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Original Article

Hylonome

Three-dimensional type I collagen co-culture systems for the study of cell-cell interactions and treatment response in bone metastases

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Abstract

The available monolayer culture systems for the study of bone metastases constitute a suboptimal simulation of the in vivo pathophysiology of bone metastases, and therefore, do not provide sufficient information to assess the morphologic evidence of bone reaction to cancer cells, the nature of cell-specific mediators of osteolysis and osteoplasia and the response to treatment. Therefore, we have developed a three-dimensional (3-D) type I collagen gel system that allows co-culture of human osteoblasts (MG-63) with cancer cells, such as MCF-7, MDA-MB-231 or ZR-75 breast cancer cells, PC-3 prostate cancer, KLE endometrial cancer cells and Calu-1 lung cancer cells. We used type I collagen purified from rat tail tendons and the 3-D system was prepared by mixing MG-63 cells with type I collagen in 24-well plates. The 3-D system was inoculated with cancer cells and processed with standard cell culture procedures. After 1 week of culture, the matrix gel was fixed with formalin and embedded in paraffin. Serial sections were stained with trichrome Masson stain and modified Masson-Goldner stain, as well as analyzed by in situ hybridization, immunohistochemistry and the TUNEL technique for semi-quantitative detection of apoptotic cell death, assessing the response to adriamycin therapy. The inoculation of PC-3 cells in this collagen matrix produced a blastic reaction, documented by an increased number of MG-63 cells and increased density of type I collagen. The human KLE cells and inoculation of cell-free media produced no reaction, while ZR-75, MCF-7 and Calu-1 cells produced local degradation of the collagen matrix. In situ hybridization revealed the expression of Insulin-like growth factor 1 (IGF-1) and urokinase-type plasminogen activator (uPA) mRNA, while immunohistochemistry detected differential expression of uPA and cathepsin D. Adriamycin induced apoptotic cell death in prostate cancer cells and estrogen receptor negative (ER-) MDA-MB-231 breast cancer cells, while adriamycin did not induce apoptosis but cytostasis in ER+ MCF-7 cells. The adriamycin-induced apoptosis was inhibited by co-culture with osteoblast-like cells (MG-63). We conclude that this 3-D culture system is a useful in vitro model allowing the analysis of local mediators of osteolytic and osteoblastic reactions to bone metastases and treatment response.

Keywords: Osteoblastic Reaction, Prostate Cancer, In Vitro Systems

Introduction

Bone corresponds to one of the most prevalent sites for the development of metastases of malignant tumors[1]. A significant extent of morbidity and mortality in patients with high-frequency and high-impact malignancies, such as breast, prostate and lung cancer, are attributed to bone metastases. Spontaneous fractures, intractable pain or hypercalcemia associated with such lesions compromise the quality of the patient's life[1]. Moreover, bone metastases generally constitute a stronghold of anti-cancer therapy-refractory tumor progression, e.g. in prostate cancer. The skeletal metastases are the first and often the only sites of disease which eventually become refractory to hormone ablation therapy[2,3], even though this modality continues to offer sustained control of the disease at the primary site, the prostate, and other extraskeletal sites, such as pulmonary metastases[4,5]. As a result of their pronounced resistance to anti-cancer therapies, bone metastases signify ominous prognosis, accelerated tumor growth and curtailed survival. Therefore, in order to achieve better therapeutic outcomes in patients with metastatic disease to the bones, it is imperative that we acquire a better understanding of the

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mechanisms responsible for the development of a bone metastatic lesion. Towards this goal, we have studied, in a 3-dimensional collagen gel culture system, the interactions of neoplastic cells with osteoblast-like cells, as well as the modulatory effects of the latter on the response of malignant cells to anti-cancer drugs.

Materials and Methods

We used an extensive panel of human cell lines derived from various osteotropic malignancies, including PC-3 prostate cancer cells, MDA-MB-231 and MCF-7 breast cancer cells, Calu-1 lung cancer cells, KLE endometrial cells, as well as MG-63 osteoblast-like cells, which express osteoblastic phenotype in an environment rich in type I collagen. Cancer cells were co-cultured with MG-63 osteoblast-like cells in 3-dimensional type I collagen gels. The co-culture experiments in such a collagen-rich milieu served to recapitulate the local microenvironment at the sites of bone metastases. PC-3, MDA-MB-231 and MCF-7 cells were exposed to adriamycin at doses ranging from 100 nM (corresponding to adriamycin peripheral blood levels in patients receiving conventional chemotherapy) to 5 μM. Control cultures of cancer cells in collagen gels were performed in the absence of MG-63 cells. 3-dimensional collagen gels were prepared, processed for Terminal deoxynucleotidyl Transferase (TdT)-mediated nick end labeling method to detect apoptosis, stained with hematoxylin-eosin and Masson-Goldner stains and analyzed, as previously described[6].

Results

The MG-63 osteoblast-like cells cultured in the 3-dimensional collagen gel system revealed a morphology consistent with osteoblast/osteocyte cells in vivo, while all cancer cell lines tested demonstrated morphological features compatible with those of the corresponding human malignant cells in pathology specimens. Moreover, in situ hybridization and immunohistochemical studies confirmed, at the mRNA and protein level, respectively, the production of uPA and IGF-1, in separate gel cultures of PC-3 and MG-63 cells, respectively. Therefore, we concluded that this type I collagen gel co-culture system could not only sustain the viability of these cells, but also provide the appropriate milieu where they could maintain their phenotypic properties. Co-culture of MG-63 cells with PC-3 cells inoculated in the collagen gel produced a reaction morphologically and histologically consistent with the osteoblastic reaction produced at the sites of bone metastases of prostate cancer. Masson-Goldner stains of the collagen gels revealed increasing numbers of osteoblast-like MG-63 cells and increased density of type I collagen around the inoculations of PC-3 cells, while the distribution of MG-63 cells at areas of the gel distant to the inoculation sites was homogeneous and similar to the one of control MG-63 cultures.

