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Review article

Subrenal capsule grafting technology in human cancer modeling and translational cancer research

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ABSTRACT

Patient-derived xenograft (PDX) cancer models with high fidelity are in great demand. While the majority of PDXs are grafted under the skin of immunodeficient mice, the Living Tumor Laboratory (LTL), using unique subrenal capsule grafting techniques, has successfully established more than 200 transplantable PDX models of various low to high grade human cancers. The LTL PDX models retain key biological properties of the original malignancies, including histopathological and molecular characteristics, tumor heterogeneity, metastatic ability, and response to treatment. The PDXs are stored frozen at early transplant generations in a resurrectable form, which eliminates continuous passaging in mice, thus ensuring maintenance of the high biologic and molecular fidelity and reproducibility of the models. The PDX models have been demonstrated to be powerful tools for (i) studies of cancer progression, metastasis and drug resistance, (ii) evidenced-based precision cancer therapy, (iii) preclinical drug efficacy testing and discovery of new anti-cancer drug candidates. To better provide resources for the research community, an LTL website (www.livingtumorlab.com) has been designed as a publicly accessible database which allows researchers to identify PDX models suitable for translational/preclinical cancer research. In summary, subrenal capsule grafting technology maximizes both tumor engraftment rate and retention of human cancer heterogeneity. Moreover, the method makes possible the recovery of PDXs from frozen stocks for further applications, thus providing a powerful platform for translational cancer research.

Contents

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1. Improvement of survival and growth of human cancer biopsies grafted under the renal capsule

The majority of PDXs has been established via subcutaneous grafting, a simple technique allowing non-invasive monitoring of tumor growth. However, the drawback of subcutaneous grafting is its low success rate. For example, van Weerden et al. (1996) reported successful grafting of only 2 out of every 150 subcutaneous prostate cancer xenografts. We attribute the low take rate in the subcutaneous graft site to paucity of the vasculature and poor blood supply. Accordingly, to improve take rate, we have used the subrenal capsule (SRC) graft site, one of the most vascular environments in the body, which also has a positive interstitial fluid pressure and a high rate of lymphatic flow (Ott and Knox, 1976). Taken together, the SRC graft site ensures an abundant supply of nutrients, hormones, growth factors and oxygen to transplanted cells and tissues even before vascularization of the graft is established (Cunha, 1976a, 1976b; Cunha et al., 1977, 1983; Bogden et al., 1979; Griffin et al., 1983; Bogden, 1985; Maenpaa et al., 1985). Fortunately, the SRC site can also accommodate tissues of a substantial size range and source (Robertson et al., 2007).

