Biochemical and Biophysical Research Communications 506 (2018) 912–917

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Metabolic targeting with recombinant methioninase combined with palbociclib regresses a doxorubicin-resistant dedifferentiated liposarcoma

Kentaro Igarashi [a, b, c], Kei Kawaguchi [a, b], Tasuku Kiyuna [a, b], Kentaro Miyake [a, b], Masuyo Miyaki [a, b], Norio Yamamoto [c], Katsuhiro Hayashi [c], Hiroaki Kimura [c], Shinji Miwa [c], Takashi Higuchi [a, b, c], Arun S. Singh [d], Bartosz Chmielowski [d], Scott D. Nelson [e], Tara A. Russell [f], Mark A. Eckardt [g], Sarah M. Dry [e], Yunfeng Li [e], Shree Ram Singh [h, ****], Sant P. Chawla [i, *****], Fritz C. Eilber [f, ***], Hiroyuki Tsuchiya [c, **], Robert M. Hoffman [a, b, *]

[a] AntiCancer, Inc, San Diego, CA, USA
[b] Department of Surgery, University of California, San Diego, CA, USA
[c] Department of Orthopaedic Surgery, Kanazawa University, Kanazawa, Japan
[d] Division of Hematology-Oncology, University of California, Los Angeles, CA, USA
[e] Department of Pathology, University of California, Los Angeles, CA, USA
[f] Division of Surgical Oncology, University of California, Los Angeles, CA, USA
[g] Department of Surgery, Yale University School of Medicine, New Haven, CT, USA
[h] Basic Research Laboratory, National Cancer Institute, Frederick, MD, USA
[i] Sarcoma Oncology Center, 2811 Wilshire Blvd, Suite 414, Santa Monica, CA, 90403, USA

ARTICLE INFO

Article history:
Received 12 October 2018
Accepted 19 October 2018
Available online 2 November 2018

Keywords:
Liposarcoma
Doxorubicin
Resistant
PDOX
Methioninase
rMETase
Palbociclib
Combination
Efficacy

ABSTRACT

Liposarcoma is the most common type of soft tissue sarcoma. Among the subtypes of liposarcoma, dedifferentiated liposarcoma (DDLPS) is recalcitrant and has the lowest survival rate. The aim of the present study is to determine the efficacy of metabolic targeting with recombinant methioninase (rMETase) combined with palbociclib (PAL) against a doxorubicin (DOX)-resistant DDLPS in a patient-derived orthotopic xenograft (PDOX) model. A resected tumor from a patient with recurrent high-grade DDLPS in the right retroperitoneum was grown orthotopically in the right retroperitoneum of nude mice to establish a PDOX model. The PDOX models were randomized into the following groups when tumor volume reached 100 mm^3: G1, control without treatment; G2, DOX; G3, PAL; G4, recombinant methioninase (rMETase); G5, PAL combined with rMETase. Tumor length and width were measured both pre- and post-treatment. On day 14 after initiation, all treatments significantly inhibited tumor growth compared to the untreated control except DOX. PAL combined with rMETase was significantly more effective than both DOX, rMETase alone, and PAL alone. Combining PAL and rMETase significantly regressed tumor volume on day 14 after initiation of treatment and was the only treatment to do so. The relative body weight on day 14 compared with day 0 did not significantly differ between each treatment group. The results of the present study indicate the powerful combination of rMETase and PAL should be tested clinically against DDLPS in the near future.

Published by Elsevier Inc.

* Corresponding author. AntiCancer, Inc, 7917 Ostrow Street, San Diego, CA, 92111, USA.
** Corresponding author. Department of Orthopaedic Surgery, Graduate School of Medicine, Kanazawa University, 13-1 Takara-machi, Kanazawa, 920-8641, Japan.
*** Corresponding author. Division of Surgical Oncology, UCLA, 10833 LeConte Avenue, Rm 54-140 CHS, Los Angeles, CA, 90095-1782, USA.
**** Corresponding author. Basic Research Laboratory, National Cancer Institute, Frederick, MD, 21702, USA.
***** Corresponding author. Sarcoma Oncology Center, 2811 Wilshire Blvd, Suite 414, Santa Monica, CA, 90403, USA.
E-mail addresses: singhshr@mail.nih.gov (S.R. Singh), santchawla@gmail.com (S.P. Chawla), fceilber@mednet.ucla.edu (F.C. Eilber), tsuchi@med.kanazawa-u.ac.jp (H. Tsuchiya), all@anticancer.com (R.M. Hoffman).

https://doi.org/10.1016/j.bbrc.2018.10.119
0006-291X/Published by Elsevier Inc.

1. Introduction

Liposarcoma (LPS) is the most common type of soft-tissue sarcoma (STS). It consists of about 15%–25% of all STS. Histologically, LPS is classified as either well-differentiated/atypical lipomatous tumors, pleomorphic, myxoid/round cell, or de-differentiated. About 40% of the LPS are well-differentiated and 10% develop into de-differentiated liposarcoma (DDLPS) [1]. DDLPS is most common in the retroperitoneum. DDLPS contains the genomic amplification of the 12q13-15 chromosomal region of the *mouse double minute 2 homolog* (*MDM2*) gene [2,3]. Recently, Asano et al. [4] found that receptor tyrosine kinase (RTK) genes were amplified in approximately one-third of DDLPS. DDLPS has the lowest survival rate among all LPS [5] and often recurs or metastasizes due to incomplete resection and resistance to radiation, or first-line chemotherapy with doxorubicin (DOX) [5–7]. Small-molecule inhibitors of MDM2 and cyclin-dependent kinase 4 (CDK4) were shown to be a treatment option for DDLPS in Phase I and II clinical trials [8–10] with limited efficacy alone on unresectable DDLPS. Therefore, transformative individualized therapy is necessary against DDLPS.

