does not take note of the prevalence and mortality rate by specific cancers (Nandakumar, 2001; Nandakumar et al., 2004; Kurkure et al., 2009). Under such circumstances, storage of important tumor tissues remains out of question. Even in urban areas due to various reasons, researchers always have difficulty in timely getting required and sufficient quantity of tumor samples for their studies. Sometime the availability and work cannot be coordinated and the small tumor tissue received from the surgeries may be wasted or just snap frozen. The snap frozen samples would be used for molecular work. However, repeated availability of the same sample is a limitation. Moreover, such samples cannot be used for growing tumors in animals and experiments thereof. Currently, to overcome such need fresh surgical tumor samples
from patients operated in the operation theatres are used. The histological features of
different tumors may be similar but tumor biology may differ because of the diverse
population and diet (Nandakumar et al., 2005).

Basic as well as clinical trials on cancer patients are essential components of the
cancer drug discovery process. The cancer drug screening historically relates to the
murine ascitic tumors such as L1210 and Carcinoma 755. In the mid 1950’s, the
National Cancer Institute (NCI) of the USA started the cancer drug screening program
with P388 and in 1970’s they added the solid tumors in the program. Subsequently,
the discovery of the Nude and SCID mice allowed the widespread use of human
tumor transplantation for anticancer research and testing (Johnson et al., 2003;
Voskoglou-Nomikos et al., 2003; Sausville and Burger, 2006; Teicher, 2006).

Because of the absence of T-cells, immuno-compromised models often develops
solid tumors and therefore are routinely used models for transplantation of human
surgical specimens and established human tumor cell lines (Kyriazis et al., 1978;
Kajiji et al., 1982; Fujii et al., 2008). 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. Traditional assays
that test the efficacy of chemotherapeutic agents evaluate the response of human
tumor cell lines and xenografts to the test drug of interest. However, these preclinical
tests neglect the normal in vivo tissue microenvironment, which most likely plays a
role in susceptibility to various drugs (Bearss et al., 2000; Song et al., 2000).
Response of the specific therapeutic regime on patients tumor can be ascertained only
if human tumor is used and not the mouse tumor (Richmond and Su, 2008). Indeed,
in vitro assays do not always predict the efficacy of a particular drug in treating a
specific cancer in the clinical setting (Waldman et al., 1997). The tissue
microenvironment is inherent in the mouse model. Consequently, the mouse tumor
response should more closely mimic the human tumor response to therapy. A close
correlation between clinical and preclinical outcome has been reported when human
tumor specimens are directly xenografted from patients to the immuno-compromised
mice. On the contrary, there has been less correlation of clinical disease outcome
when the cell lines are used as a material for xenografts. Mechanical or enzymatic
processing of tumor cells is believed to be disrupting the cell-cell communication and
the natural stromal structure. This may devoid the cells from its native three-
dimensional tissue architecture. In absence of this three-dimensional tissue
architecture, cells cannot express their full spontaneous metastatic potential and may die because of matrix detachment. Implantation of solid tumor fragments with histological intact architecture may overcome some of these limitations (Fu et al., 1991; Furukawa et al., 1993; Hoffman, 1994; An et al., 1999; Armengol et al., 2004).

Majority of the studies on human cancers are conducted using established cell lines from the well-known sources. In contrast to cell culture assays, the mouse models more accurately represent the \textit{in vivo} tumorigenesis (Van Dyke and Jacks, 2002). To enable the study of pathogenesis and molecular basis of human cancers, it is inevitable to use animal models to transplant tumor samples originated from various organs (March et al., 2001; Corey and Vessella, 2007; Sanz et al., 2009). For more than a half century the laboratory mouse has been the primary species in which experimental cancer chemotherapy have been tested (Kerbel, 2003). Mouse tumors are important in anticancer drug discovery but they are not human tumors and do not often predict what will happen in the human tumor when subjected to therapeutic response. Use of murine tumor in a syngenic animal model offer several advantages. They are easily available, low cost, have along history of use, have a strong baseline of reproducible drug response data and studies can be easily conducted using statistically significant numbers.

