Acta Med. Okayama, 2017
Vol. 71, No. 2, pp. 135–142

Acta Medica Okayama
http://escholarship.lib.okayama-u.ac.jp/amo/

Original Article

The Downregulation of the Expression of CD147 by Tumor Suppressor REIC/Dkk-3, and Its Implication in Human Prostate Cancer Cell Growth Inhibition

Akihiro Mori[a], Masami Watanabe[a,b]*, Takuya Sadahira[a], Yasuyuki Kobayashi[a], Yuichi Ariyoshi[a], Hideo Ueki[a], Koichiro Wada[a], Kazuhiko Ochiai[c], Shun-Ai Li[a,b], and Yasutomo Nasu[a]

[a]*Department of Urology, Okayama University, Graduate School of Medicine, Denistry and Pharmacentical Sciences,*
[b]*Center for Innovative Clinical Medicine, Okayama University Hospital, Okayama 700-8558, Japan,*
[c]*Department of Veterinary Nursing and Technology, Nippon Veterinary and Life Science University, Tokyo 180-8602, Japan*

The cluster of differentiation 147 (CD147), also known as EMMPRIN, is a key molecule that promotes cancer progression. We previously developed an adenoviral vector encoding a tumor suppressor REIC/Dkk-3 gene (Ad-REIC) for cancer gene therapy. The therapeutic effects are based on suppressing the growth of cancer cells, but, the underlying molecular mechanism has not been fully clarified. To elucidate this mechanism, we investigated the effects of Ad-REIC on the expression of CD147 in LNCaP prostate cancer cells. Western blotting revealed that the expression of CD147 was significantly suppressed by Ad-REIC. Ad-REIC also suppressed the cell growth of LNCaP cells. Since other researchers have demonstrated that phosphorylated mitogen-activated protein kinases (MAPKs) and c-Myc protein positively regulate the expression of CD147, we investigated the correlation between the CD147 level and the activation of MAPK and c-Myc expression. Unexpectedly, no positive correlation was observed between CD147 and its possible regulators, suggesting that another signaling pathway was involved in the downregulation of CD147. This is the first study to show the downregulation of CD147 by Ad-REIC in prostate cancer cells. At least some of the therapeutic effects of Ad-REIC may be due to the downregulation of the cancer-progression factor, CD147.

Key words: prostate cancer, REIC/Dkk-3, CD147, cell growth, p38 MAP kinase

Prostate cancer is a common disease in the male population of many developed countries, including the United States [1]. In the considerable number of cases of significant prostate cancer, the cancer's progression is often accompanied by a decreased quality of life, and death occurs early [1,2]. The current therapeutic strategies, including radical prostatectomy, androgen deprivation therapy, radiation, and cytotoxic chemotherapy, often allow recurrence and fail to stop the progression of the disease [2]. Thus, novel and effective therapeutic agents are needed to treat recurrent progressive prostate cancer.

CD147 (also known as EMMPRIN) is a cell membrane glycoprotein that was found to be the most frequently upregulated mRNA and protein in metastatic cells isolated from the bone marrow of cancer patients [3]. The protein is reported to be overexpressed in many solid tumors, including prostate cancer, indicating a key role of CD147 in the processes of tumorigenesis,

Received September 5, 2016; accepted November 10, 2016.
*Corresponding author. Phone: +81-86-235-7287; Fax: +81-86-231-3986
E-mail: mwcorrespondence@gmail.com (M. Watanabe)

Conflict of Interest Disclosures: No potential conflict of interest relevant to this article was reported.

invasion and metastasis [3-10]. CD147 is highly expressed on the surface of cancer cells and stimulates adjacent fibroblasts or cancer cells to produce matrix metalloproteinases, facilitating the invasion of the cancer cells [3]. The CD147 expression levels of prostate cancer patients have been reported to be higher than those in their normal counterparts and the elevated expression of CD147 is significantly correlated with histopathological malignancy, cancer progression and an unfavorable prognosis [4-6].

It was also reported that CD147 stimulates the expression of vascular endothelial growth factor and hyaluronan, which leads to angiogenesis, anchorage-independent growth and multidrug resistance [3]. These findings– based on both clinical studies and basic research– clearly indicate that CD147 is an essential molecule in cancer progression and is therefore a very attractive target for anti-cancer treatment [3].

