MOLECULAR ONCOLOGY 7 (2013) 428–439

available at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/molonc

Plumbagin, a medicinal plant (*Plumbago zeylanica*)-derived 1,4-naphthoquinone, inhibits growth and metastasis of human prostate cancer PC-3M-luciferase cells in an orthotopic xenograft mouse model

Bilal Bin Hafeez[a,*], *Weixiong Zhong*[b], *Joseph W. Fischer*[a], *Ala Mustafa*[a], *Xudong Shi*[c], *Louise Meske*[a], *Hao Hong*[d], *Weibo Cai*[d], *Thomas Havighurst*[e], *KyungMann Kim*[e], *Ajit K. Verma*[a]

[a]*Department of Human Oncology, Paul Carbone Comprehensive Cancer Center, School of Medicine and Public Health, University of Wisconsin, Madison, WI 53792, USA*
[b]*Department of Pathology, Paul Carbone Comprehensive Cancer Center, School of Medicine and Public Health, University of Wisconsin, Madison, WI 53792, USA*
[c]*Department of Surgery, Paul Carbone Comprehensive Cancer Center, School of Medicine and Public Health, University of Wisconsin, Madison, WI 53792, USA*
[d]*Department of Radiology, Wisconsin Institute for Medical Research, Paul Carbone Comprehensive Cancer Center, School of Medicine and Public Health, University of Wisconsin, Madison, WI 53792, USA*
[e]*Department of Biostatistics & Medical Informatics, Paul Carbone Comprehensive Cancer Center, School of Medicine and Public Health, University of Wisconsin, Madison, WI 53792, USA*

ARTICLE INFO

Article history:
Received 3 December 2012
Accepted 4 December 2012
Available online 14 December 2012

Keywords:
Plumbagin
Prostate cancer
Orthotopic xenograft model

ABSTRACT

We present here first time that Plumbagin (PL), a medicinal plant-derived 1,4-naphthoquinone, inhibits the growth and metastasis of human prostate cancer (PCa) cells in an orthotopic xenograft mouse model. In this study, human PCa PC-3M-luciferase cells (2×10^6) were injected into the prostate of athymic nude mice. Three days post cell implantation, mice were treated with PL (2 mg/kg body wt. i.p. five days in a week) for 8 weeks. Growth and metastasis of PC-3M-luciferase cells was examined weekly by bioluminescence imaging of live mice. PL-treatment significantly ($p = 0.0008$) inhibited the growth of orthotopic xenograft tumors. Results demonstrated a significant inhibition of metastasis into liver ($p = 0.037$), but inhibition of metastasis into the lungs ($p = 0.60$) and lymph nodes ($p = 0.27$) was not observed to be significant. These results were further confirmed by histopathology of these organs. Results of histopathology demonstrated a significant inhibition of metastasis into lymph nodes ($p = 0.034$) and lungs ($p = 0.028$), and a trend to significance in liver ($p = 0.075$). None of the mice in the PL-treatment group showed PCa metastasis into the liver, but these mice had small metastasis foci into the lymph nodes and lungs. However, control mice had large metastatic foci into the lymph nodes, lungs, and liver. PL-caused inhibition of the growth and metastasis of PC-3M cells accompanies inhibition of the expression of: 1) PKCε, pStat3Tyr705, and pStat3Ser727, 2) Stat3

Abbreviations: PL, plumbagin; PCa, prostate cancer; PKCε, protein kinase C epsilon; Stat3, signal transducers and activators of transcription 3.

* *Corresponding author*. Tel.: +1 608 262 1744; fax: +1 608 262 6654.
E-mail addresses: indianbilal@yahoo.com, hafeez@humonc.wisc.edu (B.B. Hafeez).

1574-7891/$ – see front matter Published by Elsevier B.V. on behalf of Federation of European Biochemical Societies.
http://dx.doi.org/10.1016/j.molonc.2012.12.001

downstream target genes (survivin and Bcl_{xL}), 3) proliferative markers Ki-67 and PCNA, 4) metastatic marker MMP9, MMP2, and uPA, and 5) angiogenesis markers CD31 and VEGF. Taken together, these results suggest that PL inhibits tumor growth and metastasis of human PCa PC3-M-luciferase cells, which could be used as a therapeutic agent for the prevention and treatment of human PCa.

Published by Elsevier B.V. on behalf of Federation of European Biochemical Societies.

1. Introduction

Prostate cancer (PCa) continues to remain the most common cancer and the second leading cause of cancer-related deaths in American men. The American Cancer Society has predicted that a total of 241,740 new cases of PCa will be diagnosed and 28,170 deaths will occur from it in the United States alone in the year of 2012 (Siegel et al., 2012). Although PCa is frequently curable in its early stage by surgical or radiation therapy, many patients present locally advanced or metastatic disease for which there are currently no curative treatment option (Albertsen, 2008; So et al., 2005). Therefore, there is an urgent need for agents, which are effective and selective, in the prevention and/or treatment of PCa metastasis.