We have developed the patient-derived orthotopic xenograft (PDOX) mouse model of cancer for discovery of transformative individualized therapy for recalcitrant cancer [11]. Our laboratory pioneered the PDOX nude mouse model with the technique of surgical orthotopic implantation (SOI) for all major cancers [12–25]. The PDOX model, developed by us, has many advantages over subcutaneous-transplant models [11,26].

Cancer cells have an elevated requirement for methionine (MET) compared to normal cells. This phenomenon is termed MET dependence [27]. MET restriction arrests tumor growth and induces a selective S/G_2-phase cell-cycle block of cancer cells *in vitro* and *in vivo* [28–32].

Recent studies suggest that MET dependence is duet to excessive use of MET for aberrant transmethylation reactions, termed the Hoffman effect [33–38], analogous to the Warburg effect for glucose in cancer [39]. The excessive and aberrant use of MET in cancer is shown in the clinic in [^{11}C]MET PET imaging, where high uptake of [^{11}C]MET results in a very strong and selective tumor signal compared to normal tissue background. [^{11}C]MET is better than [^{18}C]fluorodeoxyglucose (FDG) for PET imaging [40], suggesting MET dependence is highly cancer-specific compared to glucose dependence [41–43]. Lien et al. [44] have shown that oncogenic *PIK3CA (phosphoinositide-3-kinase, catalytic, alpha polypeptide)* promotes MET and cysteine utilization in breast cancer cells by inhibiting the cystine transporter. Further, dietary modulation of MET can alter the levels of histone methylation [45,46]. Recently, Dai et al. [47] found no change in the location of histone H3 lysine 4 trimethylation (H3K4me3) peaks under MET restriction but found that MET restriction altered the response of H3K4me3 peak width and its biology [47].

Previous studies have demonstrated that MET the cleaving enzyme, methioninase (METase), purified from *Pseudomonas putida (P. putida),* is an effective antitumor agent [48–51]. For the large-scale production of METase, the gene from *P. putida* was cloned in *Escherichia coli* (*E. coli*) and a purification protocol for recombinant METase (rMETase) has been established with high purity and low endotoxin [52–57]. Recently, we found that oral-METase (o-METase) is superior to injectable rMETase against acquired GEM resistance in pancreatic cancer (59).

Palbociclib (PAL), a CDK 4/6 inhibitor [59–61], had clinical efficacy for several tumor types [10,62–66]. PAL in combination with letrozole or fulvestran was recently approved by the US Food and Drug Administration (FDA) for breast cancer [59,66].

The present report demonstrates the efficacy of rMETase combined with palbociclib on the PDOX model of DOX-resistant DDLPS.

2. Materials and methods

2.1. Mice

Athymic *nu/nu* nude mice (AntiCancer Inc., San Diego, CA), 4–6 weeks old, were used in this study. Animal housing, their diet, surgical procedures, and imaging were performed as previously described [23–25]. The response of animals during surgery was monitored to ensure adequate depth of anesthesia. The animals were observed daily and humanely sacrificed by CO_2 inhalation if they met the humane endpoint criteria as described in our previous publication [23–25]. All animal studies were performed in accordance with the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Animals under Assurance Number A3873-1.

2.2. Patient-derived tumor

A 69-year-old male with DDLPS of the right retroperitoneum underwent radical resection with en bloc right nephrectomy. Two years after surgery, the DDLPS recurred locally and the patient underwent surgical resection at Department of Surgery, University of California, Los Angeles (UCLA). Written informed consent was obtained from the patient as part of a UCLA Institutional Review Board (IRB #10–001857)-approved protocol.

2.3. Surgical orthotopic implantation (SOI) for establishment of the PDOX model of DDLPS

A fresh sample of the tumor of the patient was obtained and transported immediately to the laboratory at AntiCancer, Inc., on wet ice [23–25]. The sample was cut into 5-mm fragments and implanted subcutaneously in nude mice. Subcutaneously-grown tumors were harvested and cut into small fragments (3–4 mm). After nude mice were anesthetized, a 20-mm skin incision was made on the left flank, and then the obliquus externus abdominis muscle was split to reach the retroperitoneum. A single tumor fragment was implanted orthotopically into the space between the left kidney and retroperitoneal fat tissue to establish a PDOX model. The wound was closed with 6-0 nylon suture (Ethilon, Ethicon, Inc., NJ, USA) [23–25].

2.4. rMETase production

The pAC-1 rMETase high expression clone was used for rMETase production. The fermentation procedure for host *E. coli* cells and the purification protocol for rMETase was as previously described: rMETase was purified by 3 different steps using columns of DEAE Sepharose FF and Sephacryl S-200HR, and ActiClean Etox, which is designed for eliminating endotoxin [57].

2.5. Treatment study design for the DDLPS PDOX

DDLPS PDOX mouse models were randomized into 5 groups of 8 mice each: G1, control without treatment; G2, DOX (3 mg/kg, intraperitoneal [i.p.] injection, weekly, for 2 weeks); G3, PAL (100 mg/kg, oral administration [p.o.], daily, for 2 weeks); G4, rMETase (100 unit/mouse, i.p., daily, for 2 weeks); G5, PAL, 100 mg/kg, p.o., daily, for 2 weeks, combined with rMETase, 100 unit/mouse, i.p., daily, for 2 weeks (Fig. 1). Tumor length and width were measured both pre- and post-treatment. Tumor volume was calculated as described [23–25]. Data are presented as mean ± SD. The tumor volume ratio is defined as described [23–25].