The disadvantages are that the tumor cells are rodent origin, express the mouse/rat homologues of the desired targets and the tumor grows pretty fast. Using the mouse tumor models we can cure many mouse tumors \textit{in vivo}. However, there is no direct correlation between response in the mouse and human patient (Kerbel, 2003; Richmond and Su, 2008). Tumor growth induction under the subcutis 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 (Fidler, 1986; Manzotti et al., 1993; Boehle et al., 2000; Press et al., 2008).

Cancer researchers need better preclinical models that more accurately predict the response of human cancer to chemotherapy. High engraftment rates of human tumor with short period of establishment are the prerequisite to make the \textit{in vivo} model feasible for cancer research. However, carcinogen induced tumors in murine models does not provide the solution for this purpose (Boehl et al., 2000; Sharpless and DePinho, 2006). For achieving the therapeutic approaches for treating the tumors, variety of human tumors are required to represent the genetic diversity that exists in Indian population. Experimental models of human cancers are important in
reconstructing the events that occur in human patients with cancers. Variety of animal models have been so far used as a tool in understanding the efficacy and finding toxicities for newly developed cancer therapeutic agents. Variety of factors decide the choice of tumor and animals model. 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 (GE) mice that are used for a specific cancer genotype. Both of these groups are unique in their qualities. However, usefulness of the GE mice for identifying the novel compounds which subsequently shows to possess significant clinical activities against appropriate cancer remains to be validated (Sharpless and DePinho, 2006; Morton and Houghton, 2007; Richmond and Su, 2008).

Cancer research centers conduct in vitro evaluation of biology of human cancers and by also using immuno-compromised animals. These mice allow established in vitro human cell lines to be propagated subcutaneously (s.c.) giving rise to solid tumors. These mice also accept the tumor biopsy pieces when transplanted directly. However, the routine practice is to develop the cell lines from the human tumor explants and then use them as seeding material to grow solid tumors. Tumors grown this way do not always accurately predict activity in the appropriate clinical histology. This may be partly due to continuously using cell lines from in vitro source for several years, which may no longer represent the original cell type. On the contrary direct comparison of patient tumor biopsy tissue with early passage xenografts have demonstrated high concordance in gene expression and even greater similarity in genomic alterations when tumors are propagated in mice (Morton and Houghton, 2007).

It has been observed that xenograft models, but not allografts of murine tumors, can be useful for predicting the phase II clinical trial performance at least for few tumor types. It has also been observed that xenograft models have been relatively accurate in identifying clinically active agents for childhood cancers. In spite of having all these advantages, suboptimal use of tumor xenografts attributes to the relatively long time required to establish the tumor heterograft and low success rate of heterograft (Morton and Houghton, 2007; Richmond and Su, 2008).
Variety of artificial and spontaneous metastatic models have been developed in experimental animals. These models have helped in characterizing metastatic cancer cells and also understanding the metastatic process. Experimental metastases studies using human tumor xenografts have so far been limited to immuno-compromised animals, especially athymic nude mice (Dore et al., 1987).

Before the discovery of the Nude/SCID mice, whole body irradiation after the thymectomy of the neonates was the choice for studying the human tumors in vivo (Fergusson et al., 1986). Hetero-transplantation of human tumor biopsies or tumor cells into Nude/SCID mice has opened new avenues for understanding the tumor processes/preclinical screening for development of newer cancer drugs. It is well known that the in vivo models use the murine rather than human tissue microenvironment. The conclusions arrived using these models represents the measurement of the tumorigenic properties such as proliferation and invasiveness, within a surrounding murine, rather than human, tissue environment (Tzukerman et al., 2003).

The 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 and is a ‘Workhorse’ of the pharmaceutical industry (Teicher, 2006 and Morton and Houghton, 2007). Recently since 2009 a hairless SCID mouse is being made available by the Charles River Laboratories, USA, for the cancer researchers (http://www.criver.com/en-US/ProdServ/ByType/ResModOver/Pages/Home2.aspx). These mice are hairless on albino background as well as lack T- and B-lymphocytes and therefore like the nude mice are convenient to transplant the tumor tissues and monitor the growth.