The reduced expression in immortalized cell (REIC) gene is identical to Dickkopf-3 (Dkk-3), and the expression of the REIC/Dkk-3 gene is significantly downregulated in a broad range of human cancer cells [11-13]. REIC/Dkk-3 is a tumor suppressor gene and is thought to be useful for gene therapy for human malignant tumors. We therefore developed adenoviral vector agents encoding a tumor suppressor REIC/Dkk-3 gene (Ad-REIC) for cancer gene therapy [12, 13]. Ad-REIC induces cancer cell-specific apoptosis in prostate cancer cells [13].

It was reported that the induction of apoptosis is dependent on the activation of c-Jun-NH2-kinase (JNK) by cellular endoplasmic reticulum (ER) stress signaling [12, 13]. Accumulating evidence indicates that the therapeutic effects of Ad-REIC are based on the induction of apoptosis, the suppression of cell growth and the inhibition of invasion in various cancer cell types [11-13]. However, the molecular mechanism underlying the therapeutic effects of Ad-REIC has not been fully clarified.

CD147 is a key molecule that promotes cancer cell growth, invasion and metastasis [3-10]. To elucidate the molecular mechanism of Ad-REIC-based gene therapy, we investigated the effects of Ad-REIC treatment on the expression of CD147 and other molecules in prostate cancer cells.

Materials and Methods

Cells and cell culture. Three Human prostate cancer cell lines (PC-3, LNCaP, and Du145) and a cervical cancer cell line (HeLa) were obtained from the American Type Culture Collection (Rockville, MD, USA). A mouse prostate cancer cell line (RM9) was kindly provided by Professor T.C. Thompson (The University of Texas, M.D. Anderson Cancer Center, Houston, TX, USA). The cells were grown in RPMI-1640 medium (Sigma, St. Louis, MO, USA) or in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% (v/v) fetal bovine serum (FBS), penicillin (100 IU/ml), and streptomycin (100 μg/ml). Human normal prostate epithelial cells (PrEC) were purchased from Lonza (Basel, Switzerland) and cultivated using the medium recommended by the supplier [13].

The generation and expansion of adenoviral vectors carrying REIC/Dkk-3 (Ad-REIC). For the development of the Ad-REIC vectors, pShuttle-SGE-REIC plasmids were digested with the restriction enzymes I-*Ceu*I and PI-*Sce*I and inserted into the Adeno-X Viral DNA (Clontech Laboratories, Mountain View, CA, USA), as described [14, 15]. Briefly, the recombinant adenoviral DNA with the full-length human REIC/Dkk-3 gene was linearized by digestion with *Pac*I and transfected into HEK293 cells. At 7-10 days after transfection, the HEK293 cells were harvested, and a viral solution was obtained by 3 freeze/thaw cycles. The recovered virus solution was used to propagate sufficient viruses in HEK293 cells for further studies. All of the virus particles were purified by CsCl density gradient ultracentrifugation and stored at −80°C.

An adenovirus vector carrying the LacZ gene (Ad-LacZ) was used as a control, as described [13, 14]. The adenoviral vectors were produced using replication-defective adenoviruses of serotype 5.

Western blotting. Western blotting was conducted as described [11, 14, 15]. The cells were seeded in flat-bottom six-well plates, cultured for 24 h in complete medium and then sampled. In the other experiments, LNCaP cells(5.0×10^5 cells) were treated with Ad-LacZ or Ad-REIC at 100 multiplicity of infection (MOI) in 0.5 ml of complete medium for 1 h. Then, 1.5 ml of fresh medium was added and the cells were cultured for 24 h. The floating dead cells were eliminated and the attached cells were lysed with lysis buffer

on ice. After the insoluble fragments were removed by centrifugation, the extracted proteins were separated on a 7.5% SDS-PAGE gel and transferred onto a polyvinylidene fluoride membrane (PVDF membranes; Millipore, Billerica, MA, USA).

