Tumor Microenvironment in Human Tumor Xenografted Mouse Models

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Abstract: Tumor microenvironment, known to exert regulatory functions on tumor cells, plays an important role when a human tumor is xenografted into immunodeficient mice. Primary human tumors xenografts represent a promising strategy to study new therapeutic's efficacy or to understand the mechanisms implicated in tumor relapse.

The development of xenografts is linked not only to the aggressivity of the tumor cells, but also to the tumor microenvironment. Tumor xenograft cell proliferation is dependent on microenvironment modifications such as angiogenesis and human blood vessel replacement, host immune cells and the presence of growth factors.

The characterisation and a better knowledge of these factors allow for a more appropriate use of xenograft animal models in the evaluation of new antitumor treatments.

In this review, we describe the different factors linked to the tumor microenvironment and their impact on the take rate when human tumors are xenografted into immunodeficient mice.

Keywords: Xenograft, tumor microenvironment, human tumor, immunodeficient mice, murine stroma, human stroma.

INTRODUCTION

Human tumor microenvironment in which tumor cells develop is composed of blood microvessels, fibroblasts and inflammatory cells (macrophages and lymphocytes). Around these components is found an extracellular matrix composed of fibers, proteoglycans, non-proteoglycans polysaccharides, growth factors, proteases, cytokines, chemokines antibody and other types of enzymes [1]. The stromal microenvironment plays a crucial role in tumorigenesis, especially in tumor progression and the aggressiveness of cancer cells, and is dependent on the interactions with immune components. Thus, tumor microenvironment exerts regulatory functions and selective pressure on cancer cells and determines the ability of the tumor to invade surrounding tissues [1].

The characterization of immune components in the tumor environment such as T-cell [2], B-cells [3], NK-cells [4] and macrophages [5] have shown their capability to infiltrate solid tumors.

Tumor proliferation is dependent upon blood supply and the interactions of tumor and endothelial cells initiate and drive this process. The growth of new capillaries from existing blood vessels, which is called angiogenesis, is mediated by a complex multistep process comprising a series of cellular events that lead to neovascularisation [6]. Angiogenesis plays a central role in various physiological processes within human body and has been found essential for tumor growth and is also a key factor in metastasis. It is due to the migration, proliferation and differentiation of endothelial cells under the influence of angiogenic factors secreted by tumor cells and stromal cells [7].

The use of preclinical models of human tumor xenografts implies changes in part of these interactions. The principle of the xenograft is based on the implantation of human tumor tissue either in subcutaneous position [8] or in an orthotopic (natural) site [9, 10] (Figure **1**). For subcutaneous models, the tumor xenograft is implanted between the dermis and underlying muscle and is typically located either on the flank, or into the footpad or on the back into the brown fat of the mouse.

The major disadvantage of this technique that sometimes fails may be due to the observation that the

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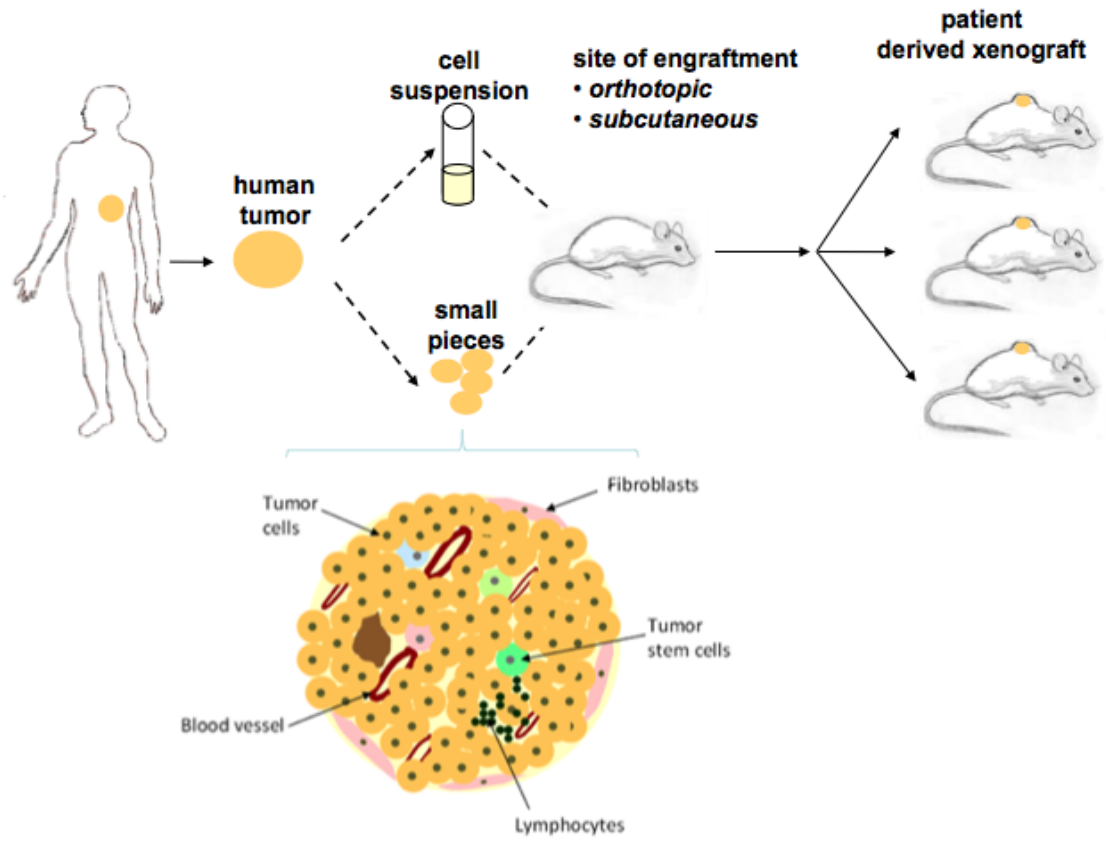

