Biomaterials 150 (2018) 150–161

Contents lists available at ScienceDirect

Biomaterials

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

Biomechanical regulation of drug sensitivity in an engineered model of human tumor

A. Marturano-Kruik [a, c], A. Villasante [a], K. Yaeger [a], S.R. Ambati [b], A. Chramiec [a], M.T. Raimondi [c], G. Vunjak-Novakovic [a, *]

[a] Department of Biomedical Engineering, Columbia University, New York 10032, USA
[b] Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10065, USA
[c] Department of Chemistry, Materials and Chemical Engineering "G Natta", Politecnico di Milano, Milano, Italy

ARTICLE INFO

Article history:
Received 4 September 2017
Received in revised form
7 October 2017
Accepted 9 October 2017
Available online 10 October 2017

Keywords:
Mechanical stimulation
Tumor microenvironment
RUNX2
Ewing sarcoma
Drug sensitivity

ABSTRACT

Predictive testing of anticancer drugs remains a challenge. Bioengineered systems, designed to mimic key aspects of the human tumor microenvironment, are now improving our understanding of cancer biology and facilitating clinical translation. We show that mechanical signals have major effects on cancer drug sensitivity, using a bioengineered model of human bone sarcoma. Ewing sarcoma (ES) cells were studied within a three-dimensional (3D) matrix in a bioreactor providing mechanical loadings. Mimicking bone-like mechanical signals within the 3D model, we rescued the ERK1/2-RUNX2 signaling pathways leading to drug resistance. By culturing patient-derived tumor cells in the model, we confirmed the effects of mechanical signals on cancer cell survival and drug sensitivity. Analyzing human microarray datasets, we showed that *RUNX2* expression is linked to poor survival in ES patients. Mechanical loadings that activated signal transduction pathways promoted drug resistance, stressing the importance of introducing mechanobiological cues into preclinical tumor models for drug screening.

1. Introduction

Developing anti-cancer drugs is a long, costly and inefficient process [1]. Although many drug candidates show promising pre-clinical results, less than 7% are approved for clinical use [2,3]. Drug safety and efficacy are currently studied *in vitro* (in cell monolayers and aggregates) and *in vivo* (in rodent models). When cultured *in vitro*, cancer cells are deprived of their native microenvironment and tend to lose the tumor phenotype due to undesired adaptation [4]. Animal models, which are considered essential for cancer research, also fail to predict the clinical outcomes [5]. To overcome these limitations, tumor features can be tailored *in vitro* using bioengineering techniques [6]. Existing, 3D models replicate some properties of bone but have not fully reproduced the structural and cellular composition of the bone microenvironment. For instance, we recently developed a bioengineered model of human bone tumor that recapitulates three-dimensional (3D) tissue context, extracellular matrix and tumor-stroma interactions [7]. In this model, cancer cells recovered their original hypoxic tumor phenotype and expression of important oncogenes. Among other factors, flow strongly affects tumor behavior and drug response, as shown using an Ewing Sarcoma 3D model cultured in a perfusion bioreactor [8]. The use of patient-derived tumor xenografts (PDXs) is also becoming a viable alternative to cultures of cancer cell lines, as they better preserve the parental tumor heterogeneity and drug responses [9]. Recent findings suggest that a PDX 3D model of prostate cancer recapitulates essential pathological properties of bone metastasis, enabling interrogation of complex tumor-stromal interactions [10].

However, critical microenvironmental cues such as mechanical signals remain elusive to study *in vivo* and are challenging to model *in vitro*. In fact, nearly every tissue in our body is subjected to mechanical forces. These forces, sensed by the cells, are transduced into biochemical signals activating intracellular pathways [11]. As a result, mechanical *stimuli* play a major role in tissue development and diseases such as cancer [12]. For instance, Ewing sarcoma (ES) – the second most frequent bone tumor in adolescents – thrives in a mechanically active microenvironment. Despite multi-modal therapy, survival rates in ES remain poor [13]. Hence, novel therapeutic strategies and translational investment are needed to increase the life expectancy of young ES patients [14].

* Corresponding author.

https://doi.org/10.1016/j.biomaterials.2017.10.020
0142-9612/

One promising approach targets a family of cell-surface receptors called receptor tyrosine kinases (RTKs). Ligand binding to these receptors activates downstream signaling pathways mediated by the extracellular-signal regulated kinase (ERK1/2). In a similar fashion, ERK1/2 is part of the mechanoregulatory circuit linking physical cues to molecular pathways in cancer cells [15]. Therefore, blocking ERK1/2 leads to reduced cell proliferation and survival in many tumors. However, despite encouraging results in ES preclinical models, the use of RTK inhibitors showed little or no effects in ES patients [16]. Recent studies have shown that mesenchymal stem cells exposure to mechanical loading stimulated ERK1/2-dependent activation of RUNX2, a transcription factor and master regulator of bone differentiation [17]. In addition to its role in osteogenesis, RUNX2 promotes cancer cell survival, invasion and drug resistance [18,19]. Given Ewing sarcoma mesenchymal features and oncogenic potential of RUNX2 in the bone, it is surprising that there is little evidence linking RUNX2 to ES.

