2. REVIEW OF LITERATURE

Digestive system, oral cavity, respiratory organs, lymphoma & leukemia and brain cancer are the top five cancers in male whereas breast, genital organs, digestive system, oral cavity and brain cancer are the top five cancers in females (Kurkure et al., 2009). Breast cancer is the leading cause of cancer among women in the world and is second most common cancer affecting women in India. Oral cavity cancer comprises cancer of lip, buccal mucosa, gingiva, floor of mouth, palate and tongue (Yeole et al., 2003). Worldwide, 23% of the total new cancer cases and 14% of the total cancer deaths in 2008 have been attributed to breast cancer (Jemal et al., 2011). Histologically intact human tumors implanted directly into the SCID mice are becoming increasingly popular. Literature suggest that such tumor grown in the mice more accurately represents the features of human tumors (Hill et al., 1991; Mueller and Reisfeld, 1991; Manzotti et al., 1993; Teraoka et al., 1995; Muleya et al., 1998; Press et al., 2008; Jin et al., 2010). Clinically relevant animal models of human cancer are important for studies of cancer biology, invasion and metastasis. Such models also play vital role in investigating new forms of prognostic diagnosis as well as therapy.

Breast cancer is one of the major lethal cancers of females. Breast carcinoma is known to metastasize to different organs of the body and it is almost impossible to predict which organ system of the body will be invaded (Lee, 1985). Most of the reports on use of human tumors for xenografts in Nude mice have emphasized the low incidence of metastasis (Kozlowski et al., 1984; Giavazzi et al., 1986; Nomura et al., 1990).

Metastasis is a primary cause of mortality in cancer patients. Metastasis is kinetic phenomenon occurring in a body system which consist of series of sequential steps and cannot be modeled in vitro (Chambers et al., 2002; Tarin, 2008). Human tumor transplanted in immuno-compromised mice leads to progressive growth, local invasion, and distant metastases mainly to lungs. The site of transplantation decides the rate of metastases. Metastasis rate in subcutaneous or intramuscularly implanted xenografts are reported low or inexistent even from tumor that are highly metastatic in patients from whom tissues were derived (Kyriazis et al., 1978; Kajiji et al., 1982; Fidler, 1990; Li et al., 2002). However, implantation of human tumor cells orthotopically in the corresponding organ in immuno-compromised mice results in much higher metastatic rate (Fu et al., 1992). The disadvantages of the orthotopic human tumor xenograft model are that the surgeries are often complex, leading to the
use of low numbers of mice per study; the models are more costly; the stroma is stillodent and the tumor lines are old; the hosts are often immuno-deficient; the tumor
growth and response are difficult to follow and therefore survival is often the end
point; and more importantly the statistics are difficult to apply (Teicher, 2006).

Liver, bone and lungs are the most frequent sites of hematogenous dissemination
of breast cancer cells. Till 2002, only Hurst et al. (1993) has described human breast
tumor metastasis to the lungs of Nude mice. Fu et al. (1993) also described metastasis
of human breast cancer to lungs of Nude mice. Sharkey and Fogh (2006) had
xenografted 1377 nude mice with 106 aggressive human tumor lines but observed
growth in only 1045 mice and metastases in only 1.5% cases. None of the sarcoma
line implanted by them in Nude mice resulted in metastases. Only breast tumor lines
showed greater frequency of metastases. Serial passage in their study did not show
formation of more malignant tumor lines as judged from the metastatic ability.

Reports have showed that implantation of tumors in the subcutaneous (s.c.) site
over the anterior aspect of the lateral thoracic wall gives higher incidence of
metastasis than did injections of same dose of tumor cells into the caudal flank region
(Kyriazis et al., 1978). Some of the reports also showed that the orthotopic
implantation of the tumor leads to more percentage of acceptance of xenografts and
metastases in different organs. Globally metastatic rate in human is as low as 0.1%
because of the metastatic inefficiency of the cancer cells due to meeting the need of
factors responsible for metastasis (Hart, 1987; Bidard and Pierga, 2008).

