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Review

Oncogenic and metastatic properties of preprotachykinin-I and neurokinin-1 genes

Arun S. Singh [a], Andrew Caplan [a], Kelly E. Corcoran [b], Joseph S. Fernandez [b], Michael Preziosi [a], Pranela Rameshwar [a,*]

[a] *Department of Medicine-Hematology/Oncology, New Jersey Medical School-University of Medicine and Dentistry of New Jersey, NJ, United States*
[b] *Graduate School of Biomedical Sciences, UMDNJ, NJ, United States*

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Abstract

Breast cancer (BC) remains the cancer with highest mortality among women in the United States. Entry of BC cells (BCCs) in bone marrow (BM) leads to poor prognosis. This review discusses studies showing interactions between BCCs and BM stroma, consequently providing BCCs with advantages of survival within BM. Myc transcription factor is investigated as a link between the transforming properties of peptides derived from the preprotachykinin-I gene (PPT-I) and Neurokinin-1 (NK1) receptor. A co-culture method previously described to model early integration of BCC in BM is used to study timeline changes of PPT-I and TGF-β using northern analyses and a bioassay, respectively. The results show changes of both genes in BCCs and BM stroma. Relevance of these changes to homeostasis in BM is discussed. Myc has been shown to link the expressions of TGF-β1 and PPT-I in BCCs. We now show a role for Myc in the expression of NK1. PPT-I and the chemokine SDF-1α induce the expression of each other through an autocrine mechanism. Since a role for Myc in SDF-1α–PPT-I axis has not been studied, we speculate on this finding, based on the cell-homing property of SDF-1α. Since Myc could be oncogenic, it might be involved in the transforming properties of PPT-I and NK1 while SDF-1α could be involved in cell-homing of BCCs through the regulation of PPT-I. The findings are discussed in the context of other related reports.

Keywords: Preprotachykinin-1; Neurokinin receptors; Breast cancer; Metastasis; Myc; TGF-β

Contents

Abbreviations: NK, neurokinin; SP, substance P; PPT-I, preprotachykinin-1; BCC, breast cancer cell; BM, bone marrow; MSC, mesenchymal stem cell; LHSC, lymphohematopoietic stem cell; SDF-1, stromal-derived growth factor-1.

* Corresponding author. UMDNJ-New Jersey Medical School, MSB, Room E-579, 185 South Orange Avenue Newark, NJ 07103, United States. Tel.: +1 973 972 0625; fax: +1 973 972 8854.
E-mail address: rameshwa@umdnj.edu (P. Rameshwar).

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1. Introduction

In 2004, more than 211,000 women were diagnosed with breast cancer (BC), and more than 40,000 died of the disease (Jemal et al., 2005). Many were diagnosed with bone marrow (BM) metastases, which is associated with morbidity. Despite improved screening, early detection, and aggressive therapies, BC could resurge within the BM after 10–20 years of mastectomy (Karrison et al., 1999). Micrometastasis of BC cells (BCCs) in stages I–III BC patients have increased risk of relapse even with complete resection of the breast (Braun et al., 2000). It appears that adjuvant chemotherapy is ineffective at removing BC deposits to BM. This problem is further compounded by potential BM toxicity posed by the dose of chemotherapy (Pantel et al., 1999). BCCs located in specific regions of the BM appear to be in the quiescent phase of the cell cycle making them resistant to conventional DNA damaging agents (Rao et al., 2004). As such, studying BC at early stages prior to their integration in BM is important for targeted/preventive therapies. The cellular milieu of the BM cavity aids the survival of BCCs in BM and might also explain why the cancer cells do not disrupt the functions of resident lympho-hematopoietic stem cells, LHSC (Bonnet, 2002; Oh et al., 2004; Moharita et al., 2004).

The proclivity of particular cancer cells for bone has been the subject of extensive debate with Paget's classic "seed and soil" hypothesis and hemodynamic arguments comprising major opinions. However, mounting evidence indicates the presence of homing mechanisms that contribute to bone metastases. Borrowing from the observation that chemokine systems attract lymphocytes and hematopoietic cells to the BM, studies have identified important chemokine/chemokine receptor pairs that guide cancer cells to their metastatic site (Chambers et al., 2002; Mundy, 2002). For instance, the CXCR4-stromal-derived growth factor-1α (SDF-1α) axis has been implicated in the metastasis of BCCs to the BM (Muller et al., 2001; Liang et al., 2005).

The expression of various integrins and cellular adhesion molecules on metastatic cells has been implicated in metastasis to specific tissues (Chambers et al., 2002). New evidence suggests that neuroendocrine-related molecules may be important in the development of endocrine cancers, BC included (Moharita et al., 2004; Shingyoji et al., 2003). The neuroendocrine-related gene, preprotachykinin-1 (PPT-I) and receptors for its peptides, neurokinin-1 (NK1), NK2 and NK3, have been implicated in BC development and metastasis to BM (Moharita et al., 2004). The PPT-I gene encodes peptides belonging to the tachykinin family (Quartara and Maggi, 1997). PPT-I has seven exons and is alternately spliced into 4 transcripts: α, β, γ, and δ. Substance P (SP) and NK-A are the major peptides derived from the PPT-I gene (Rameshwar and Gascon, 1997). NK receptors are G-protein coupled, seven transmembrane receptors (Quartara and Maggi, 1997). SP shows preference for NK1 and lesser affinity to NK2 and NK3. NK1 and NK2 are widely distributed in non-neural cells, including mammary epithelial cells, BM stromal cells and hematopoietic cells (Greco et al., 2004). Previous studies report on SP involvement in tumorigenesis and metastasis to the BM (Singh et al., 2000; Rao et al., 2004). This study focuses on mechanisms by which PPT-I, as a gene is regulated. The goal is to elucidate mechanisms by which the PPT-I gene is involved in promoting tumorigenesis and metastasis.

2. Methods

2.1. Reagents, cytokines and antibodies

Fetal calf serum (FCS) was purchased from Hyclone Laboratories (Logan, UT). Non-immune rabbit IgG were purchased from Sigma (St. Louis, MO). TGF-β1 and rabbit anti-TGF-β were purchased from R&D Systems (Minneapolis, MN). Phycoerythrin (PE)-conjugated cytokeratin mAb, PE-rat anti mouse kappa and PE-CD14 mAb were purchased from BD Bioscience (San Jose, CA). Prolyl-4-hydroxylase mAb was purchased from Dako (Glostrup, Denmark). Dynabead-Epithelial and anti-fibroblasts were purchased from Dynal Biotech (Oslo, Norway) and Miltenyi Biotec (Auburn, CA), respectively.

2.2. Cells

BM stroma was cultured from BM aspirates of healthy individuals as described (Bandari et al., 2003a). The use of BM aspirates followed the guidelines of a protocol approved by the Institutional Review Board of UMDNJ-Newark Campus. At confluence, the non-adherent cells were removed and trypsin-sensitive cells passed at least five times. α-MEM (Sigma) served as the base medium for the stromal cultures. Stromal cells were >99% positive for anti-fibroblasts as determined by flow cytometry (Rameshwar et al., 2001). T47D BCCs and MCF12A non-tumorigenic cells were purchased from American Type Culture Collection (www.atcc.org). T47D cells knockdown for PPT-I was previously described (Rao et al., 2004). The knockdown cells were established by stable