Biomaterials 77 (2016) 164–172

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

A 3D in vitro model of patient-derived prostate cancer xenograft for controlled interrogation of in vivo tumor-stromal interactions

Eliza L.S. Fong [a], Xinhai Wan [b], Jun Yang [b], Micaela Morgado [c], Antonios G. Mikos [a], Daniel A. Harrington [c], Nora M. Navone [b, **], Mary C. Farach-Carson [a, b, c, *]

[a] Department of Bioengineering, Rice University, 6500 Main Street, Houston, TX 77030, USA
[b] Department of Genitourinary Medical Oncology and the David H. Koch Center for Applied Research of Genitourinary Cancers, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA
[c] Department of BioSciences, Rice University, 6500 Main Street, Houston, TX 77030, USA

ARTICLE INFO

Article history:
Received 25 June 2015
Received in revised form
20 October 2015
Accepted 26 October 2015
Available online 9 November 2015

Keywords:
Patient-derived xenograft
Prostate cancer
Three-dimensional
Co-culture
Osteoblasts
Hydrogel
Model

ABSTRACT

Patient-derived xenograft (PDX) models better represent human cancer than traditional cell lines. However, the complex in vivo environment makes it challenging to employ PDX models to investigate tumor-stromal interactions, such as those that mediate prostate cancer (PCa) bone metastasis. Thus, we engineered a defined three-dimensional (3D) hydrogel system capable of supporting the co-culture of PCa PDX cells and osteoblastic cells to recapitulate the PCa-osteoblast unit within the bone metastatic microenvironment in vitro. Our 3D model not only maintained cell viability but also preserved the typical osteogenic phenotype of PCa PDX cells. Additionally, co-culture cellularity was maintained over that of either cell type cultured alone, suggesting that the PCa-osteoblast cross-talk supports PCa progression in bone, as is hypothesized to occur in patients with prostatic bone metastasis. Strikingly, osteoblastic cells co-cultured with PCa PDX tumoroids organized around the tumoroids, closely mimicking the architecture of PCa metastases in bone. Finally, tumor-stromal signaling mediated by the fibroblast growth factor axis tightly paralleled that in the in vivo counterpart. Together, these findings indicate that this 3D PCa PDX model recapitulates important pathological properties of PCa bone metastasis, and validate the use of this model for controlled and systematic interrogation of complex in vivo tumor-stromal interactions.

1. Introduction

Given the heavy investment in understanding cancer over the past several decades, the high drug attrition rates in oncologic drug development are disappointing [1]. The poor translation of seemingly promising preclinical findings to clinical success is partly due to the heavy reliance on cancer cell lines as tumor models for preclinical studies [2]. Recently, patient-derived xenograft (PDX) models have emerged as better surrogates of human cancer. PDX models, developed through serial propagation of patient tumor tissue in murine hosts, closely resemble the parental tumor in histology, gene expression profiles, preserved heterogeneity, and drug response [3–7]. However, use of PDX models in in vitro mechanistic studies is hampered by their poor adaptation to traditional two-dimensional tissue culture and the potential for two-dimensional culture to induce undesired adaptations [6,8]. Improved methods for in vitro PDX culture have been developed that rely on three-dimensional (3D) cell culture strategies, such as spheroid culture, or encapsulation of tumor cells within naturally derived gels such as Matrigel or collagen [9–12]. However, while spheroid cultures enable primary tumor tissue culture, the lack of a surrounding matrix prohibits control over spatial positioning of multiple cell types. Similarly, the often ill-defined and variable composition of naturally derived gels makes consistent re-creation of the engineered in vitro tumor microenvironment challenging.

In current paradigms, cancer is viewed as a complex manifestation of aberrant interactions between tumor cells and the

* Corresponding author. Department of BioSciences, Rice University, 6500 Main Street, Houston, TX 77030, USA.
** Corresponding author. Department of Genitourinary Medical Oncology and David H. Koch Center for Applied Research of Genitourinary Cancers, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA.
E-mail addresses: nnavone@mdanderson.org (N.M. Navone), farachca@rice.edu (M.C. Farach-Carson).

http://dx.doi.org/10.1016/j.biomaterials.2015.10.059
0142-9612/

surrounding stromal compartment. Accordingly, increasing emphasis is being placed on understanding the role of the host cells in the tumor microenvironment in cancer progression and metastasis and in design of therapies [13]. In prostate cancer (PCa), stromal-epithelial signaling dictates tumor cell behavior and metastasis [14–16]. Metastasizing PCa cells predominantly target bone, and the characteristic osteoblastic lesions that form reflect the functional dependency of PCa on the bone microenvironment for disease progression. Therefore, targeting the bone stroma and disrupting the PCa-stromal cross-talk can be an effective therapeutic strategy for treating or preventing bone metastases [17]. In our recent integrated clinical and preclinical studies using the MDA PCa 118b PDX model, we demonstrated that blockade of fibroblast growth factor receptor (FGFR)-dependent PCa-stromal interactions in the bone microenvironment could be an effective therapeutic strategy for a subset of PCa patients [17]. In light of these findings, we wished to further investigate the specific stromal targets and affected pathways to guide biomarker development and patient selection. However, in vitro platforms to support such mechanistic studies of PCa PDX-stromal interactions had to be created.

