Oral Oncology 48 (2012) 40–48

Contents lists available at SciVerse ScienceDirect

Oral Oncology

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

Secretion of IL-6 and IL-8 from lysophosphatidic acid-stimulated oral squamous cell carcinoma promotes osteoclastogenesis and bone resorption

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ARTICLE INFO

Article history:
Received 15 July 2011
Received in revised form 24 August 2011
Accepted 25 August 2011
Available online 16 September 2011

Keywords:
Lysophosphatidic acid
IL-6
IL-8
Osteoclastogenesis
Oral squamous cell carcinoma
RANKL

SUMMARY

Lysophosphatidic acid (LPA) is a bioactive lipid with a growth factor-like activity on a large range of cell types. Several pieces of evidence raise the possibility that LPA may play an important role in bone metastasis. Bone is a frequent metastatic site for oral cancer. However, the role of LPA in the progression of oral cancer metastasis to the bone is poorly understood. Here, we provide evidence for the role of LPA in the progression of oral cancer bone metastases and its regulatory mechanism. LPA induced the secretion of IL-6 and IL-8 in oral squamous cell carcinoma (OSCC). LPA-stimulated secretion of IL-6 and IL-8 is partly dependent on the LPA and EGF receptor (EGFR) pathways. ERK1/2 and Akt-mediated NF-κB and AP-1 were responsible for the LPA-induced IL-6 and IL-8 secretion. Moreover, conditioned medium (CM) derived from the LPA-stimulated OSCC supported osteoclast formation in bone marrow-derived macrophages (BMMs). Neutralization against both human IL-6 and IL-8 suppressed osteoclast formation induced by CM derived from the LPA-stimulated OSCC. Direct treatment with recombinant IL-6 (rIL-6) and/or soluble IL-6 receptor (sIL-6R), or IL-8 (rIL-8) reproduced the effect of the CM derived from the LPA-stimulated OSCC on osteoclast formation. In addition, CM derived from the LPA-stimulated OSCC induced receptor activator of nuclear factor (NF)-κB ligand (RANKL) expression in human osteoblasts and direct treatment with rIL-6 and/or sIL-6R or rIL-8 mimicked the effect of the CM derived from the LPA-stimulated OSCC for RANKL expression. Taken together, LPA may be a potent inducer of osteolytic factor IL-6 and IL-8 in OSCC. LPA-induced IL-6 and IL-8 exerted propound effects on RANKL expression in osteoblast and thereby promoted osteoclast formation from osteoclast precursors.

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Introduction

OSCC has a tendency to invade the maxillary and mandible bone and to cause bone damage. Despite the availability of advanced clinical diagnostic systems, a patient who has invasion into the maxillary and mandibular bone with oral cancer still remains an unresolved problem in surgery after massive facial injuries with bone loss. Consequently, it is necessary to elucidate the mechanism for bone invasion and metastasis of oral cancer. It has been reported that in osteolytic metastases, skeletal destruction is mediated primarily by osteoclasts rather than tumor cells.[1] The progression of bone metastasis requires the establishment of functional interactions between the cancer cells and bone cells. Bone destruction in patients with breast cancer metastasis is triggered by the local production of factors secreted by the tumor cells which stimulate osteoclast formation.[3,4] Tumor cells secrete parathyroid hormone-related protein (PTHrP), IL-1β, IL-6, IL-8 and IL-11, which induce the expression of RANK ligand (RANKL) in osteoblasts. The binding between RANKL and RANK, consequently enhances the proliferation and fusion of osteoclast precursors in the presence of M-CSF. As a result, osteolytic factors secreted from tumor cells enhance bone resorption by promoting osteoclastogenesis. Activated osteoclasts resorb bone, resulting in the release of factors such as TGF-β, embedded within the bone matrix. The release of TGF-β further stimulates breast cancer cells, which in turn stimulates more tumor-produced osteolytic factors. For bone metastasis, current treatments aimed to inhibit bone resorption only delay the progression of osteolytic lesions in metastatic patients.[5] Therefore, in addition to bone-derived growth factors, other endogenous sources of growth factors are probably involved in promoting tumor growth and metastasis to the skeletal bone. In this respect, LPA might be an important growth factor on the metastatic spreading of cancer in the bone.