We have compared take rates of both benign and malignant human prostate tissues in the SRC, subcutaneous, and orthotopic sites of immuno-deficient mice, and have shown that successful take rate is highest for the renal site (Wang et al., 2005). This advantage of the SRC site for developing human prostate cancer models has been confirmed by others (Priolo et al., 2010; Zhao et al., 2010). It is evident from such comparisons that, of the three graft sites, the SRC site is the most efficient for growing human prostate cancer as well as normal prostatic cells. Furthermore, the greater vascularity of the renal graft site is associated with reduced selective pressure on the various cancer subpopulations present in the original heterogeneous primary tumor sample. Given the cellular heterogeneity within a primary prostate cancer, we have postulated that the various cell types within the cancer vary significantly in their ability to tolerate the anoxia associated with the initial phases of the grafting process. For this reason, we are convinced that preservation of the cellular complexity (heterogeneity) of the original primary tumor is superior in the more vascular SRC graft site. This interpretation is supported by the high similarity observed between SRC xenografts and the parent tumors in terms of histopathology, marker expression, genetic profiles and properties such as androgen sensitivity and metastatic ability (Lee et al., 2005; Wang et al., 2005; Cutz et al., 2006; Watahiki et al., 2006; Press et al., 2008; Dong et al., 2010; Lin et al., 2014a, 2014b). These advantages of SRC xenografting indicate that this technique maximizes both tumor engraftment rate as well as the retention of the original cellular complexity of the primary tumor. Accordingly, PDXs developed in the SRC site better reflect the wide spectrum of cancer cell types in the primary tumor rather than PDXs developed in the relatively anoxic subcutaneous site, which tend to lack cellular heterogeneity. Furthermore, once SRC PDXs are well established, they can be regrafted to, for example, the subcutaneous site, which facilitates monitoring of tumor growth as affected by e.g., therapeutics, or the orthotopic site (the mouse prostate) for assessment of metastatic ability (Wang et al., 2005; Lin et al., 2010). In the Living Tumor Laboratory (www.livingtumorlab.com), we graft a variety of low to high-grade human cancers (including prostate cancer), which have been developed via SRC grafting of patients' cancer *tissue*. Non-obese Diabetic Severe Combined Immuno-Deficient (NOD/SCID) or NOD/SCID IL2 receptor gamma chain null (NSG) mice are used as hosts according to methods described in this special issue (Cunha and Baskin, this issue). A high engraftment rate (~95%) has consistently been achieved, and presently more than 200 transplantable PDXs have been established and stored frozen at various generations in a resurrectable form (Lee et al., 2005; Wang et al., 2005; Cutz et al., 2006; Watahiki et al., 2006, 2011; Press et al., 2008; Cheng et al., 2010; Dong et al., 2010; Lin et al., 2010, 2014a, 2014b; Tung et al., 2011; Choi et al., 2014; Eirew et al., 2015; Jager et al., 2015).

2. Subrenal capsule grafting technology makes the recovery of PDX models from DMSO frozen stocks feasible

Typically, once PDXs are established in mice, they are maintained by continuous serial transplantation in mice. Serially transplanted PDXs have two major problems: (1) long-term serial transplantation in mice may promote genetic and epigenetic drifts, which may affect biologic fidelity of the PDXs; (2) continuous serial transplantation has significant financial costs and labor. Accordingly, attempts were made to cryo-preserve the xenografts, and re-grow them as needed. Overall recovery rate of the frozen stocks was exceptionally low for conventional subcutaneous grafts. In contrast, in the Living Tumor Laboratory, we use the SRC grafting methodology to recover/regrow PDXs from frozen stocks with ~85% success rate (unpublished data). Thus, the PDXs are stored frozen at early generations in a resurrectable form in the lab. The SRC grafting methodology ensures a high biologic fidelity and reproducibility of the models.

3. SRC PDX models are highly similar to donors' original tumors

The SRC grafting methodology enhances the retention of important properties of the patients' malignancies as indicated, for example, by (i) retention of tumor heterogeneity, androgen and drug sensitivity (Wang et al., 2005; Lin et al., 2014a); (ii) retention of tumor progression-related properties and suitability for predicting clinical drug responses for personalized chemotherapy (Dong et al., 2010; Collins et al., 2012; Beltran et al., 2015) and (iii) retention of genetic profiles and targeted drug sensitivity (Andersen et al., 2010; Cheng et al., 2010; Dong et al., 2010; Kortmann et al., 2011; Lin et al., 2014a).

The transplantable prostate cancer PDXs, which were established by the Living Tumor Laboratory using the SRC xenograft method and NOD/SCID mice, not only retain key biological properties of the original malignancies, e.g., histopathology, growth rate, metastatic ability and response to androgen-ablation therapy (sensitivity or resistance) (Fig. 1C), but also preserve genetic/epigenetic characteristics, e.g., expression of androgen receptor (AR), prostate-specific antigen (PSA), and other molecular markers. For example, the LTL352 line, developed from a neuroendocrine prostate tumor, retained its pathological signiture and expressed the neuroendocrine markers of the original tumor, namely CD56, chromogranin, synaptophysin and neuron-specific enolase. Array Comparative Genomic Hybridization analysis of this tumor line identified a 5′-deoxy-5′-methylthioadenosine phosphorylase deletion, which was also found in the original tumor (Collins et al., 2012). Similarly, the