Figure 1: Principe of human tumor xenograft into immunodeficient mice. After resection, human tumor tissue can be xenografted into immunodeficient mice after tissue dissociation either as cell suspension or as small solid tumors specimen. Serial passages can be thus established into mice. The human tumor initially grafted into mice contains a heterogeneous population of tumor cells, tumor stem cells, inflammatory cells, blood vessels as well as fibroblast and extracellular matrix.

subcutaneous microenvironment is not relevant to the organ site of primary or metastatic disease. Human tumor xenografts which are implanted orthotopically can reproduce the organ environment in which the tumor grows, so that the effect of the tumor on its microenvironment can be modulated [11]. In this model, the tumor xenograft is either implanted or injected into the equivalent organ from which the cancer originated, or where metastases are found in patients [12]. To avoid xenorejection and allow an efficient transplantation, nude athymic (nu/nu) or severe combined immunodeficient (*scid/scid*) mice are used. Human tumor tissue can be then serially transplanted into mice. To avoid any infection and contamination, mice are handled under aseptic conditions including the wearing of gloves, gowns and shoe coverings.

Primary human tumor xenografted into immunodeficient mice represents a promising modality to study the therapeutic efficacy of new drugs [13, 14], associations of drugs and mechanisms of molecular or cellular response to treatment [15].

The xenograft method shows both advantages and disadvantages. Among the advantages, the tumor xenografts are 1) easy to use, 2) relatively inexpensive comparing to genetic modified murine models [16], 3) able to reproduce the heterogeneity of the initial patient tumor, thereby allowing the study of tumor cell subpopulations [17, 4] potentially proposed as a personalized therapeutic to anticipate personalized anticancer treatment [18-20].

Among the disadvantages, we can mention that 1) to allow xenotransplantation, immunodeficient mice are used and therefore, the important interactions between the different types of immune cells and cancer cells during tumor initiation and maintenance are excluded [17] and 2) a selection pressure is induced by the host animal, and the human stroma are gradually lost [21]. The differences observed are probably due to changes in tumor microenvironment resulting from engraftment in immunocompromised mice [22]. Tumor microenvironment is characterized by properties such as low extracellular pH, low glucose concentration, necrosis and hypoxia, known to induce genetic

instability and alteration in gene expression in tumors cells [1].

When human tumors are grafted into animals, the tumor microenvironment is influenced by different factors, among them being the human tumor stroma, the mouse strain, the site of xenografts, and the progressive human blood vessels replacement. These factors could induce phenotypic and genotypic modifications on tumors cells. In the next sections the role of these different factors will be discussed.

1. Human Stroma Versus Mouse Stroma

Human stroma is replaced with murine stroma during successive passages of the tumors within mice and could alter the original composition of the tumor [17, 22]. Stromal microenvironment modifications such as angiogenesis, inflammatory cells, extracellular matrix composition, and expression of growth factors in the stromal compartment influence progression of tumor cells.