Following the transfer, the PVDF membranes were blocked for 1 h with 5% nonfat milk powder, 6% glycine and 0.1% Tween-20 in Tris buffered saline (TBS). The membranes were incubated for 1 h at room temperature with the primary antibodies; CD147 (EPR4052, abcam, Cambridge, UK), REIC/Dkk-3 (mouse monoclonal, raised in our laboratory), and the following antibodies from Cell Signaling Technology (Danvers, MA): Phospho-p38 MAPK (cat.#4511), Phospho-p44/42 MAPK (Erk1/2) (#4370), Phospho-JNK (#9251), Phospho-c-Jun (#2361), c-Myc (#9402), Phospho-GSK-3β (#9323) and β-Actin (#4967). After three washes in TBS supplemented with 0.1% Tween-20, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. The bound antibodies were visualized by the enhanced chemiluminescence detection method (ECL kit, Amersham Pharmacia Biotech, Chandler, AZ, USA) using medical X-ray film [11].

The in vitro cell growth assay. LNCaP human prostate cancer cells (5.0×10^5 cells) were seeded in flat-bottom six-well plates and cultured for 24 h. The cells were treated with Ad-LacZ or Ad-REIC at 100 MOI in 0.5 ml of complete medium for 1 h, and 1.5 ml of fresh medium was then added and the cells were incubated for 24 h. Following the elimination of floating dead cells by medium exchange, the attached cells were detached by trypsin. The number of cells was then determined with a disposable hemocytometer.

Statistical analysis. The data are shown as the mean ± standard deviation. The unpaired Student's *t*-test was used to analyze the significance of differences between pairs of groups. *P*-values < 0.05 were considered significant.

Results

The expression of CD147 in normal prostate epithelial cells and prostate cancer cells. We examined the expression of CD147 protein in prostate - derived cells including human normal prostate epithelial cells (PrEC). The CD147 antibody used was confirmed to react with both human and mouse CD147 protein in our preliminary studies. Three bands of CD147 protein were observed at the molecular size of 40-60 kDa (Fig. 1); this is consistent with previous reports, which stated that the protein indicates plural bands of different molecular sizes [3]. Relatively strong CD147 expression was observed in the LNCaP cancer cells and weak expression was detected in the PC-3, Du145 and HeLa cells (Fig. 1). The expression level in the PrEC and mouse prostate cancer RM9 cells was undetectable or minimal.

Ad-REIC treatment suppressesed the CD147 expression in human prostate cancer LNCaP cells. We used Western blotting to determine the expression of CD147 protein in the LNCAp cells after Ad-REIC treatment (Fig. 2). The infectious efficiency of Ad-REIC was confirmed to be nearly 100% in our preliminary studies. REIC/Dkk-3 protein was strongly expressed after Ad-REIC treatment, and the bands were recognized at molecular sizes of 60-70 kDa. The CD147 expression in the LNCaP cells that underwent the control treatment was significantly downregulated by Ad-REIC.

Ad-REIC significantly inhibited the cell growth of LNCaP prostate cancer cells. To investigate the anti-proliferative effects of Ad-REIC, we treated the cultured LNCaP cell with the agent. Apoptosis was significantly induced in the Ad-REIC-treated cells compared to the controls. After the floating dead cells were removed, the attached viable cells were counted for the *in vitro* cell growth assay. The results showed that the number of viable cells was significantly reduced by Ad-REIC treatment compared to the controls (Fig. 3). The results indicate that the Ad-REIC agent significantly

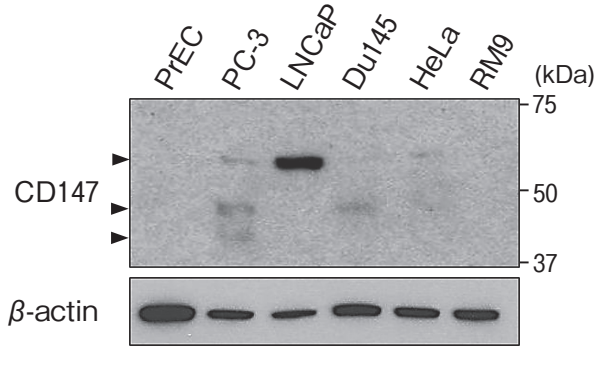

Fig. 1 The expression of CD147 in human and mouse prostate cancer cells as determined by Western blotting. Human normal prostate epithelial cells (PrEC) and the prostate cancer cell lines were examined. The β-actin band is shown as a loading control.