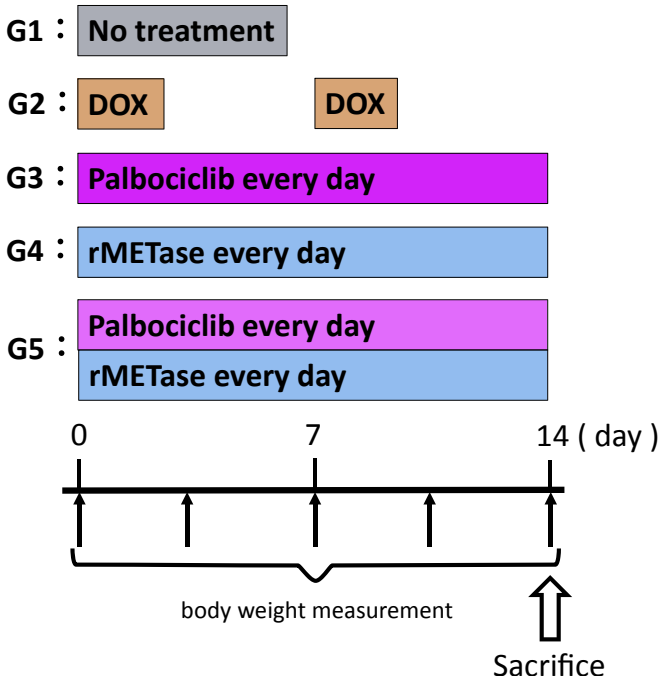

Fig. 1. Treatment schema.

2.6. Effect of treatment on tumor histology

All experimental protocols and data were collected as described [23–25]. Fresh tumor samples were fixed in 10% formalin and embedded in paraffin before sectioning and staining. Tissue sections (3 μm) were deparaffinized in xylene and rehydrated in an ethanol series. Hematoxylin and eosin (H&E) staining was performed according to standard protocol. Histological examination was performed with a BHS system microscope. Images were acquired with INFINITY ANALYZE software (Lumenera Corporation, Ottawa, Canada).

2.7. Statistical analysis

SPSS statistics version 21.0 was used for all statistical analyses (IBM, New York City, NY). Significant differences for continuous variables were determined using the Student's t-test. Both line graphs and bar graphs expressed mean values and error bars show standard deviation (SD). A probability value of P was calculated between a control group and each treatment group. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of treatment on tumor growth

On day 14 after treatment initiation, all treatments significantly inhibited tumor growth compared to untreated control except DOX: (DOX: $p = 0.76$; rMETase: $p = 0.05$; PAL: $p < 0.05$; PAL combined with rMETase $p<0.001$) on day 14 after initiation. PAL combined with rMETase was significantly more effective than either DOX ($p < 0.001$), rMETase alone ($p < 0.01$) or PAL alone ($p < 0.01$). The combination of PAL and rMETase significantly regressed tumor volume on day 14 after initiation of treatment ($p = 0.01$) (Fig. 2A and B) and was the only treatment to do so.

3.2. Effect of treatment on body weight

The relative body weight on day 14 compared with day 0 did not significantly differ between any treatment group (Fig. 3).

3.3. Effect of treatment on tumor histology

High-power microscopy of the original patient tumor demonstrated spindle cells with hyperchromatic, enlarged nuclei. Mitotic figures and atypical cells are present (Fig. 4A). High-power microscopy of the untreated PDOX tumor showed similar features including spindle cells with hyperchromatic and enlarged nuclei. Mitotic figures, including atypical forms are also present (Fig. 4B). PDOX tumors treated with DOX were comprised of viable cells without apparent necrosis or inflammatory changes (Fig. 4C). PDOX tumors treated with rMETase show changes in cancer cell shape and necrosis (Fig. 4D). PDOX tumors treated with PAL show changes in cancer cell shapes and fibrosis (Fig. 4E). PDOX tumors treated with PAL combined with rMETase show extensive necrosis (Fig. 4F).

4. Discussion

DDLPS is one of the most lethal malignancies with lowest survival rate among all types of LPS [5] and often recurs or metastasizes because of lack of curative treatments. Therefore, transformative individualized therapy is needed for DDLPS. In the present study, we show that a combination of rMETase and PAL is effective against DDLPS. The strong efficacy of the PAL-rMETase combination is important and the mechanism will be further studied in the future.

Bollard et al. [65] found that PAL inhibited human liver-cancer cell growth by promoting a reversible cell-cycle arrest and was effective either alone or in combination with sorafenib against hepatocellular carcinoma (HCC) [65]. PAL in combination with fulvestrant significantly enhanced progression-free survival compared to fulvestrant alone in patient with hormone receptor positive, human epidermal growth factor receptor 2 (HER2)–advanced breast cancer (ABC) progressing during prior endocrine therapy [67]. In a randomized Phase 2 clinical trial, Finn et al. [66] reported that PAL together with letrozole works as a first-line treatment of patients with advanced, oestrogen receptor-positive and HER2-negative breast cancer. Haines at al [68]. reported that PAL together with the mitogen-activated protein kinase (MEK) inhibitor PD0325901 effectively inhibit Kras-mutant non-small cell lung cancer (NSCLC) with enhanced progression-free survival compared to animals treated with either drug alone. PAL inhibited the growth of DOX-resistant PDOX model of Ewing's sarcoma [69].