It is well known that the human tumors have a slower growth rate than most of
the rodent tumors. Therefore, in order to get the metastases, the human tumor
xenografted mice need to be maintained for a substantial more duration of time. Even
gross and histopathological examination of the possible organs of metastases should
be careful. Use of PCR method is an added advantage of confirming the visible as
well as micro metastases in different organs. Reports suggest that the metastasis can
be diagnosed by use of PCR with a human specific base arrangement of the \( \beta \)-globulin
gene (Teraoka et al., 1995; Elkas et al., 2002; Komatsubara et al., 2002).

In order to select the metastatic variants of tumor cells in vivo, traditionally two
modalities are used. One is to implant the tumor cells into orthotopic site and let the
spontaneous metastasis to develop. The metastatic colonies are isolated and again re­
injected orthotopically. Another option is injection of tumor cell into the circulation to
produce experimental metastasis. In the first option one has to isolate the distinct
tumor colonies and then inject into the animals. Implantation of tumor lines derived from metastases is reported to grow faster than cells isolated from primary tumors (Giavazzi et al., 1986; Bruns et al., 1999). Generally the mice die before the sizable tumor colonies are achieved. Therefore, getting sizable colonies of metastatic tumor is difficult. It has been observed that the metastases are often seen histologically as 'micro-metastases'. In case of metastases, culturing the metastatic tumor cells and then injecting the sizable number orthotopically into the mice is the only option of selecting the metastatic variants.

Orthotopic implantation of the tumor cells is an appropriate site to grow the tumor and get the metastases. However, metastases are also reported to arise from non-orthotopic sites (Fu et al., 1991). Human breast cancer is one of the most difficult tumors to grow in immuno-compromised mice. The success rate of xenograft acceptance of human breast cancer varies from 7-20% (Li et al., 2002). Breast cancer is reported to metastasize to lungs, lymph node and bones and less frequently liver, brain and adrenal medulla (Xie et al., 1992; Fu et al., 1993; Hurst et al., 1993; Visonneau et al., 1998; Francia et al., 2011).

Virginie et al. (2007) have reported that prior xenografts leads to better efficient cell line establishment compared to direct establishment from fresh tumors. They reported metastasis of human colon cancer to liver when injected in Nude mice. Naomoto et al. (1987) have also reported that colon cancer xenografts metastasize to lungs, intra-peritoneal (i.p.) cavity and liver when injected in Nude mice. Whereas Kyriazis et al. (1978) have reported that human larynx and colon cancer xenografts metastasize to lymphatic vessels, perivascular lymphatic spaces and regional lymph nodes. They opined that Human tumor grow exceedingly well when transplanted intra-peritoneal (i.p.) and imitate more closely the biological characteristics of malignant growth as compared to subcutaneous (s.c.) route.

Xie et al. (1992) have reported that human melanoma, bladder carcinoma and breast cancer xenografts show metastasis in lungs of Nude as well as SCID mice and concluded that metastatic capacity of human tumor cells appears to be better expressed in SCID mice than Nude. Visonneau et al. (1998) have reported systemic spread of breast cancer xenografts to lungs, peripheral blood, lymph node and bone marrow. Rofstad (1995) have reported that human melanoma shows organ specific metastatic pattern when injected in Nude mice. The organs of preference were lungs, lymph node, brain and peritoneal organs. Bryzgalov et al. (1996) have reported that
human melanoma xenograft metastasizes to lungs when injected in Nude or beige/Nude mice.

