Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Fluorescence-guided surgery of prostate cancer bone metastasis

Shinji Miwa, MD, PhD,[a,b,c] *Yasunori Matsumoto, MD,*[b] *Yukihiko Hiroshima, MD, PhD,*[a,b] *Shuya Yano, MD,*[a,b] *Fuminari Uehara, MD,*[a,b] *Mako Yamamoto, PhD,*[a,b] *Yong Zhang, MD,*[a] *Hiroaki Kimura, MD, PhD,*[c] *Katsuhiro Hayashi, MD, PhD,*[c] *Norio Yamamoto, MD, PhD,*[c] *Michael Bouvet, MD,*[b,*] *Naotoshi Sugimoto, MD, PhD,*[d] *Hiroyuki Tsuchiya, MD, PhD,*[c] *and Robert M. Hoffman, PhD*[a,b]

[a] *AntiCancer, Inc, San Diego, California*
[b] *Department of Surgery, University of California, San Diego, San Diego, California*
[c] *Department of Orthopedic Surgery, Kanazawa University Graduate School of Medical Sciences, Kanazawa, Ishikawa, Japan*
[d] *Department of Physiology, Kanazawa University Graduate School of Medical Sciences, Kanazawa, Ishikawa, Japan*

ARTICLE INFO

Article history:
Received 12 February 2014
Received in revised form
16 March 2014
Accepted 16 May 2014
Available online 23 May 2014

Keywords:
Prostate cancer
Bone metastasis
GFP
Fluorescence-guided surgery
Bright-light surgery
Nude mice
Zoledronic acid

ABSTRACT

Background: The aim of this study is to investigate the effectiveness of fluorescence-guided surgery (FGS) of prostate cancer experimental skeletal metastasis.

Materials and methods: Green fluorescent protein-expressing PC-3 human prostate cancer cells (PC-3-green fluorescent protein) were injected into the intramedullary cavity of the tibia in 32 nude mice. After 2 wk, 16 of the mice underwent FGS; the other 16 mice underwent bright-light surgery (BLS). Half of BLS and FGS mice (8 mice in each group) received zoledronic acid (ZOL). Weekly fluorescence imaging of the mice was performed. Six weeks after surgery, metastases to lung and inguinal lymph node were evaluated by fluorescence imaging.

Results: The percentage of residual tumor after BLS and FGS was $9.9 \pm 2.2\%$ and $0.9 \pm 0.3\%$, respectively ($P < 0.001$). FGS reduced recurrent cancer growth compared with BLS ($P < 0.005$). Although FGS alone had no significant effect on inguinal lymph node metastases, lung metastasis or disease-free survival (DFS), ZOL in combination with FGS significantly increased DFS ($P = 0.01$) in comparison with the combination of BLS and ZOL. ZOL reduced lymph node metastases ($P = 0.033$) but not lung metastasis.

Conclusions: FGS significantly reduced recurrence of experimental prostate cancer bone metastasis compared with BLS. The combination of FGS and ZOL increased DFS over BLS and ZOL. ZOL inhibited lymph node metastasis but not lung metastasis.

* *Corresponding author*. Department of Surgery, Moores UCSD Cancer Center, 3855 Health Science Drive #0987, La Jolla, CA 92093-0987. Tel.: +1 858 822 6191; fax: +1 858 822 6192.
E-mail address: mbouvet@ucsd.edu (M. Bouvet).
0022-4804/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2014.05.049

1. Introduction

Bone metastasis is a devastating outcome of many types of cancer including prostate. The revolution in small-animal imaging enabled by fluorescent proteins has enabled imaging of bone metastasis in real time [1]. In an orthotopic model of H460-green fluorescent protein (GFP) human lung cancer, metastases were visualized by GFP fluorescence in the contralateral lung, plural membrane, and widely throughout the skeletal system including the skull, vertebra, femur, tibia, pelvis, and bone marrow of the femur and tibia [2].

In-mouse models of GFP-expressing LOX human and B16 mouse melanoma, extensive bone and bone marrow metastases of B16F0 were visualized by GFP expression when the animals were sacrificed 3 wk after cell implantation. Metastases for both cell lines were visualized in many organs, including the brain, lung, pleural membrane, liver, kidney, adrenal gland, lymph nodes, skeleton, muscle, and skin by GFP fluorescence [3].