Liu et al. [70] reported that deprivation of MET and cystine (Cys) together inhibited the growth of glioma cells via inducing ROS and autophagy pathways. Li et al. [71] found hyaluronic acid-modified polyamidoamine dendrimer G5-entrapped gold nanoparticles delivering METase strongly inhibited gastric cancer tumor growth by disrupting the mitochondrial function of cluster of differentiation 44 (CD44)-positive gastric cancer stem cell. Recently, we reported that combining o-rMETase and caffeine with first-line chemotherapy DOX regressed the DOX-resistant synovial sarcoma (SS) in a PDOX model [72]. rMETase together with gemcitabine (GEM) was significantly more effective compared to either agent alone in a GEM-resistance PDOX model of pancreatic cancer [58]. rMETase in combination with DOX could overcome first-line DOX-resistance in a PDOX model of undifferentiated spindle cell sarcoma (USCS) [24].

PAL targets CDK4/6, which plays a role in the cell cycle [25,69]. MET restriction, such as with rMETase, results in a cancer-cell selective S/G_2 phase cell-cycle block [22,73,74] that may play an

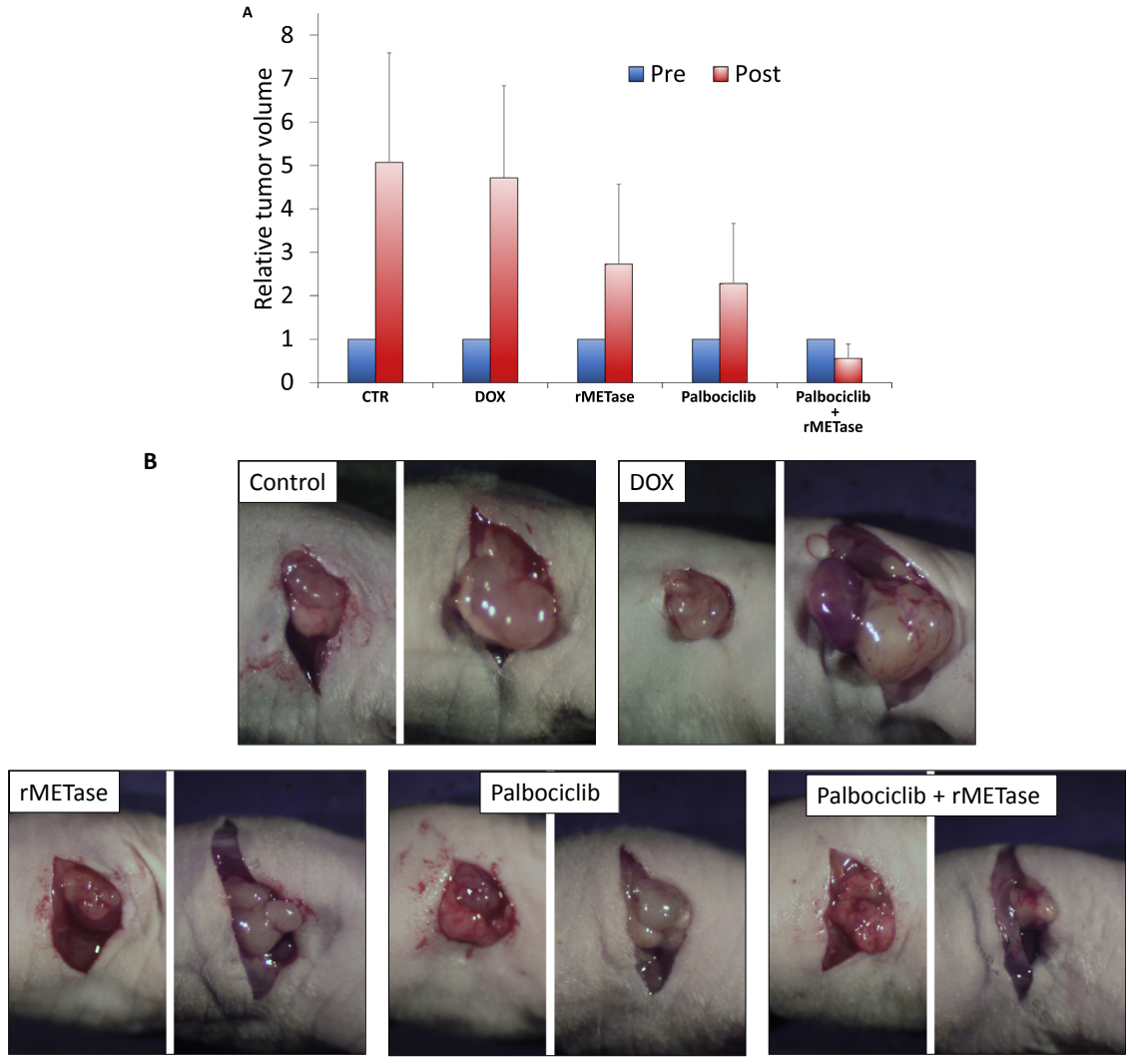

Fig. 2. (A) Quantitative treatment efficacy of the DDLPS PDOX models. Bar graphs show tumor volume measurements at pre- and post-treatment. N = 8 mice/group. (B) Photos of representative DDLPS PDOX mouse models, before (left panels) and after (right panels) treatment.