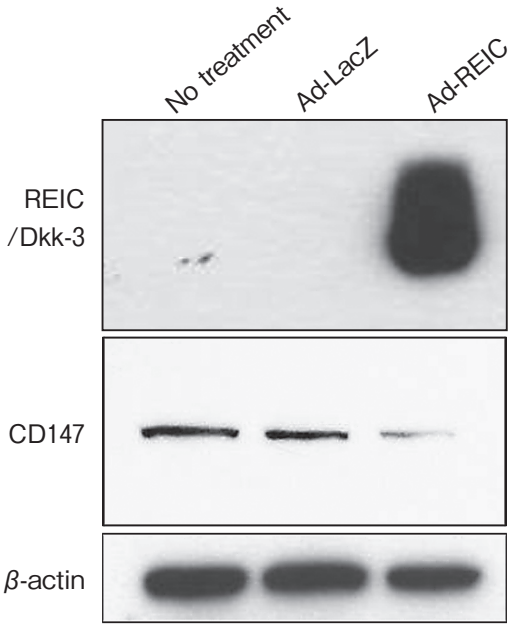

Fig. 2 The Ad-REIC treatment suppressed the expression of CD147 in human prostate cancer LNCaP cells. The cells were treated with Ad-LacZ or Ad-REIC at 100 MOI in 0.5 ml of complete medium for 1 h, and then 1.5 ml of fresh medium was added and the cells were cultured for 24 h. The expression levels of REIC/Dkk-3 and CD147 were analyzed by Western blotting. The β-actin band is shown as a loading control.