Some differences observed in gene expression breast cancer xenografts seem to be due to the loss of human stromal genes [21]. Modifications on tumor stroma were also observed by other teams. Thus, Chou *et al.* showed in the colorectal cancer xenografts, that the human stroma, vasculature, and hematopoietic elements were systematically replaced by murine analogues while the carcinoma component persisted [23].

Stromal microenvironment is thus a determinant for a malignant growth. Alteration in the stromal microenvironment in a rat model was sufficient to promote malignant transformation of human prostatic epithelial cells appearance of carcinoma-associated fibroblasts (CAFs), and was associated with additional genetic alterations and changes in gene expression [24, 25].

2. Mouse Strain

Xenograft tumor models were developed extensively after the identification of athymic nude mutant mouse with a deletion in the FOXN1 gene [26]. Lack of the thymus in homozygotes *nude* mice leads to defect in the immune system, such as T lymphocytes (Figure **2**). In these mice the lymphocyte population is composed almost entirely of B-cells. Intact humoral immunity in nude mice reduces the efficiency of tumor formation after xenografts. These mouse strains have proven to be useful for the establishment of xenograft tumors both from patient's tumor samples and established human cancer cell lines [27, 28].

The *SCID* mouse harbours a point mutation in chromosome 16 in the CB-17 inbred mouse strain showing defects in DNA repair. This results in the interruption of lymphocyte maturation and a deficit in circulating, mature, functional T and B-cells. However, these mouse strains possess an intact innate immune system with normal numbers of monocytes/ macrophages, natural killer (NK) cells and granulocytes leading sometimes to elimination of tumor xenografted cells over time [29]. SCID/Bg mice lack B-cell, T-cell, and NK cell function entirely, but show enhanced macrophage populations [30-32] (Figure **2**). *SCID* mice with mutations in the *Il2r*γ locus have significantly improved the survival of human tissues such as peripheral blood monocytes, hematopoietic cells [33] and diverse tumor cell types such as lung [34] or ovarian tumor xenografts [26, 33].

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Experiments using the *SCID* mice have demonstrated that engraftment of a human tumor microenvironment is preserved for a limited period of time [29, 33, 35].

3. Site of Xenograft

The site of implantation is important because of the microenvironment. Subcutaneous engraftment allows easy assessment of tumor size but does not replicate the natural tumor microenvironment, which contributes to tumor progression and could modulate therapeutic response [17]. Thus, the main limit of subcutaneous engraftment is the lack or reduced potential of