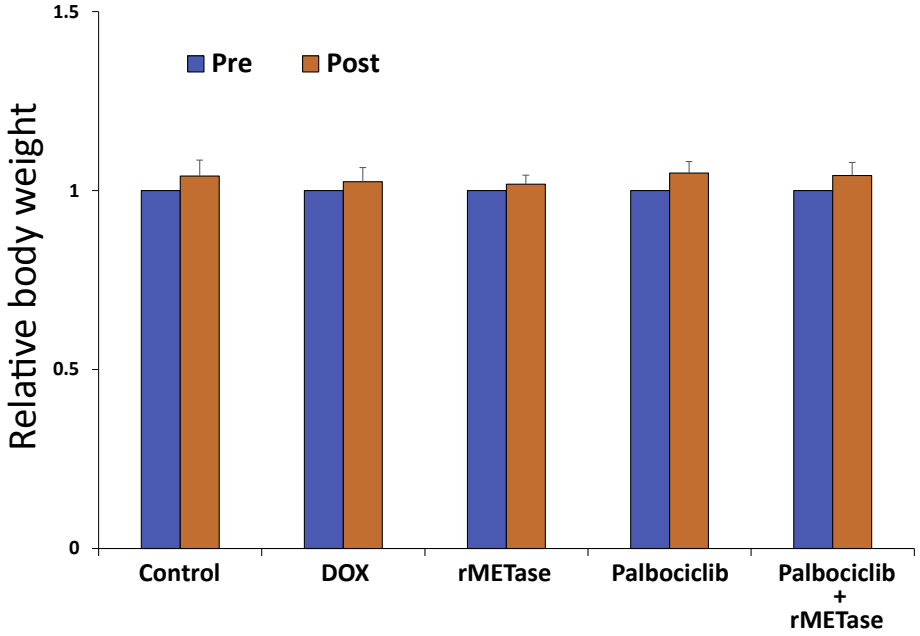

Fig. 3. Effect of treatments on body weight of the DDLPS PDOX mouse models. Bar graphs show body weight in each group at pretreatment and 2 weeks after initiation of drug administration.

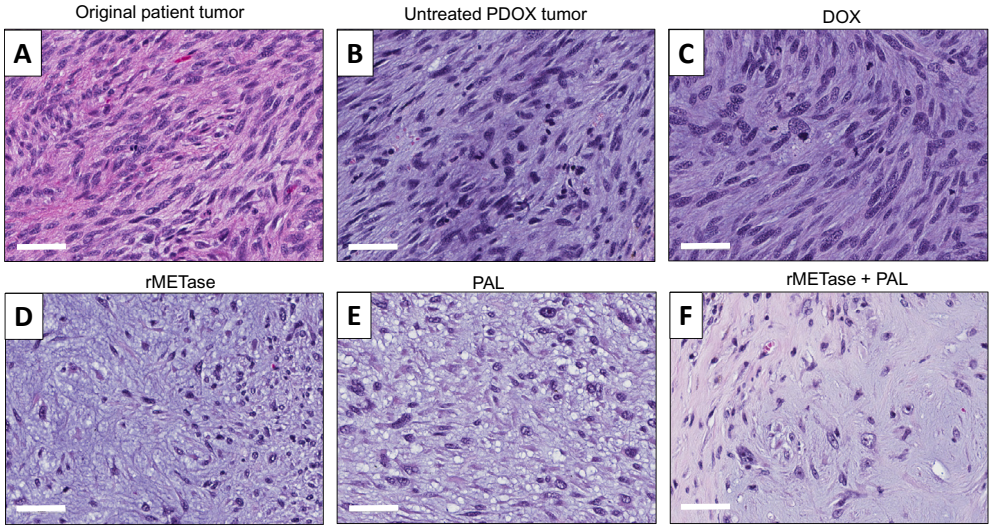

Fig. 4. Effect of treatment on the DDLPS PDOX models. H&E-stained section of the original patient tumor (A), untreated PDOX tumor (B), PDOX tumor treated with DOX (C), PDOX tumor treated with rMETase (D) and PDOX tumor treated with PAL (E) and PDOX tumor treated with both rMETase and PAL. White scale bars: 50 μm.

important role in combination with PAL, to effect tumor-regression observed in the present study. In summary, the results of the present study suggest that the combination of rMETase and PAL could be developed clinically.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest

The authors declare that there are no potential conflicts of interest.

Acknowledgement

This paper is dedicated to the memory of A. R. Moossa, M.D., and Sun Lee, M.D.

