Development and characterization of a novel human 3D model of bone metastasis from breast carcinoma *in vitro* cultured

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Abstract

Breast cancer frequently metastasizes to the skeleton causing significant morbidity. Here, we set-up a novel and advanced *ex vivo* model by using fresh tissue from human vertebral bone metastasis from breast carcinoma patients able to retain the tumor microenvironment and tumor cells heterogeneity.

Introduction

Breast cancer is the most frequent malignancy in women with an estimation of 2.1 million new diagnoses in 2018.[1] Even though primary tumors are usually efficiently removed by surgery, 20–40% of patients will develop metastases in distant organs. Bone is one of the most frequent site of metastases from advanced breast cancer, accounting from 55 to 58% of all metastases.[2] Currently, none of the therapeutic strategies used to manage breast cancer bone metastasis are really curative. Tailoring a suitable model to study and evaluate the disease pathophysiology and novel advanced therapies is one of the major challenges that will predict more effectively and efficiently the clinical response. Preclinical traditional models, *i.e.* two-dimensional cell culture systems and animal models, have been largely used as they can provide standardization and simplicity, moreover, further advancements have been made with 3D cultures, by spheroids and artificial matrices, patient derived xenografts and microfluidics. Despite these models recapitulate numerous aspects of tumor complexity, they do not completely mimic the clinical native microenvironment. Thus, to fulfill this need, in our study we developed a new, advanced and alternative model of human breast cancer bone metastasis as potential biologic assay for cancer research.

Materials and Methods

The study involved breast cancer bone metastasis samples obtained from three female patients undergoing wide spinal decompression through a posterior approach, with removal of both pedicles of the affected vertebra as well as of the metastatic area causing the epidural compression, followed by posterior stabilization. Samples were cultured in a TubeSpin Bioreactor on a rolling apparatus under hypoxic conditions (to mimic the microenvironment encountered in the bone marrow by metastatic cells spreading from breast cancer) at time 0 and for up to 40 days and evaluated for viability by the Alamar Blue test, gene expression profile, histology and immunohistochemistry.

Results

Results showed the maintenance and preservation, at time 0 and after 40 days of culture, of the tissue viability, biological activity, as well as molecular markers, *i.e.* several key genes involved in the complex interactions between the tumor cells and bone able to drive cancer progression, cancer

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Key words: Bone metastasis; breast carcinoma; *ex vivo* model.

Topic: In vitro models: 2D cell cultures, microfluidics, and organoids.

Acknowledgments: This work was supported by grants from IRCCS Istituto Ortopedico Rizzoli (Ricerca Corrente) and by the Project ERA-NET Euronanomed III "Next generation antibacterial nanostructured osseointegrated customized vertebral replacement - NANOVERTEBRA".

Disclosures: The authors declare that they have no conflict of interest.

Conference presentation: This paper was presented at the Third Centro 3R Annual Meeting - L'era delle 3R: modelli *in silico, in vitro* e *in vivo* per promuovere la ricerca traslazionale - 30 September - 1 October 2021, Evento online organizzato dal Politecnico di Torino.

Received for publication: 9 July 2021.
Accepted for publication: 7 September 2021.

Biomedical Science and Engineering 2021; 4(s1):165
doi:10.4081/bse.2021.165

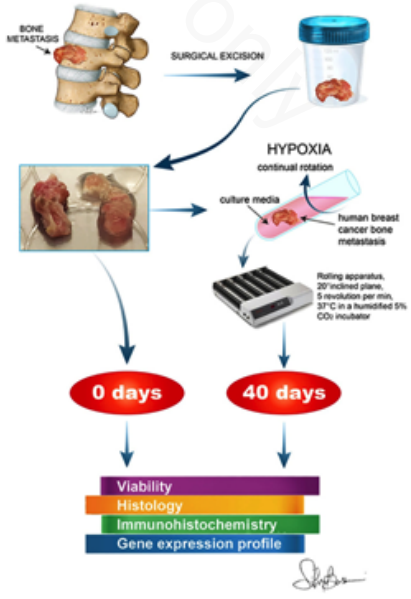

Figure 1. Set-up of *ex vivo* assay.