Abbreviations: LPA, lysophosphatidic acid; OSCC, oral squamous cell carcinoma; RANKL, receptor activator of nuclear factor (NF)-κB ligand; CM, conditioned medium; TRAP, tartrate-resistant acid phosphatase.

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doi:10.1016/j.oraloncology.2011.08.022

LPA was first implicated in human carcinogenesis by the observation that LPA is present at elevated levels in the ascites of ovarian cancer patients.[6–8] It has been reported that LPA exhibits multiple cellular effects on proliferation, migration and survival of many cell types.[9] In animal models, the role of LPA in the progression of breast cancer bone metastasis has been reported.[10,11] Aggregation of human blood platelets upon thrombin activation is an important source of LPA, and bone-residing breast cancer cells induce platelet aggregation with the release of LPA from the activated platelets. Platelets-derived LPA then stimulates the skeletal tumor growth and cytokine-mediated bone destruction. However, the exact pathophysiological roles of LPA in OSCC-mediated bone destruction remains poorly understood.

Interleukin-6 (IL-6) and Interleukin-8 (IL-8) are known to influence osteoclast formation and bone resorption.[3,12] LPA stimulates production of IL-6 and IL-8 in ovarian cancer cell.[13] In the case of oral cancer, IL-8 was detected at higher concentrations in saliva and IL-6 was detected at higher concentrations in the serum of patients with OSCC, suggesting that IL-6 and IL-8 are potential biomarkers for oral cavity and oropharyngeal OSCC.[14] However, the mechanism of LPA-induced IL-6 and IL-8 secretion from OSCC and its regulation are unknown.

The aim of this study was to investigate the role of LPA in OSCC-mediated bone destruction. We provide experimental evidence showing that LPA, derived from an endogenous source in the tumor and bone environment, stimulated the production and secretion of both IL-6 and IL-8 from OSCC cells, and these cytokines enhanced osteoclast formation and bone destruction.

Materials and methods

Materials

Oleoyl-2-hydroxy-sn-3-glycerol-3-phosphate·Na (LPA) was purchased from BIOMOL (Plymouth Meeting, PA). Dulbecco's modified Eagle's medium/F12 (DMEM/F12) and fetal bovine serum (FBS) were purchased from Gibco BRL (Rockville, MD). Cholera toxin (CTX), hydrocortisone, insulin, transferrin and triiodothyronine (T3) from Sigma–Aldrich (St. Louis, MO). Pertussis toxin (PTX), PD98059, SB203580, SP600125, LY294002, AG1478 and PD158780 were purchased from Calbiochem (La Jolla, CA). Recombinant mouse RANKL was purchased from Koma Biotech Inc. (Seoul, Korea). Recombinant mouse macrophage-colony stimulating factor (M-CSF), Quantikine human IL-6 and CXCL8/IL-8 ELISA kit, recombinant human IL-6 and CXCL8/IL-8, neutralizing anti-hIL-6, anti-hCXCL8/IL-8 and anti-RANKL, recombinant human EGF were purchased from R&D System Inc. (Minneapolis, MN). Recombinant human sIL-6Rα was purchased from PEPROTECH (Rocky Hill, NJ). 3-[[[4-4[4-[[[1-(2-chlorophenyl)ethoxy]carbonyl]amino]-3-methyl-5-isoxazoly]phenyl]methyl]thio]propanoic acid (Ki16425) was purchased from Cayman Chemicals (Ann Arbor, MI). The following antibodies were purchased from their respective sources: total/phospho form of Akt, p38 MAPKs, ERK1/2, JNK and IKK (Cell Signaling Technology, Denver, MA); phosphor-EGFR (clone 3G3.2) and EGFR (MILLIPORE, Billerica, MA); p50, p65, Lamin C and normal rabbit IgG (Santa Cruz Biotechnology, CA).