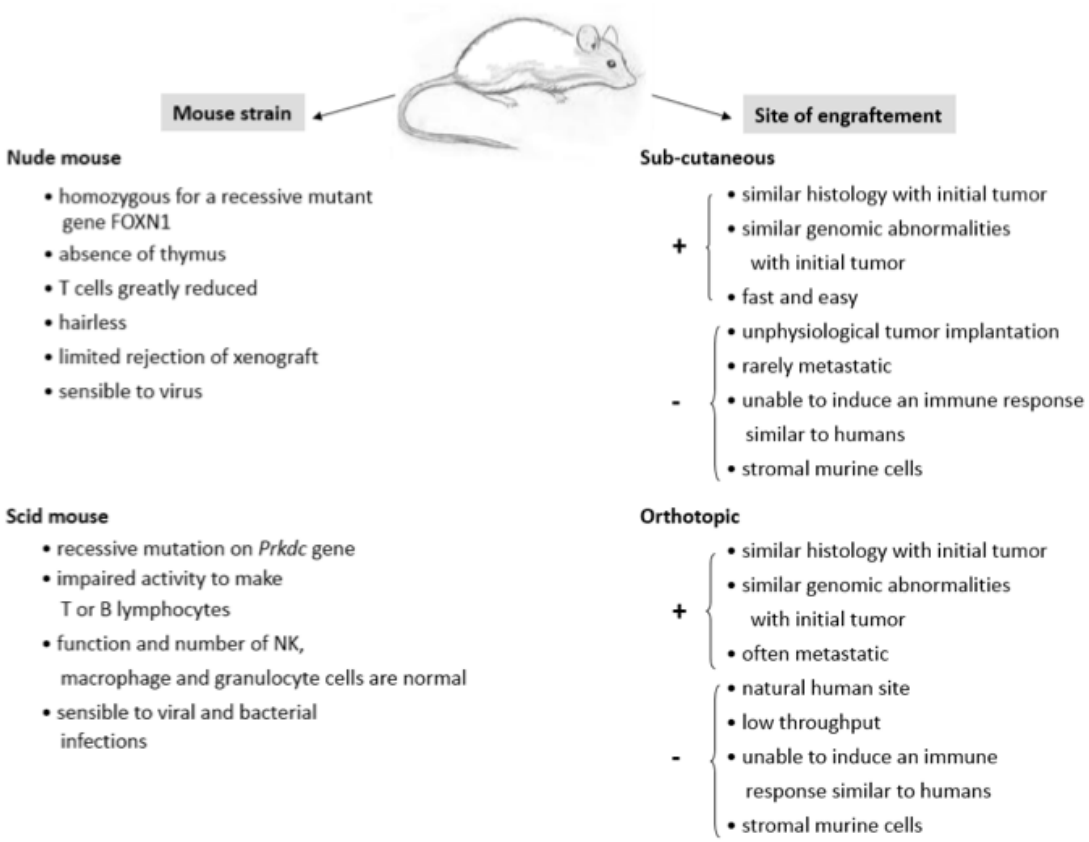

Figure 2: Advantages (+) and disadvantages (-) of tumor xenograft depending on the site of engraftment and characteristics of mouse strain used for xenograft procedure.

metastasis compared to orthotopic sites where an increased rate was observed [36] (Figure **2**).

Orthotopic tumor xenograft models provide a more biologically relevant context to study the disease and tumor-host interactions. Orthotopic transplantation models may mimic the biologic behaviour of the primary tumor but this procedure is more difficult to perform [37]. Some studies have demonstrated that the orthotopic implantation of human tumor show a superior growth and metastasis as compared to subcutaneous position. This capacity of metastasis of implanted tumors cells into mice depends also on the properties of tumor cells [38].

A modality for mimicking human tumor microenvironment is to humanize mice models [39, 40]. Thus, in order to reproduce a natural microenvironment researchers either administered low doses of estradiol to mice [32, 41] or "humanized" the mammary fat pad of mice with immortalized human fibroblast [42, 43]. Human breast tumors, expressing or not expressing estrogens receptor (ER^+ or ER^-) grown in the fat pad of severe combined immunodeficient SCID/Beige and non-obese diabetic (NOD)/SCID /IL2-receptor null (NSG) mice, yielded stably transplantable xenografts at rates of 21% and 19%, respectively [32]. Primary outgrowth and stable take rate in these mice were not statistically different under estradiol supplementation. ER^- and ER^+ xenografts were propagated in the presence of estradiol pellets suggesting that estradiol supplementation stimulates growth of breast cancer xenografts. ER^+ tumor graft remained dependent on estrogen for tumor growth. The stimulatory effect of estradiol on ER^- tumor growth at least could due to an ERα-mediated effect on bone marrow-derived myeloid cells that promote angiogenesis and tumor growth [32, 44].

High take rate was also observed by DeRose *et al.,* using Matrigel coated tumor tissue and implanted into the epithelium-free fat pad of NOD/SCID mice supplemented with estradiol [41]. Kuperwasser *et al*. have developed a protocol for the establishment of human mammary stroma within the mouse mammary fat pad. The "humanizing" of the mammary fat pad of mice by introducing immortalized human fibroblast cell line before transplantation, showed an increased efficiency of xenografting into NOD/SCID mice. Their results showed that stroma provide a proper

environment for the development of human mammary epithelium [45]. This model is applied for understanding normal human breast development or breast tumorigenesis [42]. Fibroblasts contribute to the maintenance of the structural framework of most tissues. Human sub-peritoneal fibroblasts and cancer cells interactions create microenvironment enhancing tumor progression and metastasis of human colorectal cancer cells when injected subcutaneously into *SCID* mice [43].

4. Tumor Xenograft Stability

When human tumor is xenografted into mice, the tumor tissue undergoes a selective pressure of tumor cells induced by the new environment. The validity of xenograft studies is highly dependent on the phenotypic and genotypic stability of the models. A fundamental assumption in using human tumor xenografts as model for preclinical anticancer drug development is that the xenograft closely resembles the corresponding primary tumor.

Previous studies have analyzed the similarity of xenograft models to primary tumors by comparing specific biological phenotypes of the primary tumor, such as tumorigenicity [46], tumor volume [47] or DNA index [48].