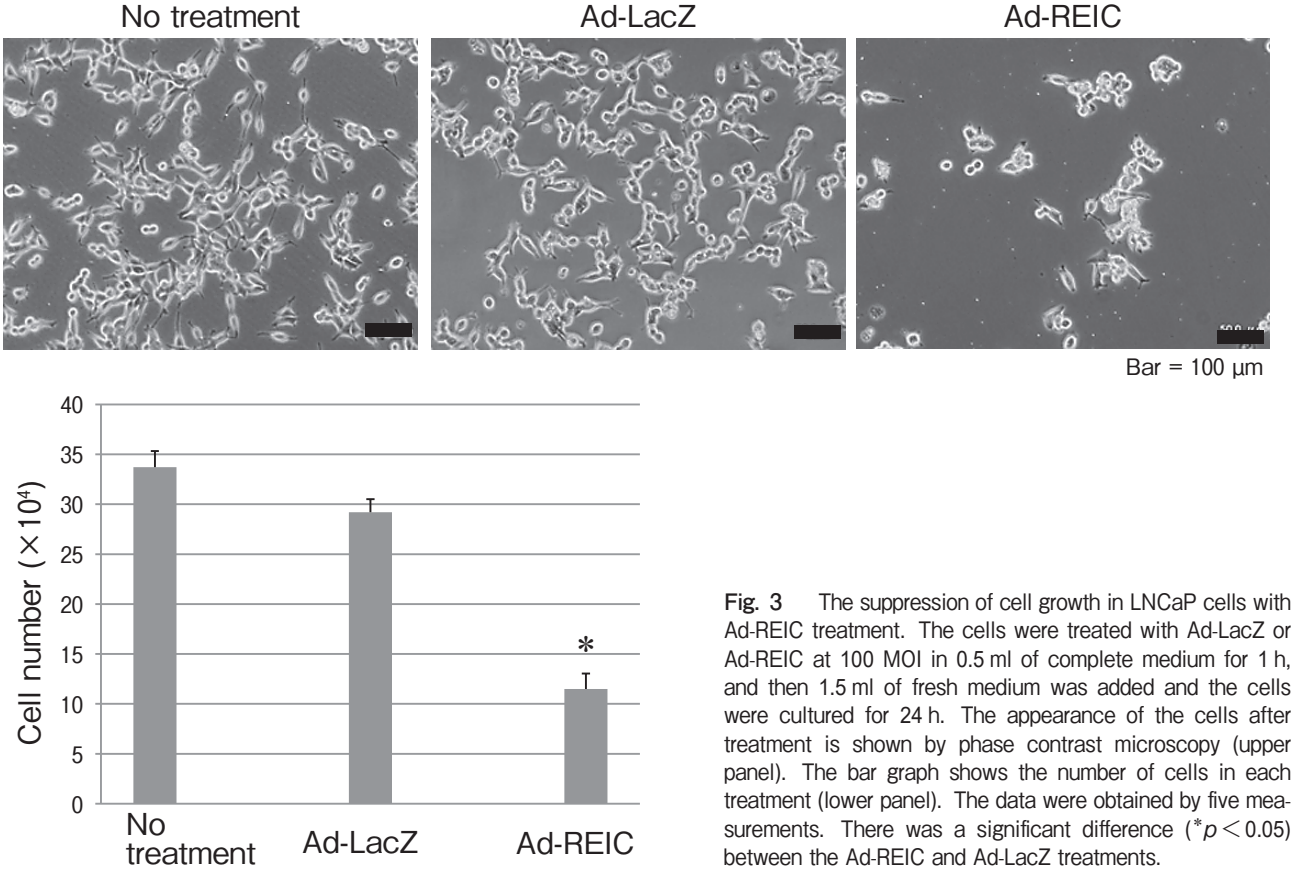

Fig. 3 The suppression of cell growth in LNCaP cells with Ad-REIC treatment. The cells were treated with Ad-LacZ or Ad-REIC at 100 MOI in 0.5 ml of complete medium for 1 h, and then 1.5 ml of fresh medium was added and the cells were cultured for 24 h. The appearance of the cells after treatment is shown by phase contrast microscopy (upper panel). The bar graph shows the number of cells in each treatment (lower panel). The data were obtained by five measurements. There was a significant difference ($^{*}p < 0.05$) between the Ad-REIC and Ad-LacZ treatments.

inhibited the cell growth of the prostate cancer cells.

Ad-REIC suppressed the expression of CD147 in a MAPK signaling- and c-Myc-independent manner. With regard to the expression of the CD147 gene, some signaling pathways are reported to affect or regulate its transcription [16-19]. Since the expression of CD147 is positively controlled by p38-, Erk1/2- and JNK-dependent MAPK signaling and the c-Myc protein transcription factor [16-19], we investigated the association between the CD147 expression level and the levels of phosphorylated MAPKs or c-Myc after Ad-REIC treatment. We also focused on the levels of phosphorylated JNK and its downstream molecule, phosphorylated c-Jun, because the JNK-dependent signaling that occurs via these proteins is considered to be an important step in the induction of apoptosis by Ad-REIC [4,5]. We used Western blotting to compare the expression levels of the indicated factors between Ad-LacZ and Ad-REIC treatment (Fig. 4).

The LNCaP cells were confirmed to express the REIC/Dkk-3 protein following Ad-REIC treatment. Unexpectedly, no positive association was observed between the expression of CD147 and the possible CD147 regulators of MAPK signaling and c-Myc expression. We also examined the levels of phosphorylated GSK-3β, a protein regulated by the c-Myc signaling, but no positive associations was observed between the expression and the downregulation of CD147. As for phosphorylated p38, MAPK signaling was noticeably upregulated after Ad-REIC treatment, indicating that the intrinsic cell survival response against the agent induced apoptotic signaling.

These findings indicated that the Ad-REIC agent downregulates the expression of CD147 without inhibiting MAPK signaling or suppressing c-Myc. In addition, no increases in JNK or c-Jun phosphorylation were observed with Ad REIC treatment, indicating that JNK signaling had not been significantly activated in the LNCaP prostate cancer cells.