Cell culture

YD-10B and HSC-3 OSCC cell lines were cultured in DMEM/F12 (3:1 ratio) medium supplemented with 10% FBS, 1×10^{-10} M CTX, 0.4 mg/ml hydrocortisone, 5 μg/ml insulin, 5 μg/ml transferring, and 2×10^{-11} M triiodothronine (T3) in a humidified atmosphere of 5% CO_2 at 37 °C. The human osteoblast hFOB1.19 cell line was cultured in the medium consisting of phenol red-free DMEM/F12 (1:1 ratio) with 10% FBS. Mouse BMMs were isolated from the tibiae of 4-week-old ICR mice by Histopaque density gradient centrifugation and were cultured in α-MEM containing 10% FBS and 30 ng/ml M-CSF in a humidified atmosphere of 5% CO_2 at 37 °C. For treatment of LPA, biological specific inhibitors or CM, cells were starved with serum-free media before addition.

RT-PCR analysis

Total RNA was isolated with the TRIzol® reagent and then single stranded cDNA was transcribed by priming with oligo-dT with Promega's reverse transcription system (Madison, WI). PCR amplification of the cDNA was done in a reaction mixture containing *Taq* polymerase (Takara, Shiga, Japan). The primer sequences, product sizes and annealing temperatures for each gene are shown in Table 1. PCR products were electrophoresed on a 2% agarose gel. The obtained bands were visualized with the Quantity One software and the Gel Doc 2000 system (Bio-Rad Laboratories, CA).

Table 1
Primer sequences, product sizes and annealing temperatures for RT-PCR.

Target gene	Primer sequence	Size of products (bp)	Annealing temp. (°C)
IL-6	Sence: 5′-CATCCTCGACGGCATCTCAGC-3′ Antisence: 5′-TTGGGTCAGGGGTGGTTATTG-3′	332	55
IL-8	Sence: 5′-ATGACTTCCAAGCTGGCCGT-3′ Antisence: 5′-CCTCTTCAAAAACTTCTCCACACC-3′	283	55
RNAKL	Sence: 5′-GCCAGTGGGAGATGTTAG-3′ Antisence: 5′-TTAGCTGCAAGTTTTCCC-3′	486	55
PTHrP	Sence: 5′-AACTCGCCTCCAACCTGCGC-3′ Antisence: 5′-CGCTCGGGACCTCCTCTGGT-3′	212	60
OPG	Sence: 5′-GGGGACCACAATGAACAAGTTG-3′ Antisence: 5′-AGCTTGCACCACTCCAAATCC-3′	409	57
IL-6R	Sence: 5′-TGAGCTCAGATATCGGGCTG-3′ Antisence: 5′-CGTCGTGGATGACACAGTCA-3′	855	60
CXCR1	Sence: 5′-TTTGTTTGTCTTGGCTGC-3′ Antisence: 5′-CCAAGAACTCCTTGCTGAC-3′	532	58
GAPDH	Sence: 5′-CCGCCTACTGCCCACTGCCACCAC-3′ Antisence: 5′-TCCATCCACTATGTCAGCAGGTCC-3′	420	55
LPA_1	Sence: 5′-GAGAGGCACATTACGGTTTTCC-3′ Antisence: 5′-CATTTCTTTGTCGCGGTAGGAG-3′	552	57
LPA_2	Sence: 5′-CATCATGCTTCCCCGACAACG-3′ Antisence: 5′-GGGCTTACCAAGGATACGCAG-3′	381	57
LPA_3	Sence: 5′-ATCATGGTTGTGGTGTACCT-3′ Antisence: 5′-GACTGCACCTTGGCTAATAC-3′	487	57

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