References

[1] A. Crago, S. Singer, Clinical and molecular approaches to well-differentiated and dedifferentiated liposarcoma, Curr. Opin. Oncol. 23 (2011) 373–378.
[2] M.B. Binh, X. Sastre-Garau, L. Guillou, G. de Pinieux, et al., MDM2 and CDK4 immunostainings are useful adjuncts in diagnosing well-differentiated and dedifferentiated liposarcoma subtypes: a comparative analysis of 559 soft tissue neoplasms with genetic data, Am. J. Surg. Pathol. 29 (10) (2005) 1340–1347.
[3] S. Lokka, A.H. Scheel, S. Dango, K. Schmitz, et al., Challenging dedifferentiated liposarcoma identified by MDM2-amplification, a report of two cases, BMC Clin. Pathol. 28 (2014) 14–36.
[4] N. Asano, A. Yoshida, S. Mitani, E. Kobayashi, et al., Frequent amplification of receptor tyrosine kinase genes in welldifferentiated/dedifferentiated liposarcoma, Oncotarget 8 (8) (2017) 12941–12952.
[5] R.L. Jones, C. Fisher, O. Al-Muderis, et al., Differential sensitivity of liposarcoma subtypes to chemotherapy, Eur. J. Canc. 41 (2005) 2853–2860.
[6] J.A. Livingston, D. Bugano, A. Barbo, H. Lin, et al., Role of chemotherapy in dedifferentiated liposarcoma of the retroperitoneum: defining the benefit and challenges of the standard, Sci. Rep. 7 (1) (2017), 11836.
[7] A. Italiano, et al., Advanced well-differentiated/dedifferentiated liposarcomas: role of chemotherapy and survival, Ann. Oncol. 23 (2011) 1601–1607.
[8] J.J. Luke, D.R. D'Adamo, M.A. Dickson, M.L. Keohan, et al., The cyclin-dependent kinase inhibitor flavopiridol potentiates doxorubicin efficacy in advanced sarcomas: preclinical investigations and results of a phase I dose-escalation clinical trial, Clin. Canc. Res. 18 (2012) 2638–2647.
[9] I. Ray-Coquard, J.Y. Blay, A. Italiano, A. Le Cesne, et al., Effect of the MDM2 antagonist RG7112 on the P53 pathway in patients with MDM2-amplified, well-differentiated or dedifferentiated liposarcoma: an exploratory proof-of-mechanism study, Lancet Oncol. 13 (2012) 1133–1140.
[10] M.A. Dickson, G.K. Schwartz, M.L. Keohan, S.P. D'Angelo, et al., Progression-free survival among patients with well-differentiated or dedifferentiated liposarcoma treated with CDK4 inhibitor palbociclib: a phase 2 clinical trial, JAMA Oncol 2 (7) (2016) 937–940.
[11] R.M. Hoffman, Patient-derived orthotopic xenografts: better mimic of metastasis than subcutaneous xenografts, Nat. Rev. Canc. 15 (2015) 451–452.
[12] X. Fu, P. Le, R.M. Hoffman, A metastatic-orthotopic transplant nude mouse model of human patient breast cancer, Anticancer Res. 13 (1993) 901–904.
[13] X. Fu, R.M. Hoffman, Human ovarian carcinoma metastatic models constructed in nude mice by orthotopic transplantation of histologically-intact patient specimens, Anticancer Res. 13 (1993) 283–286.
[14] X. Wang, X. Fu, R.M. Hoffman, A new patient-like metastatic model of human lung cancer constructed orthotopically with intact tissue via thoracotomy in immunodeficient mice, Int. J. Canc. 51 (1992) 992–995.
[15] Y. Hiroshima, Y. Zhang, N. Zhang, et al., Establishment of a patient derived orthotopic xenograph (PDOX) model of HER-2-positive cervical cancer expressing the clinical metastatic pattern, PloS One 10 (2015), e0117417.
[16] T. Murakami, T. Kiyuna, K. Kawaguchi, et al., The irony of highly effective bacterial therapy of a patient-derived orthotopic xenograft (PDOX) model of Ewing's sarcoma, which was blocked by Ewing himself 80 years ago, Cell Cycle 16 (2017) 1046–1052.
[17] X. Fu, J.M. Besterman, A. Monosov, et al., Models of human metastatic colon cancer in nude mice orthotopically constructed by using histologically intact patient specimens, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 9345–9349.
[18] C.A. Metildi, S. Kaushal, G.A. Luiken, et al., Fluorescently-labeled chimeric anti-CEA antibody improves detection and resection of human colon cancer in a patient-derived orthotopic xenograft (PDOX) nude mouse model, J. Surg. Oncol. 109 (2014) 451–458.
[19] T. Furukawa, T. Kubota, M. Watanabe, et al., Orthotopic transplantation of histologically intact clinical specimens of stomach cancer to nude mice: correlation of metastatic sites in mouse and individual patient donors, Int. J. Canc. 53 (1993) 608–612.
[20] X. Fu, F. Guadagni, R.M. Hoffman, A metastatic nude-mouse model of human pancreatic cancer constructed orthotopically with histologically intact patient specimens, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 5645–5649.
[21] Y. Hiroshima, A.A. Maawy, M.H. Katz, et al., Selective efficacy of zoledronic acid on metastasis in a patient-derived orthotopicxenograph (PDOX) nude-mouse model of human pancreatic cancer, J. Surg. Oncol. 111 (2015) 311–315.
[22] K. Igarashi, K. Kawaguchi, T. Kiyuna, et al., Patient-derived orthotopic xenograft (PDOX) mouse model of adult rhabdomyosarcoma invades and recurs after resection in contrast to the subcutaneous ectopic model, Cell Cycle 16 (2017) 91–94.
[23] K. Kawaguchi, T. Higuchi, S. Li, Q. Han, et al., Combination therapy of tumor-targeting Salmonella typhimurium A1-R and oral recombinant methioninase regresses a BRAF-V600E-negative melanoma, Biochem. Biophys. Res. Commun. 503 (4) (2018) 3086–3092.
[24] K. Igarashi, K. Kawaguchi, S. Li, Q. Han, et al., Recombinant methioninase in combination with doxorubicin (DOX) overcomes first-line DOX resistance in a patient-derived orthotopic xenograft nude-mouse model of undifferentiated spindle-cell sarcoma, Cancer Lett. 417 (2018) 168–173.
[25] T. Kiyuna, Y. Tome, T. Murakami, K. Kawaguchi, et al., Trabectedin arrests a doxorubicin-resistant PDGFRA-activated liposarcoma patient-derived orthotopic xenograft (PDOX) nude mouse model, BMC Canc. 18 (1) (2018) 840.