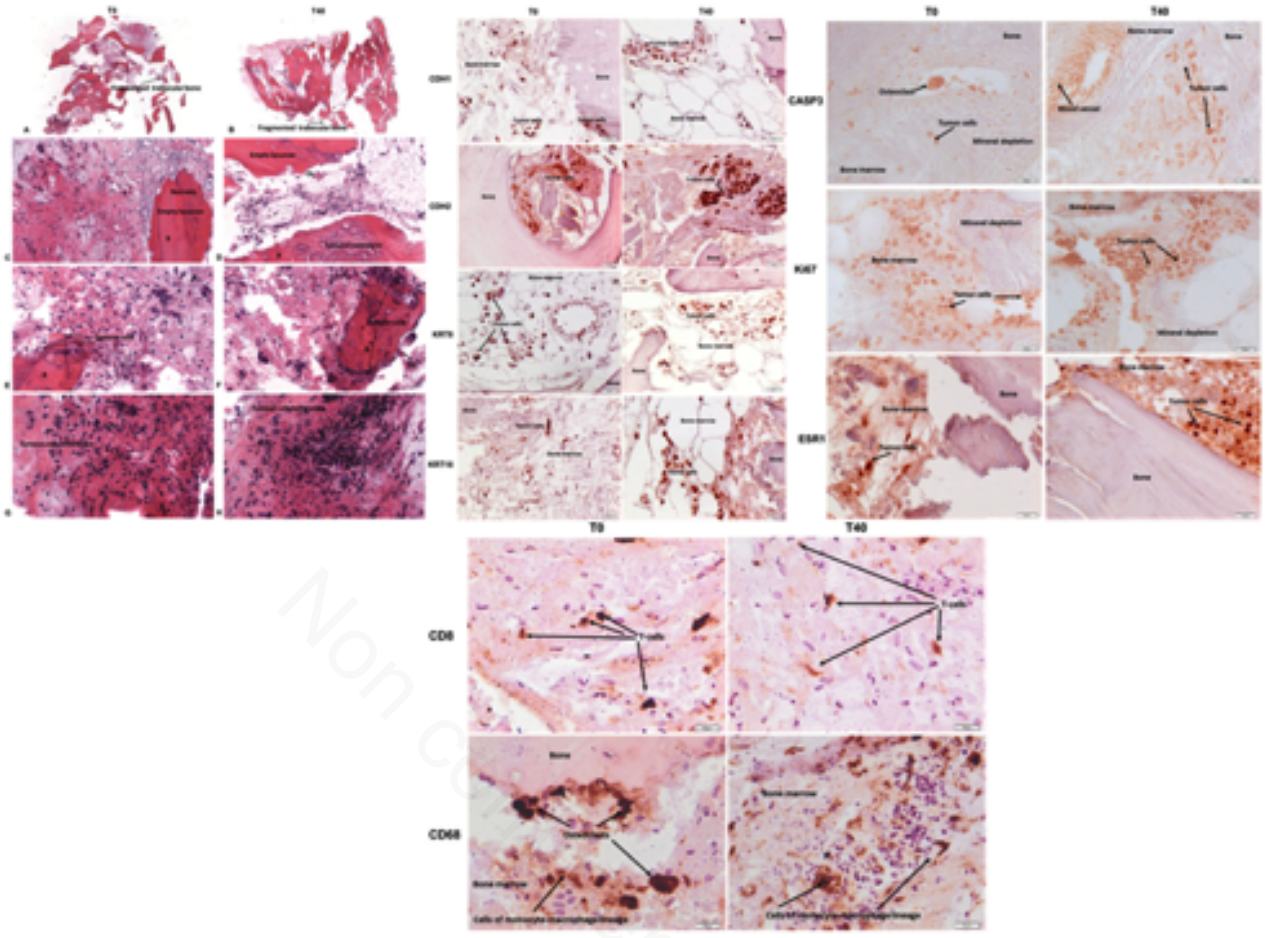

Figure 2. H/E and immunohistochemical staining of breast cancer bone metastases fragments at T0 and at T40.

aggressiveness and metastasis to bone (IL10, IL1β, MMP1, MMP7, PTH1R, PTH2R, TNF, ACP5, SPI1, VEGFA, CTSK, TGF-β). A good tissue morphological and microarchitectural preservation with the presence of lacunar osteolysis, fragmented trabeculae locally surrounded by osteoclast cells and malignant cells and an intense infiltration by tumor cells in bone marrow compartment in all examined samples. Histomorphometrical data on the levels of bone resorption (Oc.S/BS) and bone apposition (Ob.S/BS) parameters remained constant between T0 and T40 for all analyzed patients. Additionally, immunohistochemistry showed homogeneous expression and location of CDH1, CDH2, KRT8, KRT18, Ki67, CASP3, ESR1, CD8 and CD68 between T0 and T40, thus further confirming the invasive behavior of breast cancer cells and indicating the maintaining of the metastatic microenvironment and the development of a reliable, reproducible and cost-effective advanced model (Figure 1 and 2).

Discussion and Conclusions

The novel tissue culture, set-up in this study, has significant advantages in comparison to the pre-existent 3D models: the tumor environment is the same of the clinical scenario, including all cell types as well as the native extracellular matrix; it can be quickly set-up employing only small samples of breast cancer bone metastasis tissue in a fast, simple, ethically correct (after Ethical Committee approval and patients' consent) and cost-effective manner; it bypasses and/or decreases the necessity to use more complex preclinical model, thus reducing the ethical burden following the 3R principles, the guiding principles aimed at replacing/reducing/refining (3R) animal use and their suffering for scientific purposes; it can allow the study of the interactions within the breast cancer bone metastasis tissue over a relatively long period of up to 40 days; it preserves over a relatively long period of time the tumor morphology and architecture, allowing also the evaluation of different biological factors, parameters and activities. These advantages and unique features were confirmed by the monitoring of tissue viability, tissue histomorphology and micro-architecture, antigen location e positivity and gene expression for up to 40 days. Therefore, the study provides for the first time the feasibility and rationale for the use of a human-derived advanced alternative model for cancer research and testing of drugs and innovative strategies, taking into account patient individual characteristics and specific tumor subtypes so predicting patient specific responses.

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