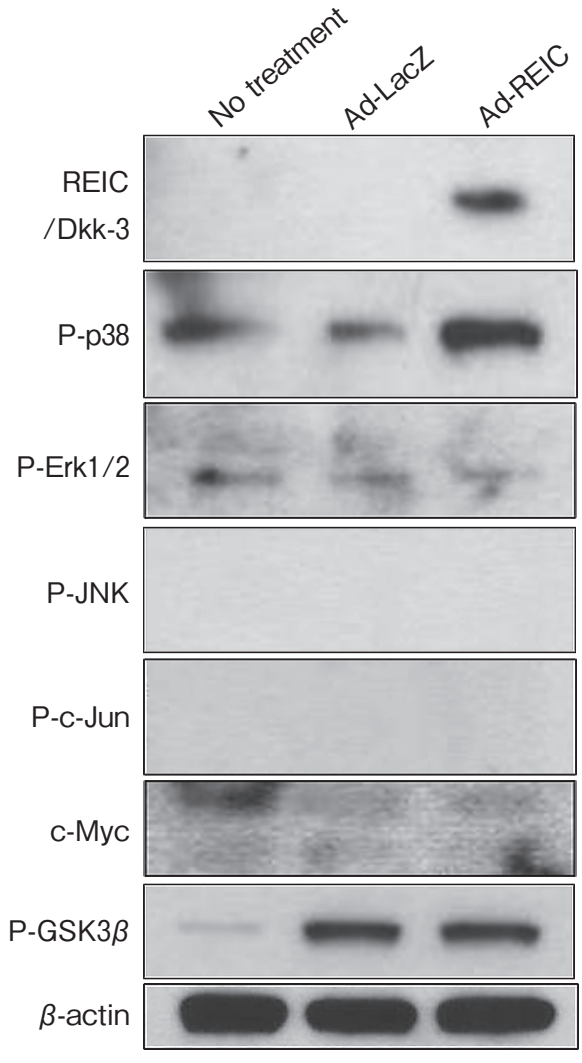

Fig. 4 The expression levels of the factors that may be responsible for the expression of CD147 were investigated after Ad-REIC treatment. Because the expression of CD147 is reported to be positively regulated by p38-, Erk1/2- and JNK-dependent MAPK signaling and the expression of c-Myc, we analyzed the levels of these related factors by Western blotting. The cells were treated with Ad-LacZ or Ad-REIC at 100 MOI in 0.5 ml of complete medium for 1 h, and then 1.5 ml of fresh medium was added and the cells were cultured for 24 h. The β-actin band is shown as a loading control. P-: phosphorylated.

Discussion

Accumulating evidence supports the concept that CD147 protein is a key player in the development and progression of cancer, including its proliferation, invasion and metastasis [3-10]. Several research groups have proposed that CD147 serves as an important regulator of cell proliferation by promoting the reprogramming of glucose metabolism, including glycolysis [20-24]. Glucose provides the precursors for the chemical constituents that are required to build the macromolecules that are essential for cell division and proliferation [22]. Thus, the downregulation or blocking of CD147 can robustly reduce glycolysis, and the targeting of

CD147 using pharmacological agents could be an attractive cancer therapeutic strategy [22, 23, 25-27].

The result of the resent study revealed the suppressive effects of Ad-REIC treatment on CD147, a cancer-progression factor, and on the cell growth of prostate cancer LNCaP cells. This is the first study to indicate a link between the downregulation of CD147 and the therapeutic effects of the Ad-REIC agents.

REIC/Dkk-3 has emerged as a novel tumor suppressor in the development and progression of cancer. Its abundant expression in normal tissues is frequently abolished in solid cancer types and hematological malignancies due to epigenetic suppression with aberrant promoter methylation [28]. The Ad-REIC agents reduced the tumor volume of prostate cancer PC-3 and malignant mesothelioma 211H xenografts in nude mouse models, and this was attributed to the activation of JNK with its phosphorylation leading to the induction of apoptosis [12, 13, 28]. We therefore examined the levels of phosphorylated JNK and c-Jun in the present study; however, no significant change was observed in the prostate cancer LNCaP cells after Ad-REIC treatment.

The induction of apoptosis and the suppression of cell growth was indeed observed in the LNCaP cells after treatment with Ad-REIC agents, indicating that the JNK activation pathway might not be used for the anti-proliferative signaling in some cancer cell lines. These results suggest that other anti-cancer mechanisms are involved in the effects of Ad-REIC agents and that the downregulation of CD147 could be an important therapeutic effect of these agents.

The original Ad-REIC agent of GMP (Good Manufacturing Practice) was evaluated in a phase I/IIa trial of *in situ* gene therapy for patients with prostate cancer [29]. Patients with high-risk localized prostate cancer were enrolled, and the inhibitory effects against cancer recurrence after radical prostatectomy were assessed. The continuous induction of apoptosis was reported in Ad-REIC-injected lesions, and an apparent reduction of the tumor volume was observed in some patients. A significantly favorable outcome was observed, with respect to biochemical recurrence-free survival, in the higher-dose treatment groups compared to the lower-dose groups. The results of the present study suggest that the therapeutic effects observed in the above trial's patients may at least partially depend on the suppression of the CD147 oncoprotein by Ad-REIC. Further studies using cancer-bearing mouse models are necessary to investigate the Ad-REIC mediated downregulation of CD147 *in vivo*.