In the interdisciplinary study reported here, we addressed this need by developing and validating an in vitro PCa PDX model that accurately reflects PCa-stromal interactions in bone for controlled mechanistic studies. We demonstrate for the first time, to our knowledge, an engineered tumor microenvironment consisting of clinically relevant PDX tumor cells and osteoblastic cells, co-encapsulated within a well-defined 3D hyaluronan (HA) hydrogel. Osteoblasts play a key role in promoting PCa progression in bone [18]. Ubiquitous in the extracellular matrix of connective tissues, HA is a building block for the fabrication of hydrogel matrices designed to mimic HA-rich tissues such as the bone marrow, where bone metastatic PCa cells reside [19,20]. Indeed, we previously reported the first demonstration of using a 3D scaffold-based approach to culture PDX cells in vitro in unmodified HA hydrogels [21]. Prior to development of our 3D hydrogel approach, it was difficult to culture PCa PDX cells in vitro for experimentation given their poor viability on tissue culture plastic. In this work, to enable the culture of osteoblastic cells with PCa PDX cells, HA was specifically modified with integrin-binding peptides and cross-linked with matrix metalloproteinase (MMP)-degradable peptides. Plain, unmodified HA hydrogels are not capable of supporting the attachment and spreading of encapsulated osteoblastic cells. Incorporation of these peptides is an approach that was previously developed to enable cell-mediated remodeling of synthetic 3D hydrogels for tissue regeneration applications [22–24]. This PDX co-culture model maintains key phenotypic tumor markers; recapitulates the in vivo structural arrangement of osteoblasts with respect to tumor cells; mimics many elements of the previously observed FGFR-mediated tumor-stromal cross-talk for PDX tumor cells grown in bone, including cross-talk involving FGFR1 and fibroblast growth factor 9 (FGF9) [17,25]; and offers a robust platform for in vitro drug evaluation. This model addresses the critical unmet need in PCa research and drug discovery for platforms that support the controlled interrogation of complex in vivo tumor-stromal interactions as discrete units in vitro.

2. Materials and methods

2.1. Study design

The primary objective of this study was to develop an auxiliary model to the in vivo PCa PDX model that supports controlled mechanistic studies of tumor-stromal interactions in vitro. We cultured PCa PDX cells and osteoblastic cells within a well-defined 3D hydrogel matrix and characterized the tumor architecture, viability, phenotype, as well as the biochemical interaction between the two cell populations. These findings were then validated against the corresponding PCa PDX in vivo model [17]. We also evaluated whether [1] standard molecular biology tools (such as gene knockdown) can be employed to manipulate cell–cell interactions for mechanistic investigations and [2] the effect of dovitinib in vivo [17] can be at least in part, reflected in this in vitro PCa PDX model.

2.2. Synthesis and characterization of thiolated HA

Sulfhydryl groups were incorporated in HA (620 kDa, Genzyme, Cambridge, MA) by reacting HA with a disulfide-containing dihydrazide compound, followed by reduction with dithiothreitol, using a previously reported method [20,26]. The degree of modification (35–43%) in thiolated HA was measured [20,26] by ^{1}H nuclear magnetic resonance, and the lyophilized product was stored at −20 °C under argon prior to use.

2.3. Synthesis of acrylated peptides

Cell-adhesive peptide GRGDS (GenScript USA Inc., Piscataway, NJ) with C-terminal amidation was reacted with acrylate-PEG-SVA (3400 g/mol, Laysan Bio Inc., Arab, AL) at a molar ratio of 1.2:1 in HEPBS buffer (20 mM HEPBS [Santa Cruz Biotechnology, Inc., Dallas, TX], 100 mM NaCl, 2 mM $CaCl_2$, and 2 mM $MgCl_2$) adjusted to pH 8.0 using 0.1 N NaOH [27]. The reaction was allowed to run overnight at 4 °C on a shaker protected from light and then dialyzed for 2 days against ultrapure water using a 3500 Da MWCO dialysis membrane (Spectrum Laboratories Inc., Rancho Dominguez, CA) before lyophilization for an additional 2 days. MMP-degradable peptide, KGGGPQG↓IWGQGK (GenScript USA Inc.) with N-terminal acetylation, herein referred to as PQ (↓ marks the MMP-cleavable site), was reacted with acrylate-PEG-SVA at a molar ratio of 1:2.5 using the same protocol. Conjugation of acrylate-PEG to the peptides was verified by high-performance liquid chromatography (Vydak C_{18} 218TP54 column, Varian Prostar solvent delivery module and UV–vis detector) and MALDI-TOF (Bruker AutoFlex II). The lyophilized solids were stored at −20 °C prior to use.

2.4. MDA PCa 118b PCa PDX in vivo propagation and processing

The MDA PCa 118b PDX [25] was routinely maintained as subcutaneous tumors in CB-17 SCID mice (Charles River). Propagation of tumors in mice was conducted under approval by the Institutional Animal Care and Use Committee of The University of Texas MD Anderson Cancer Center. Following harvest, the tumors were processed for hydrogel encapsulation as previously described [21]. To obtain sections of MDA PCa 118b PDX in bone for histologic characterization, tumor-bearing femurs were prepared and processed as previously described [25].

2.5. Cell culture

MC 3T3-E1 (ATCC, Manassas, VA) cells were maintained in alpha-MEM (Life Technologies, Grand Island, NY) containing 10% (v/v) FBS and in the presence of 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C and 5% CO_2.

2.6. Preparation of cell-laden hydrogel constructs

To prepare the MDA PCa 118b-only constructs, following our previously established protocol [21], tumor cell aggregates that formed in suspension were mixed with thiolated HA dissolved in PBS to 10 mg/mL. Each MDA PCa 118b-only construct was prepared