In contrast to co-culture gels involving PC-3 cells, the inoculation of MDA-MB-231 and MCF-7 breast cancer cells, and Calu-1 lung cancer cells induced reactions morphologically reminiscent of the osteolytic lesions that accompany the bone metastases of the respective neoplasms, in vivo. Areas adjacent to inoculated cells manifested local degradation of the collagen matrix, while inoculations of KLE human endometrial cancer cells or control inoculations of cell-free medium caused no identifiable significant morphological change, either lytic or blastic, in the collagen gel.

We, therefore, documented that the collagen gel system for co-culture of osteoblast-like cells with cancer cells is a reproducible in vitro model that recapitulates the in vivo setting of cancer cell metastases to bones and yields a local reaction which may be blastic or lytic, depending on the specific type of cancer cell inoculated in the collagen gel.

Having established the specificity and reproducibility of our in vitro model for bone metastases and having documented the ability of prostate cancer cells to induce osteoblastic reactions in this model, we investigated whether the presence of osteoblasts could, in turn, modulate the response of cancer cells to anti-cancer drugs. When PC-3 cells were cultured in a collagen gel, separately from MG-63, exposure to adriamycin induced extensive apoptosis. In contrast, co-culture of MG-63 cells with PC-3 cells neutralized adriamycin-induced apoptosis and, in fact, led to pronounced proliferation of PC-3 cells, whose number increased by 40-50% compared to control cultures without the presence of MG-63. Interestingly, IGF-1 partially neutralized the adriamycin-induced apoptotic death of PC-3 cells, in the absence of MG-63.

Therefore, it was concluded that osteoblasts protect prostate cancer cells from apoptosis induced by cytotoxic chemotherapeutic agents. This protective effect, which was mediated primarily by IGF-1, is consistent with the long standing clinical observation that bone metastases of prostate cancer are eventually becoming resistant to pro-apoptotic anti-cancer therapies, such as androgen ablation therapy or cytotoxic chemotherapy, even though they may have initially responded to hormonal manipulations.

The ability of osteoblasts to modulate the response of cancer cells to anti-cancer therapies was also confirmed in breast cancer cells. MG-63 cells were shown to alter the effects of adriamycin on cell cycle and apoptosis of estrogen receptor negative (ER-) MDA-MB-231[8] and estrogen receptor positive (ER+) MCF-7 breast cancer cells[9]. Adriamycin can arrest MCF-7 and MDA-MB-231 cells at the G2/M phase in the cell cycle and inhibit cell growth. In addition, adriamycin arrested the MCF-7 cells at the G1/G0 phase and induced apoptosis of MDA-MB-231 cells. Co-culture with MG-63 (or exogenous TGFβ1) partially neutralized the adriamycin-induced cytotoxic death of MDA-MB-231 cells, but enhanced adriamycin-induced blockade of MCF-7 cells at the Gl/G0 phase. Exogenous IGF-1 partially neutralized the adriamycin-cytotoxicity/

cytostasis of MDA-MB-231 cells. MG-63 osteoblast-like cells inhibited growth of MCF-7 cells, while they promoted growth and rescued from adriamycin-induced apoptosis the MDA-MB-231 cells in the type I collagen co-culture system. These data suggested that osteoblast-derived growth factors, such as IGF-1 and TGFβ can modify the response of not only prostate, but also breast cancer cells to cytotoxic chemotherapy, however, in a differential manner, depending on the estrogen receptor (ER) status, rescuing (ER-) breast cancer cells from chemotherapy-induced programmed cell death, while enhancing the (chemotherapy-induced) arrest of (ER+) MCF-7 cells at the Gl/G0 phase.

Discussion

Osteoblasts can not only facilitate the proliferation of malignant cells, but may also protect these cells from pro-apoptotic anti-cancer drugs. This protection is manifested clinically by the establishment of hormonal therapy - or cytotoxic chemotherapy - resistant growth which may be considered as a major culprit for the curtailed survival of patients developing bone metastases. In view of our findings on the pivotal role of IGF-1 in mediating this protective effect of osteoblasts on cancer cells, we have developed a novel therapeutic approach, with the aim to enhance the efficacy of conventional anti-cancer drugs, used in the setting of bone metastases.

This approach is termed “anti-survival factor therapy”, which is administered in combination with e.g. androgen ablation therapy for prostate cancer. It involves dexamethasone and somatostatin analogs administration with the aim to suppress both the paracrine, osteoblast-derived and endocrine, liver-derived IGF-1, which is the major survival factor mediating the protection that prostate cancer cells enjoy in the bone microenvironment. This novel approach has already yielded encouraging preliminary results at a phase II clinical trial[10].

Therefore, our 3-dimensional collagen gel co-culture system, a valuable tool for the study of cell-cell interactions at the milieu of bone metastases, allowed the reliable and reproducible assessment of response of metastatic cells to standard anti-cancer therapies and facilitated the translation of our results into a potentially significant novel therapeutic approach in the management of bone metastatic disease.

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