Although the tight association between CD147 and tumorigenesis has recently been a topic of focus, the signaling pathway by which the expression of CD147 is regulated has not been fully elucidated. In a coculture experiment involving MCF-7 breast cancer cells and macrophages, CD147 was upregulated in cocultured cancer cells in a JNK- and NF-κB-dependent manner; this led to the increased invasive capacity of the cancer cells [16]. Other researchers investigated the role of the MAPK signaling pathway in the downregulation of CD147 that was induced by a plant compound in PMA (Phrbol 12-myristate 13-acetate)-induced THP-1 cells. They demonstrated that the agent inhibited the expression of CD147 through the inhibition of both ERK1/2 and p38 MAPK signaling pathways [17].

As for the MAPK signaling-dependent expression of CD147, it was also reported that a selective inhibitor of COX 2 induced the expression of CD147 via the activation of p38, which promoted the invasion of PANC-1 pancreatic cancer cells [18]. In terms of the transcription of the CD147 gene, the mechanisms and roles of c-Myc protein in the expression of CD147 were intensively investigated in human breast cancer cells. The CD147 core promoter region for transcription factor-binding sites was examined and binding sites for c-Myc were identified in this 217-bp region [19]. It was suggested that c-Myc could bind to the special binding motif of the promoter region to activate the transcription of CD147 [19]. In the present study, we investigated the involvement of the MAPK signaling pathway and c-Myc protein in the downregulation of CD147, and we did not observe the expected association between the currently examined signaling pathways and the downregulation of CD147 (Fig. 5). It is conceivable that another type of signaling is involved in the downregulation that was induced by treatment with Ad-REIC.

In conclusion, our findings suggest that a novel therapeutic mechanism is involved in the effects of Ad-REIC. The suppression of the CD147 oncoprotein could be an important action of the agent in addition to the previously reported JNK phosphorylation that occurs via ER stress signaling. Based on the significance of CD147 in the progression of cancer, the further elucidation of the signaling pathway that regulates the

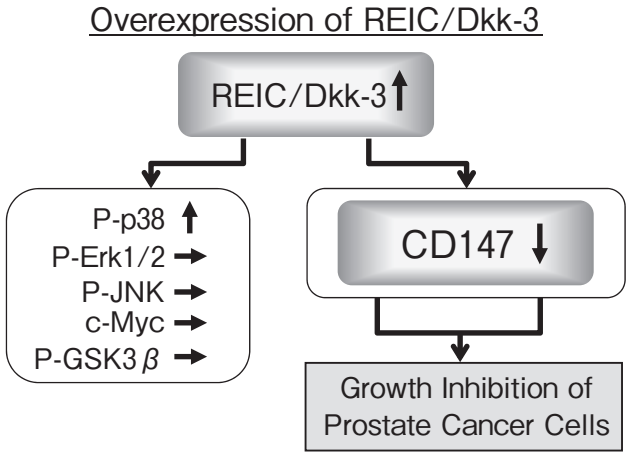

Fig. 5 The downregulation of the CD147 protein by the Ad-REIC-mediated overexpression of REIC/Dkk-3. We investigated the responsible pathways for the downregulation of CD147 by Ad-REIC treatment. No positive associations were observed between the signaling pathways (MAPKs, c-Myc expression and its related GSK-3β) that were examined and the downregulation of CD147, indicating that other types of signaling could be involved in the downregulation. We also suggest that the downregulation of CD147 could be an important anti-cancer effect of Ad-REIC.

expression of CD147 can be expected to make this molecule a more attractive therapeutic target.

Acknowledgments. This study was supported by scientific research grants (JSPS Kakenhi grant nos. JP15H04297, JP15H04974, JP15K10590 and JP16K11004) from Japan's Ministry of Education, Culture, Sports, Science and Technology and the Takeda Science Foundation. We thank Ms. Fusaka Oonari (Okayama University) for her valuable assistance. Okayama University and Momotaro-Gene Inc. are applying for patents for the Ad-REIC agents. Drs. M.W. and Y.N. are the creators of the agents and own stock in Momotaro-Gene Inc.

