

Bisphosphonates suppress insulin-like growth factor 1-induced angiogenesis via the HIF-1 α /VEGF signaling pathways in human breast cancer cells

Xudong Tang^{1,2}, Qunzhou Zhang¹, Shihong Shi¹, Yun Yen³, Xiangyong Li², Yuefei Zhang⁴, Keyuan Zhou², Anh D. Le¹

¹Center for Craniofacial Molecular Biology, University of Southern California School of Dentistry, Los Angeles, CA

²Institute of Biochemistry and Molecular Biology, Guangdong Medical College, Zhanjiang, Guangdong 524023, People's Republic of China

³Department of Clinical and Molecular Pharmacology, City of Hope National Medical Center, Duarte, CA

⁴Department of Otorhinolaryngology, the First Affiliated Hospital of Guangdong Medical College, Zhanjiang, Guangdong 524023, People's Republic of China

Adjunctive chemotherapy with bisphosphonates has been reported to delay bone metastasis and improve overall survival in breast cancer. Aside from its antiresorptive effect, bisphosphonates exhibit antitumor activities, *in vitro* and *in vivo*, via several mechanisms, including antiangiogenesis. In this study, we investigated the potential molecular mechanisms underlying the antiangiogenic effect of non-nitrogen-containing and nitrogen-containing bisphosphonates, clodronate and pamidronate, respectively, in insulin-like growth factor (IGF)-1 responsive human breast cancer cells. We tested whether bisphosphonates had any effects on hypoxia-inducible factor (HIF)-1 α /vascular endothelial growth factor (VEGF) axis that plays a pivotal role in tumor angiogenesis, and our results showed that both pamidronate and clodronate significantly suppressed IGF-1-induced HIF-1 α protein accumulation and VEGF expression in MCF-7 cells. Mechanistically, we found that either pamidronate or clodronate did not affect mRNA expression of HIF-1 α , but they apparently promoted the degradation of IGF-1-induced HIF-1 α protein. Meanwhile, we found that the presence of pamidronate and clodronate led to a dose-dependent decrease in the newly-synthesized HIF-1 α protein induced by IGF-1 in breast cancer cells after proteasomal inhibition, thus, indirectly reflecting the inhibition of protein synthesis. In addition, our results indicated that the inhibitory effects of bisphosphonates on the HIF-1 α /VEGF axis are associated with the inhibition of the phosphoinositide 3-kinase/AKT/mammalian target of rapamycin signaling pathways. Consistently, we demonstrated that pamidronate and clodronate functionally abrogated both *in vitro* and *in vivo* tumor angiogenesis induced by IGF-1-stimulated MCF-7 cells. These findings have highlighted an important mechanism of the pharmacological action of bisphosphonates in the inhibition of tumor angiogenesis in breast cancer cells.

Breast cancer is the leading cancer affecting millions of women worldwide with aggressive osteolytic bone metastases in the advanced diseases^{1,2} and longstanding morbidity or skeletal complications, including bone pain, pathological fracture, hypercalcemia, spinal cord or nerve root compression syndrome.³

Bisphosphonates are synthetic analogs of inorganic pyrophosphate, containing a phosphorus-carbon-phosphorus (P-C-P) backbone and variable side chains that determine the specific potency for inhibition of bone resorption.^{4,5} Bisphosphonates that lack a nitrogen functional group in the R2 side chain (such as clodronate) condense with an

Key words: bisphosphonates, breast cancer, IGF-1, HIF-1 α , VEGF, PI-3K/Akt, angiogenesis

Abbreviations: 4E-BP1: eukaryotic initiation factor 4E (eIF)-binding protein 1; CHX: cycloheximide; HIF-1: hypoxia-inducible factor-1; HUVEC: human umbilical vascular endothelial cells; IGF-1: insulin-like growth factor-1; mTOR: mammalian target of rapamycin; p70S6K: phosphorylated MW. 70,000 ribosomal protein S6 kinase; PI-3K: phosphoinositide 3-kinase; VEGF: vascular endothelial growth factor. Additional Supporting Information may be found in the online version of this article.

The first two authors contributed equally to this work.

Grant sponsor: National Institute of Health Research; **Grant numbers:** 1S11 AR47359, R03 CA128099; **Grant sponsor:** Oral and Maxillofacial Surgery Foundation Research Support; **Grant number:** OMSF002894; **Grant sponsor:** National Natural Science Foundation of China; **Grant numbers:** 30672741, 30872944

DOI: 10.1002/ijc.24710

History: Received 18 Mar 2009; Accepted 22 Jun 2009; Online 30 Jun 2009

Correspondence to: Anh D. Le, Division of Surgical, Therapeutic and Bioengineering Sciences, Center for Craniofacial Molecular Biology, University of Southern California School of Dentistry, Health Sciences Campus, 2250 Alcazar Street, CSA103, Los Angeles, California 90033, USA, Fax: +323-442-2981, E-mail: anhle@usc.edu; or Keyuan Zhou, Institute of Biochemistry and Molecular Biology, Guangdong Medical College, Zhanjiang, Guangdong 524023, People's Republic of China, E-mail: kyz@gdmc.edu.cn