Our objective was to develop a bioengineered model of Ewing sarcoma that incorporates the application of mechanical loadings to investigate the role of RUNX2 in ES cells drug sensitivity. We hypothesized that the exposure of ES cells to mechanical forces, stimulates ERK1/2-dependent expression of RUNX2, altering RTK inhibitors efficacy. To test this hypothesis, we analyzed RUNX2 expression in ES tumor samples and ES cell lines. ES cell lines or patient-derived ES xenografts were grown in a previously validated biomimetic 3D matrix [20]. The 3D tissue models were cultured in the bioreactor and exposed to external forces of physiologically relevant types and magnitudes, with static controls. The ERK1/2-RUNX2 transduction mechanism was studied by measuring gene and protein expression. Drug sensitivity to RTK inhibitors was assessed by analyzing cell phenotype, apoptosis and proliferation, with emphasis on the effects of mechanical forces on the ERK1/2-RUNX2 signaling pathway.

2. Materials and methods

2.1. Drugs and chemicals

Sorafenib was purchased from Santa Cruz Biotechnology. Doxorubicin, sunitinib, and imatinib were purchased from Sigma Aldrich. U0126 was purchased from Cell Signaling Technology.

2.2. Cell lines

Ewing sarcoma cell lines SK-N-MC (HTB-10) and RD-ES (HTB-166) were purchased from the American Type Culture Collection (ATCC) and cultured according to the manufacturer's specifications using ATCC-formulated EMEM or RPMI-1640 medium respectively, supplemented with 10% (v/v) Hyclone fetal bovine serum (FBS) and 1% penicillin/streptomycin (Gibco).

2.3. Patient-derived cancer cells

Processing of the patients' samples, expansion, and isolation of the patient-derived xenografts were conducted as in our previous studies [21]. Briefly, de-identified samples of the patients' tumor tissue were collected under a protocol approved by the Memorial Sloan Kettering Cancer Center (MSKCC) Institutional Review Board (IRB). The patient-derived xenograft was established by engrafting and expanding the patient's tumor tissue in NSG mice (second passage). Single cell suspensions were achieved by physical disruption and digestion of the explanted xenografts using collagenase type IV (Gibco). Early cell cultures (PS3 cells, $p < 3$) were characterized, validated, and tested for mycoplasma contamination at MSKCC core facility. The cells were cultured in Dulbecco's Modified Eagle's Medium and supplemented with 10% FBS, L-glutamate, and antibiotics penicillin/streptomycin.

2.4. 3D matrix preparation

3D porous matrices were prepared from collagen 1 and hyaluronic acid solutions using a freeze-drying technique as in our previous studies [20]. A low molecular weight (10–20 kDa) Sodium Hyaluronate (Lifecore) was dissolved in distilled water to obtain a 1% (wt/v) solution. Four parts of Collagen 1 (8–11 mg/ml in 0.02 N acetic acid, Corning) were mixed with one part of HA solution (4:1). 75 μl of the solution was dispensed into a 3 mm high x 4 mm diameter well serving as a mold for scaffold formation, frozen at −40 °C for 4 h, and lyophilized under vacuum of <100 mTorr at −40 °C for 12 h. Sublimation of ice crystals formed in the frozen mixture results in the formation of interconnected pores within the 3D matrix. The collagen 1 − HA 3D matrices were cross-linked with a water-soluble carbodiimide and soaked in 95% ethanol containing 33 mM 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (Sigma Aldrich Co. Ltd.) and 6 mM *N*-hydroxysuccinimide (Sigma Aldrich) for 4 h at 25 °C. After crosslinking, the porous scaffolds were washed in distilled water (5 min × 10 times) and freeze-dried overnight.

2.5. 3D cell culture

Each matrix was seeded with 4 million cells. To this end, 12 scaffolds and 24 ml of cell suspension containing 2×10^6 cells/ml were placed into a 50 ml Falcon tube on an orbital shaker (3 h at 37 °C and 5% CO_2) Cell-seeded matrices were then transferred to non-treated 24-multiwell plates (Nunc) and cultured in 1.5 ml of medium at 37 °C and 5% CO_2 for 48 h to allow the cells to attach to matrix. These cultures were established using ES cell lines (SK-*N*-MC and RD-ES) and patient-derived xenografts (PS3 cells).

2.6. Bioreactor

A bioreactor developed in our previous studies [22,23] was used to subject 3D cell cultures to dynamic compressive loading, by the vertical motion of plungers that were in contact with the tissues placed into standard 24 well-plates. A linear actuator and a stepper motor were used to control the displacement magnitude, and the stimulation frequency and waveform. Live monitoring of the motion was achieved using a linear variable differential transformer. To maintain the viability, the culture chamber was filled with culture medium, and all experiments were conducted within an incubator at 37 °C and 5% CO2.

Porous scaffolds (3 mm high x 4 mm diameter cylinders), were prepared from collagen I and hyaluronic acid solutions using a freeze-drying technique. Cells were seeded into porous scaffolds (2×10^6 cells/ml, 2 ml/scaffold) and allowed to attach. The resulting tissue constructs were placed in the bioreactor and subjected to cycles of dynamic mechanical stimulation.

2.7. Mechanical stimulation protocol

3D tumor tissues were placed into the bioreactor and subjected to unconfined, dynamic compressive loading, applied periodically. Specifically, the compressive strains of 1, or 10% were applied using a sinusoidal waveform at a 0.25 Hz frequency. Each day, 1800 loading cycles were applied over 2 h of stimulation. The protocol included 2 h of bioreactor culture each day, where the 3D cultures (static and stimulated group), placed in regular tissue culture dishes, had top and bottom surfaces in contact with bioreactor parts. The stimulated group was exposed to dynamic loading,