[26] R.M. Hoffman, in: W.B. Coleman, G.J. Tsongalis (Eds.), Patient-derived Mouse Models of Cancer, Humana Press, New York, 2017.
[27] R.M. Hoffman, Development of recombinant methioninase to target the general cancer-specific metabolic defect of methionine dependence: a 40-year odyssey, Expet Opin. Biol. Ther. 15 (2015) 21–31.
[28] H. Guo, V.K. Lishko, H. Herrera, et al., Therapeutic tumor-specific cell cycle block induced by methionine starvation *in vivo*, Cancer Res. 53 (1993) 5676–5679.
[29] R.M. Hoffman, S.J. Jacobsen, Reversible growth arrest in simian virus 40-transformed human fibroblasts, Proc. Natl. Acad. Sci. U. S. A. 77 (1980) 7306–7310.
[30] D.M. Kokkinakis, M.A. von Wronski, T.H. Vuong, et al., Regulation of O6-methylguanine-DNA methyltransferase by methionine in human tumour cells, Br. J. Canc. 75 (1997) 779–788.
[31] D.M. Kokkinakis, S.C. Schold Jr., H. Hori, et al., Effect of long-term depletion of plasma methionine on the growth and survival of human brain tumor xenografts in athymic mice, Nutr. Canc. 29 (1997) 195–204.
[32] I.R. Miousse, J. Tobacyk, C.M. Quick, A. Jamshidi-Parsian, et al., Modulation of dietary methionine intake elicits potent, yet distinct, anticancer effects on primary versus metastatic tumors, Carcinogenesis 39 (9) (2018) 1117–1126.
[33] R.M. Hoffman, R.W. Erbe, High *in vivo* rates of methionine biosynthesis in transformed human and malignant rat cells auxotrophic for methionine, Proc. Natl. Acad. Sci. U.S.A. 73 (1976) 1523–1527.
[34] P.H. Stern, J.O. Mecham, C.D. Wallace, R.M. Hoffman, Reduced free-methionine in methionine-dependent SV40-transformed human fibroblasts synthesizing apparently normal amounts of methionine, J. Cell. Physiol. 117 (1983) 9–14.
[35] P.H. Stern, C.D. Wallace, R.M. Hoffman, Altered methionine metabolism occurs in all members of a set of diverse human tumor cell lines, J. Cell. Physiol. 119 (1984) 29–34.
[36] R.M. Hoffman, Altered methionine metabolism, DNA methylation and oncogene expression in carcinogenesis. A review and synthesis, Biochim. Biophys. Acta 738 (1984) 49–87.
[37] D.W. Coalson, J.O. Mecham, P.H. Stern, R.M. Hoffman, Reduced availability of endogenously synthesized methionine for S-adenosylmethionine formation in methionine-dependent cancer cells, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 4248–4251.
[38] P.H. Stern, R.M. Hoffman, Elevated overall rates of transmethylation in cell lines from diverse human tumors, In Vitro 20 (1984) 663–670.
[39] O. Warburg, On the origin of cancer cells, Science 123 (1956) 309–314.
[40] K. Palanichamy, K. Thirumoorthy, S. Kanji, N. Gordon, et al., Methionine and kynurenine activate oncogenic kinases in glioblastoma, and methionine deprivation compromises proliferation, Clin. Canc. Res. 22 (14) (2016) 3513–3523.
[41] R.M. Hoffman, Is DNA methylation the new guardian of the genome? Mol. Cytogenet. 10 (2017) 11.
[42] R.M. Hoffman, The wayward methyl group and the cascade to cancer, Cell Cycle 16 (2017) 825–829.
[43] W. Xu, L. Gao, A. Shao, et al., The performance of 11C-Methionine PET in the differential diagnosis of glioma recurrence, Oncotarget 8 (2017) 91030–91039.
[44] E.C. Lien, L. Ghisolfi, R.C. Geck, J.M. Asara, A. Toker, Oncogenic PI3K promotes methionine dependency in breast cancer cells through the cystine-glutamate antiporter xCT, Sci. Signal. 10 (510) (2017) pii: eaao6604.
[45] N. Shiraki, et al., Methionine metabolism regulates maintenance and differentiation of human pluripotent stem cells, Cell Metabol. 19 (2014) 780–794.
[46] S.J. Mentch, M. Mehrmohamadi, L. Huang, X. Liu, et al., Histone methylation dynamics and gene regulation occur through the sensing of one-carbon metabolism, Cell Metabol. 22 (5) (2015) 861–873.
[47] Z. Dai, S.J. Mentch, X. Gao, S.N. Nichenametla, J.W. Locasale, Methionine metabolism influences genomic architecture and gene expression through H3K4me3 peak width, Nat. Commun. 9 (1) (2018) 1955.
[48] V.K. Lishko, O.V. Lishko, R.M. Hoffman, The preparation of endotoxin-free L-methionine-alpha-deamino-gamma-mercaptomethane-lyase (L-methioninase) from Pseudomonas putida, Protein Expr. Purif. 4 (1993) 529–533.
[49] V.K. Lishko, O.V. Lishko, R.M. Hoffman, Depletion of serum methionine by methioninase in mice, Anticancer Res. 13 (1993) 1465–1468.
[50] Y. Tan, J. Zavala Sr., M. Xu, et al., Serum methionine depletion without side effects by methioninase in metastatic breast cancer patients, Anticancer Res. 16 (1996) 3937–3942.
[51] Y. Tan, J. Zavala Sr., Q. Han, et al., Recombinant methioninase infusion reduces the biochemical endpoint of serum methionine with minimal toxicity in high-stage cancer patients, Anticancer Res. 17 (1997) 3857–3860.
[52] Y. Tan, M. Xu, X. Tan, et al., Overexpression and large-scale production of recombinant L-methionine-alpha-deamino-gamma-mercaptomethane-lyase for novel anticancer therapy, Protein Expr. Purif. 9 (1997) 233–245.