Current genome profiling studies indicate the retention of molecular characteristics that define tumor type. The study by Whiteford *et al.* [49] using analysis of cDNA-expression profiles demonstrates that, xenografts derived can cluster accurately with their human counterparts. Similarly, direct comparison of patient tumor biopsy tissue with early-passage xenografts demonstrates high concordance in gene expression and even greater similarity in genomic alterations when tumors are propagated in mice. Genomic and phenotypic stability between patient tumor tissue and corresponding xenograft was studied in different lesions such as oesophageal and gastro-esophageal junction [50], breast [32, 51], lung [52], kidney [9], gynecological tumors [53], uveal melanoma [54] and colorectal cancer [23].

We have demonstrated that the xenograft models of aggressive human RCC are clinically relevant, showing a good histological and molecular stability and are suitable for studies of basic biology and response to therapy [55].

Cancer involves dynamic changes in the genome, is the result of several complex events and it is characterized by uncontrolled cell proliferation. Its development is dependent not only on the changes occurring within the transformed cells, but also on the interactions of the cells with their microenvironment. The majority of our current understanding of carcinogenesis comes from the *in vitro* analysis of late-stage tumor tissue removed from cancer patients. While this has elucidated many genomic changes experienced by cancer cells, it provides little information about the factors influencing early-stage cancer development *in vivo*.

The stability of the ranking between model system and primary tumor therefore suggests that the xenograft gene expression database is an effective tool also for marker discovery.

5. Human Blood Vessels Replacement

The production of angiogenic factors in the local microenvironment of tumors contributes to the development of a vascular network with immature microvessels. It has been suggested that implanted tumors may vary in the degree to which the original human vasculature survives [56-58]. In the human tumors engrafted into immunodeficient mice, the human vessels as part of the original tumor did not survive and were no longer detectable at the time of first passage (15-25 weeks). Thus, after passage, the vessels supporting the growth of these tumors are of murine origin. The loss of the human vessels and vascularization by host vessels occurred more rapidly in a colon tumor (by 3 weeks) than in a mesothelioma (by 9 weeks), this replacement being dependent upon the tumor type [59]. These results support that the successful engraftment and growth of patient tumor xenografts depends on recruitment of new vessels from the murine host [59]. In subcutaneous xenografts of prostate and renal cell carcinoma studies, 80% of the vessels in primary xenografts of benign and malignant tissue of both organs were lined with human endothelial cells through a 30-day study period [56]. A similar study on colorectal cancer xenografts found that the human vasculature rapidly disappeared from growing colorectal xenografts. So, that by day 10, 50% of the vasculature was murine, by day 20, it was predominantly murine and by day 30, no human vessels were detectable [57]. The fate of the human vessels into the tumor xenograft is related to individual tumor types and the time point at which the engrafted specimens are examined. The regulation of the angiogenic process and molecular mechanisms that determine persistence or disappearance of human

endothelial cells in tumor contexts is different. Thus after implantation of human renal cell and prostate carcinoma primary xenografts from biopsy specimens, human endothelial cells were rapidly substituted by their murine counterparts (nearly 50% by day 10 after implantation [57]. Prostate cancer primary xenografts transplanted into athymic nude mice showed that the majority of the vessels were lined with human endothelial cells through the day 30 [56], while in other primary xenografts of fresh surgical specimens prostate cancer tissue the burst of angiogenesis by endogenous human blood vessels occurs between days 6-14 after transplantation into SCID mice pre-implanted with testosterone pellets. In this model, the androgen mediated angiogenesis was induced by up-regulation of VEGF-A expression in the stromal compartment [60]. Some reports showed a kind of “mosaic” of vessels partially lined by human tumor cells [61] and “vascular mimicry” in which blood cells are seen in channels lined by tumor cells but not endothelial cells [62]. The evaluation of endothelial cell species (*i.e*. murine or human) on xenograft tumor is important also to evaluate response to therapy such as antiangiogenics [63]. The origin of endothelial cells has a direct impact on xenograft tumor growth and response to treatment with the chemotherapeutic drug cisplatin or with the anti-angiogenic drug sunitinib [64].

In conclusion, tumor xenograft proliferation is dependent not only on the aggressivity of the tumors cells but also on the human and mouse microenvironment and its interaction with its components when engrafted into mice.

The characterisation of the different factors related to tumor microenvironment may help to understand the role of each of them in the development of human tumor xenografts into immunodeficient mice. The knowledge of these factors could be a prerequisite to elaborate a human tumor-like animal models for the molecular studies of responses to human cell therapies.

COMPETING INTERESTS

The authors declare no conflict of interest.

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