Tumor specimens from human patients are often difficult to procure for research; however, it is much easier to prorogate the tumor in immuno-compromised mice and then investigate. The advantage of use of human tumor xenograft is that the tumor cells are human origin; many of the tumors can be repeatedly grown in immuno-compromised animals; vide variety of cell lines are available; have a long history of use; have a strong baseline of drug response data; and the host i.e. immuno-compromised mice are easily available for tumor transplantation. The disadvantages are that the host animals are costly to procure and maintain, the stromal components of the tumor is rodent origin and the tumor can also be grown in the non-natural sites
too. Currently Indian researchers have ATCC, USA; NCI, USA; and NCCS, India as major sources of availability of human tumor cell lines or xenografts. Besides, certain organizations have also developed their own human tumor xenograft banks which provide either testing services or tumor samples for researchers. Some of them are Ontario Tumor Bank, Ontario Institute for Cancer Research, Toronto, Canada (http://oicr.on.ca/); Fox Chase Cancer Centre, Philadelphia, USA (http://www.fccc.edu/); The Swedish National Biobank Program, Sweden (http://www.biobanks.se/); Sunnybrook Health Sciences Center, Toronto Canada (http://sunnybrook.ca/); CNIO Tumor Bank Unit, Spanish National Cancer Research Centre, Madrid, Spain (http://www.cnio.es/ing/); Crown Bio Sciences Inc., California, USA (http://www.crownbio.com/); Iran Tumor Bank, Tehran, Iran (http://www.irantumorbank.com/); MIR Preclinical Services, Michigan, USA (http://www.molecularimaging.com/); Charles River Laboratories, USA (http://www.criver.com/en-US/ProdServ/ByType/Discovery/Pages/about.aspx) etc. No such sources are available in India for the fresh tumor samples which are stored without putting them into the tissue culture medium.

Studies conducted to understand the biology of the cancer cells involves necessarily the use of immuno-compromised mice. Several such studies have been published achieving number of breakthroughs in understanding the biology of cancers as well as development of new anticancer drugs. Nude mice are reported to rarely develop metastases when grafted with human tumor cells (Dore et al., 1987; Hill et al., 1991; Williams et al., 1993). However, one of the important addition of knowledge by way of use the immuno-compromised mice for study of human tumor is that it yielded the information of metastasis to different organs (Kozlowski et al., 1984; Fidler, 1986; Dore et al., 1987; Williams et al., 1993; Priolo et al., 2010). There are reports wherein scientists have reported metastases in different parts of the body of the mice. This information is not always possible in human patient unless otherwise operated or detailed post mortem is conducted after deaths. Most of the subcutaneously developed tumor xenografts are surrounded by the capsule and have little scope to invade and disseminate even when highly aggressive tumors have served as the source of the xenografts.

Mice and rats often develop benign and malignant cancers spontaneously. The incidence may vary from strain to strain. These spontaneous tumors do not always metastasize to different organs or have mild local tissue invasion and therefore the
incidence is very rare. However, majority of the data generated on the metastatic behavior of cancer is reported to be derived from studies in rodent models (Giavazzi et al., 1986; Morikawa et al., 1988). Metastasis, which cannot be modeled in vitro, is a multistep process which involves tumor cell invasion from the primary site, intravasation and extravasation of the circulatory system, arrest of cells, angiogenesis and growth at a distant site (Fidler, 1990; Chambers et al., 2002; Steeg, 2006; Gupta and Massague, 2006; Sahai, 2007; Tarin, 2008). However, having primary tumors as a source of cancer is clearly a pre-requisite for establishing metastasis. If the tumor cells are able to survive the blood stream, they must successfully arrest at a secondary stage site, cross the vascular barrier and migrate into the extra-vascular connective tissue (Havens et al., 2008). Cancer progression with resultant metastasis requires genetic changes that permits tissue invasion at the site of the primary tumor, entry into the vasculature, localization to metastatic site, survival and proliferation in the microenvironment of the metastatic organs (Rosol et al., 2003). Metastasis can occur in different organs and in different anatomical locations within the same organs (Fidler, 1989).