PL is a quinoid (5-hydroxy-2-methyl-1,4-napthoquinone) constituent isolated from the roots of the medicinal plant *Plumbago zeylanica* L. (also known as Chitrak) (Sandur et al., 2006). PL has also been found in *Juglans regia* (English Walnut), *Juglans cinerea* (butternut and white walnut) and *Juglans nigra* (blacknut) (Sandur et al., 2006). The root of *P. zeylanica* has been used in traditional Indian and Chinese systems of medicine for more than 2500 years for the treatment of various types of ailments (Sandur et al., 2006). PL has been shown for its potential health benefits including neuroprotective (Son et al., 2010) and anti-cancer property against various types of cancers (Padhye et al., 2012 and references therein). PL, fed in the diet (200 ppm), inhibits azoxymethane-induced intestinal tumors in rats (Courboulin et al., 2012). PL inhibits ectopic growth of human breast cancer (Sugie et al., 1998), non-small cell lung cancer (Hsu et al., 2006), melanoma (Wang et al., 2008), and ovarian (Sinha et al., 2012) cells in athymic nude mice. We have shown that PL inhibits ultraviolet radiation-induced development of squamous cell carcinomas (Ravindra et al., 2009). We including others have also reported its apoptosis inducing and growth inhibitory effects against pancreatic cancer (Hafeez et al., 2012a; Lai et al., 2012) and PCa (Aziz et al., 2007a; Gomathinayagam et al., 2008; Powolny and Singh, 2008) cells. Recently, we have reported that PL inhibits prostate tumor growth in transgenic adenocarcinoma of mouse prostate (TRAMP) mice (Hafeez et al., 2012b). However, no study has demonstrated anti-metastasis potential of PL against human PCa. We present in this communication, for the first time, that PL administration inhibits metastatic growth of human PCa PC-3M-luciferase cells in an orthotopic xenograft mouse model. PL-caused inhibition of the growth and metastasis of PC-3M-luciferase cells accompanies inhibition of the expression of PKCε, pStat3-Tyr705, and pStat3Ser727, Stat3 downstream target genes (survivin and Bcl_{xL}), proliferative markers (Ki-67 and PCNA), metastatic markers (MMP2, MMP9 and uPA), angiogenesis markers (CD31 and VEGF) and induction of iNOS expression.

2. Materials and methods

2.1. Antibodies

Monoclonal or polyclonal antibodies specific for actin, CD31, E-Cadherin, iNOS, Ki-67, MMP9, MMP2, PKCε, PCNA, survivin, total Stat3, uPA, and VEGF were purchased from Santa Cruz Biotechnology, (Santa Cruz, CA). Monoclonal antibodies specific for pStat3Tyr705, and pStat3Ser727 were obtained from BD Biosciences (San Jose, CA). PL (Practical grade, purity: 99.80% HPLC, molecular weight: 188.18) was purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Orthotopic xenograft

PC-3M-luciferase cells were obtained from Caliper Life Sciences (Hopkinton, MA). Six weeks old male athymic nude mice, purchased from Harlan Laboratory (Madison, WI), were housed under pathogen-free environment with a 12 h light/12 h dark schedule and fed with an autoclaved diet and water *ad libitum*. To establish orthotopic xenografts in mice, PC-3M-luciferase cells (2.0×10^6) were suspended in 20 μl of HBSS media and directly implanted into the anterior lobe of the prostate. Three days later, 16 mice were randomly divided into two groups. One group of mice ($n = 8$) was treated with an i.p. injection of PL (2 mg/kg body weight in 0.1 ml PBS, once a day and 5 days per week for 8 weeks). Control mice ($n = 8$) were treated the same with vehicle (0.1 ml PBS). The mice were maintained at the AAALAC-accredited Animal Resources Facility of the University of Wisconsin. All of the protocols were approved by the University's Research Animal Resources Committee in accordance with the NIH Guideline for the Care and Use of Laboratory Mice.

2.3. Bioluminescence imaging

Mice of both the groups were imaged weekly using an IVIS Spectrum scanner (formerly Caliper Life Sciences now PerkinElmer, Waltham, MA). In brief, 200 μl of substrate D-luciferin (3.0 mg) in PBS was injected i.p. in each mouse at 10 min prior to imaging. Images were quantified and normalized by using vendor software (Living Image® 4.0). Regions-of-interest (ROI) of the same size and shape were used for all mice throughout the study. The bioluminescence images were quantified by measuring the total photons over the prostate region and the average photon flux within the ROI were presented as photons/second/cm^2/sr (sr denotes steradian). To determine the metastasis into the distant organs, entire excised organs (liver, lymph nodes, lungs, and bone) were kept in 6-well plates, imaged and quantified as described above.

Download English Version:

https://daneshyari.com/en/article/2145713

Download Persian Version:

https://daneshyari.com/article/2145713

Daneshyari.com