Inohara et al. (1992) have observed that head and neck cancer xenografts metastasize to lung only and it retains the histological characteristics of both benign as well as malignant tumors. Bastide et al. (2002) have reported PC3 xenografts metastasize in lungs, liver, spleen, adrenal gland, kidney, lymph node and diaphragm. Whereas Olden (1990) have observed that subcutaneous injections of PC3 cell lines in Nude mice metastasizes to lymph nodes and lungs but not to the skeleton while tail vein injection of PC3 cells leads to skeletal metastases. Elkas et al. (2002) have reported no growth of ovarian cancer in Nude mice. However, xenografts were established in SCID mice and all xenografts retained histologic similarity to their original human tumors. Metastases were observed in bowel mesentery and on diaphragm. Farre et al. (2002) have studied the differences in growth between s.c. and orthotopically implanted tumor and their metastases. They opined that implantation site of xenografts changes tumor growth by altering apoptotic or cell cycle regulation in a tumor specific manner. They reported human pancreatic tumor xenografts metastasizes to liver. Corti et al. (1999) reported that human large cell carcinoma becomes metastatic to the lungs of Nude mice after s.c. injections. Crnalic et al. (1997) have reported that osteosarcoma xenografts metastasize to lungs, lymph node and liver when injected in Nude mice. Kawata et al. (1994) have reported that the dissemination of tumor cells to various tissues in SCID mice were in a manner analogous to tumors in patients with leukemia/lymphoma, whereas tumors in Nude mice were not as widely disseminated and grew mainly as ascites. Kubota et al. (1993) have reported that acceptance rate of colon cancer xenografts in SCID mice was higher than Nude mice. However, the growth rate was found similar in Nude as well as SCID mice.

Nude/SCID mice are frequently used model system for the transplantation of human surgical specimens. Majority of the times, tumor explants are used for making the cell lines in tissue culture laboratories which in turn are used for the further animal experiments. However, fresh surgical tumor specimens are also used directly to grow the tumor in Nude/SCID mice and then for experimentation (Press et al., 2008; Jin et al., 2010). During the process of injecting the tumor cell lines in animals or serial transplantation of tumor from animal to animal, there are chances of contaminating the tumor with the host cell. Handling different cell lines at a time in the tissue culture
laboratory, prone the contamination of these cell lines. Whereas, dissecting host cell along with the tumor is a prime reason for contaminating the tumor with the host cell. Use of such contaminated cells/ tumor samples pose a challenge in the reproducibility of the research (Pathak et al., 1999). Therefore, it is of utmost importance to characterize the tumor to ensure that it is originated from the starting material (Gras et al., 2000 and Morton and Houghton, 2007). Granting agencies are now insisting on authentication of the cells or xenografts used in research. Besides, the scientific journals are also insisting the authors to address the authentication within submitted manuscript (Nims et al., 2010). There are several methods including enzyme polymorphisms, HLA typing, karyotyping and DNA polymorphisms which can be used to authenticate the human cell lines. With the use of PCR based short tandem repeat (STR) analyses, it is possible to find out the contamination of the tumor with the host cells (Masters et al., 2001).

Correlation between cell free nucleic acid levels in plasma and cancer was first established by Leon et al., 1977. With the advancement in the PCR-based technologies, detection of such circulating tumor cells is possible (Pathak et al., 2006; Cabral et al., 2010; Kumar et al., 2010). Tumor cells are capable of shedding nucleic acids into the blood stream. Variety of causes such as lysis of cancer cells in circulation, cell necrosis, apoptosis, spontaneous active release of DNA by tumor are proposed for detection of circulating extracellular DNA in the blood (Pathak et al., 2006).

One living metastatic tumor cell lodged in the distant organs in the animal can eventually grow and kill the host. Therefore, to cure the experimental xenografts, it is necessary to diagnose metastasis of even few cells. The successful diagnosis of the experimental metastasis is a basis to find the new anticancer molecules which can kill every cell in the mouse, regardless of the number, anatomic distribution with treatment that spares the host.

In view of this, attempts were made to establish xenografts of the fresh human tumor samples from Indian population using immuno-compromised mice. For this purpose, tumors grown in the SCID mice were stored in -20° C refrigerators. Since the tumors grown in these mice are directly frozen down in liquid nitrogen (LN₂), these tumors would serve as excellent samples as primary tumors for the researchers in the future. Availability of these human tumors would be made possible at any time in any size without putting the tumor in tissue culture medium.
Our approach in this study was to avoid disruption of tumor integrity, directly implant the tumor subcutaneously and let the tumor grow to the sizable size of 15 mm or more. Such model generally reflects the original properties of the human tumor and could be of great value in establishing the tumor xenografts and understanding the metastatic properties of the tumors. Such models are important for development of new drugs and treatment strategy for the metastasis. With this objective, we have conducted the study to establish the xenografts of histologically intact tumor tissues, to understand the metastatic pattern of these tumors and also response to standard anticancer drug.