Mere oncogenic transformation of a cell is not sufficient for metastatic competence. All oncogenic driven mouse models of cancer do not always develop distant metastases. Transformed cells therefore must acquire additional abilities to overcome the natural barriers against metastasis (Nguyen et al., 2009). The formation of secondary lesions is the result of sequence of selective events. Distant organ infiltration and colonization are general steps that primary cells need to acquire to metastasize. All metastatic tumor cells need to complete these events. The presence of tumor cells in the circulation does not on its own constitute metastasis because most of them are rapidly destroyed. Factors responsible for the destruction of the circulating tumor cells include trauma from shear forces in the circulation, impaction in the capillary bed, restrictive force in the host environment, apoptosis and attack by the host defense system. For this reason very large number of cells must be released from the primary tumor to form the metastases to the distant organs (Fidler, 1970; Price, 1990; Chambers et al., 2002; Sahai, 2007; Nguyen et al., 2009).

Despite significant improvement in diagnosis, surgical techniques, general patient care, and local and systemic adjuvant therapy, most deaths from cancer are due to metastasis that are resistant to conventional therapies (Lee, 1985; Fidler, 2003; Minn et al., 2005; Nemati et al., 2010). Prevention of metastasis has always been of more
interest to academic scientists than the practicing oncologists (Wang et al., 1994). Metastatic tumor cells can enter period of dormancy at any stage of colonization. Metastases from certain histological types of cancer can occur after more than 10 years of treatment for the primary tumor. Possible reasons may be that such tumor cells lack vascular connections and may enter a viable but non dividing phase (Steeg and Theodorescu, 2008).

It is observed that although the human tumors may grow in immuno-compromised mice, it may fail to produce metastasis. This may be due to the fact that the small tumors are encapsulated and probably prolonged time is required for transplanted tumors to grow and metastasize (Ingle and Hosetti, 2010). Studies have shown that malignant human tumors xenografted into orthotopic organs of the immuno-compromised mice closely mimic the natural characteristics of human tumor, are highly vascularised, grow progressively and produce distant metastasis. On the contrary, implantation of the same tumor at an ectopic organ does not lead to similar angiogenesis or production of metastasis (Bruns et al., 1999; Nemati et al., 2010). Unlike the injection of single cell suspension using enzymatic disruption, implantation of solid tumor fragment also overcome the disruption of cell-cell communication and natural stromal structures by maintaining native three-dimensional tissue architecture (Furukawa et al., 1993; An et al., 1999; Armengol et al., 2004).

Metastasis is a kinetic phenomenon occurring in a body system which involves series of sequential steps and cannot be modeled in vitro (Chambers et al., 2002 and Tarin, 2008). It is a phenomenon where malignant cells freed itself from the cluster and evolve new adaptive changes which enable it to move into the circulation and settle in the distant organs of molecular determinants. Interaction of metastatic cells with environment in different organs decides the fate of metastasis. The main steps in the formation of a metastasis can be described as shown in the fig. 1.1. (adopted from Fidler, 2003) as cellular transformation and tumor growth. Growth of neoplastic cells must be progressive, with nutrients for the expanding tumor mass initially supplied by simple diffusion. Extensive vascularization must occur if a tumor mass is to exceed 1-2 mm in diameter. The synthesis and secretion of angiogenic factors establish a capillary network from the surrounding host tissue. Local invasion of the host stroma by some tumor cells occurs by several parallel mechanisms. Thin-walled venules, such as lymphatic channels, offer very little resistance to penetration by tumor cells and provide the most common route for tumor-cell entry into the circulation.
Detachment and embolization of single tumor cells or aggregates occurs next, most circulating tumor cells being rapidly destroyed. After the tumor cells have survived the circulation, they become trapped in the capillary beds of distant organs by adhering either to capillary endothelial cells or to sub-endothelial basement membrane that might be exposed. Extravasations occur probably by mechanisms similar to those that operate during invasion. Proliferation within the organ parenchyma completes the metastatic process. To continue growing, the micro-metastases must develop a vascular network and evade destruction by host defense (Fidler, 2003).