Noninvasive imaging could also visualize bone metastasis throughout the skeleton in nude mice in models of cancer-expressing GFP. Imaging was with either a trans-illuminated epifluorescence microscope or a fluorescence light box and thermoelectrically cooled color charge-coupled device camera. The depth to which metastasis and micrometastasis could be imaged depended on their size [4].

In an orthotopic model of PC-3 human prostate cancer cells green fluorescent protein (PC-3-GFP) human prostate cancer, many metastases were visualized throughout the skeleton, including the skull, rib, pelvis, femur, and tibia. The central nervous system, including the brain and spinal cord, was also involved with tumor, as visualized by GFP

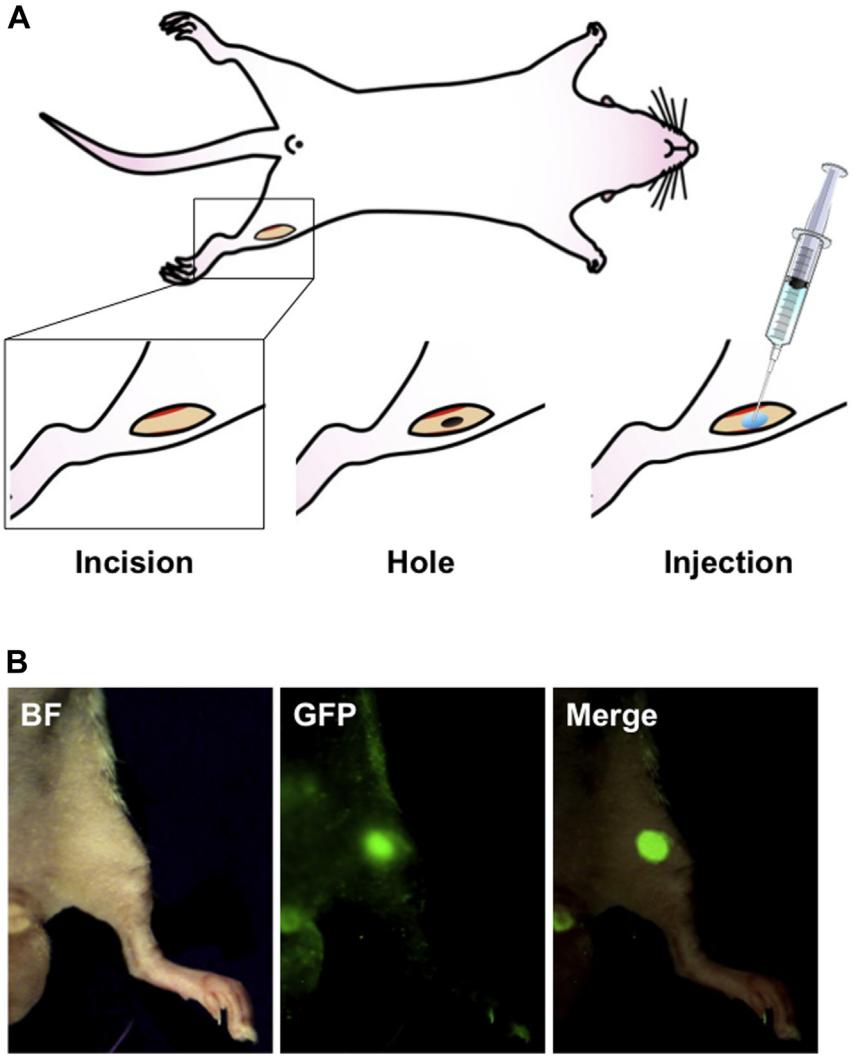

Fig. 1 – Orthotopic mouse model of human prostate cancer experimental metastasis to bone. (A) Implantation of PC-3-GFP human prostate cancer cells in tibia. The tibial tuberosity was exposed and a hole was made for the implantation of cancer cells. GFP-expressing PC-3 human prostate cancer cells, combined with an equal volume of Matrigel, were then injected into the intramedullary cavity of the left tibia. (B) Two weeks after implantation of PC-3-GFP cells, the fluorescent tumors were observed with the OV100 Small Animal Imaging System. (Color version of figure is available online.)