[53] H. Inoue, K. Inagaki, M. Sugimoto, et al., Structural analysis of the L-methionine gamma-lyase gene from Pseudomonas putida, J. Biochem. 117 (1995) 1120–1125.
[54] H. Hori, K. Takabayashi, L. Orvis, et al., Gene cloning and characterization of Pseudomonas putida L-methionine-alpha-deamino-gamma-mercapto-methane-lyase, Cancer Res. 56 (1996) 2116–2122.
[55] T. Takakura, T. Ito, S. Yagi, et al., High-level expression and bulk crystallization of recombinant L-methionine γ-lyase, an anticancer agent, Appl. Microbiol. Biotechnol. 70 (2006) 183–192.
[56] T. Takakura, A. Takimoto, Y. Notsu, et al., Physicochemical and pharmacokinetic characterization of highly potent recombinant L-methionine γ-lyase conjugated with polyethylene glycol as an antitumor agent, Cancer Res. 66 (2006) 2807–2814.
[57] D. Kudou, S. Misaki, M. Yamashita, et al., Structure of the antitumour enzyme L-methionine γ-lyase from Pseudomonas putida at 1.8Å resolution, J. Biochem. 141 (2007) 535–544.
[58] K. Kawaguchi, K. Miyake, Q. Han, S. Li, et al., Oral recombinant methioninase (o-rMETase) is superior to injectable rMETase and overcomes acquired gemcitabine resistance in pancreatic cancer, Cancer Lett. 432 (2018) 251–259.
[59] N.C. Turner, J. Ro, F. André, S. Loi, S. Verma, et al., Palbociclib in hormone-receptor-positive advanced breast cancer, N. Engl. J. Med. 373 (3) (2015) 209–219.
[60] C.J. Sherr, D. Beach, G.I. Shapiro, Targeting CDK4 and CDK6: from discovery to therapy, Cancer Discov. 6 (4) (2016) 353–367.
[61] K.T. Flaherty, et al., Phase I, dose-escalation trial of the oral cyclin-dependent kinase 4/6 inhibitor PD 0332991, administered using a 21-day schedule in patients with advanced cancer, Clin. Canc. Res. 18 (2012) 568–576.
[62] G.K. Schwartz, P.M. LoRusso, M.A. Dickson, S.S. Randolph, M.N. Shaik, K.D. Wilner, R. Courtney, P.J. O'Dwyer, Phase I study of PD 0332991, a cyclin-dependent kinase inhibitor, administered in 3-week cycles (Schedule 2/1), Br. J. Canc. 104 (12) (2011) 1862–1868.
[63] J.P. Leonard, et al., Selective CDK4/6 inhibition with tumor responses by PD0332991 in patients with mantle cell lymphoma, Blood 119 (2012) 4597–4607.
[64] U. Asghar, A.K. Witkiewicz, N.C. Turner, E.S. Knudsen, The history and future of targeting cyclin-dependent kinases in cancer therapy, Nat. Rev. Drug Discov. 14 (2) (2015) 130–146.
[65] J. Bollard, V. Miguela, M. Ruiz de Galarreta, et al., Palbociclib (PD-0332991), a selective CDK4/6 inhibitor, restricts tumour growth in preclinical models of hepatocellular carcinoma, Gut 66 (7) (2017) 1286–1296.
[66] R.S. Finn, J.P. Crown, I. Lang, K. Boer, et al., The cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with letrozole versus letrozole alone as first-line treatment of oestrogen receptor-positive, HER2-negative, advanced breast cancer (PALOMA-1/TRIO-18): a randomised phase 2 study, Lancet Oncol. 16 (1) (2015) 25–35.
[67] A. Rocca, R. Maltoni, S. Bravaccini, C. Donati, D. Andreis, Clinical utility of fulvestrant in the treatment of breast cancer: a report on the emerging clinical evidence, Cancer Manag. Res. 10 (2018) 3083–3099.
[68] E. Haines, T. Chen, N. Kommajosyula, Z. Chen, G.S. Herter-Sprie, L. Cornell, K.K. Wong, G.I. Shapiro, Palbociclib resistance confers dependence on an FGFR-MAP kinase-mTOR-driven pathway in KRAS-mutant non-small cell lung cancer, Oncotarget 9 (60) (2018) 31572–31589.
[69] T. Murakami, A.S. Singh, T. Kiyuna, et al., Effective molecular targeting of CDK4/6 and IGF-1R in a rare FUS-ERG fusion CDKN2A-deletion doxorubicin-resistant Ewing's sarcoma in a patient-derived orthotopic xenograft (PDOX) nude-mouse model, Oncotarget 7 (2016) 47556–47564.
[70] H. Liu, W. Zhang, K. Wang, X. Wang, et al., Methionine and cystine double deprivation stress suppresses glioma proliferation via inducing ROS/autophagy, Toxicol. Lett. 232 (2) (2015) 349–355.
[71] Y.F. Li, H.T. Zhang, L. Xin, Hyaluronic acid-modified polyamidoamine dendrimer G5-entrapped gold nanoparticles delivering METase gene inhibits gastric tumor growth via targeting CD44+ gastric cancer cells, J. Canc. Res. Clin. Oncol. 144 (8) (2018) 1463–1473.
[72] T. Higuchi, K. Kawaguchi, K. Miyake, Q. Han, Y. Tan, H. Oshiro, N. Sugisawa, Z. Zhang, S. Razmjooei, N. Yamamoto, K. Hayashi, H. Kimura, et al., Oral recombinant methioninase combined with caffeine and doxorubicin induced regression of a doxorubicin-resistant synovial sarcoma in a PDOX mouse model, Anticancer Res. 38 (10) (2018) 5639–5644.
[73] S. Yano, S. Li, Q. Han, et al., Selective methioninase-induced trap of cancer cells in S/G_2 phase visualized by FUCCI imaging confers chemosensitivity, Oncotarget 5 (2014) 8729–8736.
[74] S. Yano, K. Takehara, M. Zhao, et al., Tumor-specific cell-cycle decoy by *Salmonella typhimurium* A1-R combined with tumor-selective cell-cycle trap by methioninase overcome tumor intrinsic chemoresistance as visualized by FUCCI imaging, Cell Cycle 15 (2016) 1715–1723.