Metastasis is a primary cause of mortality in cancer patients. Metastasis can occur in different organs and in different anatomical locations within the same organs (Fidler, 1989). Passive aspects like access to blood vessels, blood flow pressure, trapping of cancer cells in capillaries; and active aspects like expression of molecules required for anchoring and growth of new blood vessels in secondary sites are important steps involved in metastasis. In short the process of metastasis involves invasion of the tumor microenvironment, migration through the extracellular matrix, blood vessel disruption, embolism through vascular or lymphatics, establishment of a pre-metastatic niche, induction of cell adhesion molecule expression in endothelial cells, extravasations, micro-metastasis dormancy and establishment of a new growth in distant sites (Steeg, 2006; Bidard and Pierga, 2008).

Globally metastatic rate in humans is as low as 0.1% because of the metastatic inefficiency of the cancer cells due to meeting the need of above factors (Hart, 1987; Bidard and Pierga, 2008). In Nude mice the success rate of growth and metastases of all types of human tumor is low. Recent studies have proved that human tumors grow readily in SCID mice. A model in which human tumor xenograft metastasizes from subcutaneous site resembles more closely to cancer-bearing human patient and might be useful for preclinical investigations of new treatment modalities (Hill et al., 1991; Mueller and Reisfeld, 1991; Manzotti et al., 1993; Teraoka et al., 1995). Human tumor transplanted in immuno-compromised mice leads to progressive growth, local invasion, and distant metastases primary to lungs. The site of transplantation decides the rate of metastases.

The size of the tumor growth in the animal is directly proportional to the number of malignant cells in the tumor mass. One living tumor cell in the animal can kill the host. Therefore, to cure the experimental xenografts, it is necessary to kill every cell in the mouse, regardless of the number, anatomic distribution with treatment that
spares the host. The rate of killing of the tumor cells by the anticancer drugs should be faster than the proliferation rate of the tumor cells, if the complete cure is to be approached. Tumor cells which are not killed by the anticancer agent regain the growth again and proliferate at the same rate as the original tumor grows in animals of the control groups (Teicher, 2006).

Earlier, antitumor activity in murine ascitic leukemia models was assessed on the basis of percent mean or median increase in life span, net log₁₀ cell kill, and long-term survivors of the mice. Treatment with test agents can be initiated either before tumor development or after a tumor growth appears. If the treatment begins the day after or on the day of tumor cells/ tissue implantation, the experiment is considered as a ‘tumor growth inhibition study’. If treatment begins when an established tumor nodule (50-200 mm³) is present, the experiment is considered as a ‘tumor growth delay study’. Activity in a tumor growth delay shows a stronger evidence of clinical potential than activity in a tumor growth inhibition (Teicher, 2006). One of the requirements of these assays is that drugs be administered at doses producing tolerable normal tissue toxicity, so that the response of the tumor to the treatment can be observed for a relatively long period of time.

Treatment of cancer in humans has moderate effectiveness unless detected early. Paclitaxel is well known anticancer drug used for treatment of solid tumors (Sarosy and Reed, 1993; Chou et al., 1998; Li et al., 1999; Bearss, 2000; Scripture et al., 2005; Nakayama et al., 2009). Therefore, in the present study, Paclitaxel is used as a standard anticancer drug to test the response of brain, breast and oral cavity tumors to this drug.
Fig. 1.1. Main steps involved in the metastasis process.

a. Cellular transformation and tumor growth. b. Vascularization of a tumor mass. c. Local invasion of the host stroma by some tumor cells. d. Detachment and embolization of single tumor cells or aggregates in the blood vessels, some of them are trapped in the vascular bed of target organs. The tumor cells adhere to the vessel wall. e. Extravasation of the tumor cells and establishment of microenvironment required for growth. f. Proliferation of the tumor cells within the organ parenchyma by formation of new vessels.

Reproduced from Fidler et al., 2003.