References

1. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, Feuer EJ and Thun MJ: Cancer statistics, 2005. CA Cancer J Clin (2005) 55: 10–30.
2. Mano R, Eastham J and Yossepowitch O: The very-high-risk prostate cancer: a contemporary update. Prostate Cancer Prostatic Dis (2016) Sep 13. In press. doi: 10.1038/pcan.2016.40.
3. Nabeshima K, Iwasaki H, Koga K, Hojo H, Suzumiya J and Kikuchi M: Emmprin (basigin/CD147): matrix metalloproteinase modulator and multifunctional cell recognition molecule that plays a critical role in cancer progression. Pathol Int (2006) 56: 359–367.
4. Madigan MC, Kingsley EA, Cozzi PJ, Delprado WJ, Russell PJ and Li Y: The role of extracellular matrix metalloproteinase inducer protein in prostate cancer progression. Cancer Immunol Immunother (2008) 57: 1367–1379.
5. Han ZD, Bi XC, Qin WJ, He HC, Dai QS, Zou J, Ye YK, Liang YX, Zeng GH, Chen ZN and Zhong WD: CD147 expression indicates unfavourable prognosis in prostate cancer. Pathol Oncol Res (2009) 15: 369–374.
6. Zhong WD, Liang YX, Lin SX, Li L, He HC, Bi XC, Han ZD, Dai QS, Ye YK, Chen QB, Wang YS, Zeng GH, Zhu G, Zhang Z, Chen ZN and Wu CL: Expression of CD147 is associated with prostate cancer progression. Int J Cancer (2012) 130: 300–308.
7. Fang F, Wang L, Zhang S, Fang Q, Hao F, Sun Y, Zhao L, Chen S, Liao H and Wang L: CD147 modulates autophagy through the PI3K/Akt/mTOR pathway in human prostate cancer PC-3 cells. Oncol Lett (2015) 9: 1439–1443.
8. Liang YX, Mo RJ, He HC, Chen JH, Zou J, Han ZD, Lu JM, Cai C, Zeng YR, Zhong WD and Wu CL: Aberrant hypomethylation-mediated CD147 overexpression promotes aggressive tumor progression in human prostate cancer. Oncol Rep (2015) 33: 2648–2654.
9. Liang YX, Lu JM, Mo RJ, He HC, Xie J, Jiang FN, Lin ZY, Chen YR, Wu YD, Luo HW, Luo Z and Zhong WD: E2F1 promotes tumor cell invasion and migration through regulating CD147 in prostate cancer. Int J Oncol (2016) 48: 1650–1658.
10. Fang F, Qin Y, Hao F, Li Q, Zhang W, Zhao C, Chen S, Zhao L, Wang L and Cai J: CD147 modulates androgen receptor activity through the Akt/Gsk-3β/β-catenin/AR pathway in prostate cancer cells. Oncol Lett (2016) 12: 1124–1128.
11. Hirata T, Watanabe M, Kaku H, Kobayashi Y, Yamada H, Sakaguchi M, Takei K, Huh NH, Nasu Y and Kumon H: REIC/Dkk-3-encoding adenoviral vector as a potentially effective therapeutic agent for bladder cancer. Int J Oncol (2012) 41: 559–564.
12. Watanabe M, Nasu Y and Kumon H: Adenovirus-mediated REIC/Dkk-3 gene therapy: Development of an autologous cancer vaccination therapy (Review). Oncol Lett (2014) 7: 595–601.
13. Abarzua F, Sakaguchi M, Takaishi M, Nasu Y, Kurose K, Ebara S, Miyazaki M, Namba M, Kumon H and Huh NH: Adenovirus-mediated overexpression of REIC/Dkk-3 selectively induces apoptosis in human prostate cancer cells through activation of c-Jun-NH2-kinase. Cancer Res (2005) 65: 9617–9622.
14. Watanabe M, Sakaguchi M, Kinoshita R, Kaku H, Ariyoshi Y, Ueki H, Tanimoto R, Ebara S, Ochiai K, Futami J, Li SA, Huang P, Nasu Y, Huh NH and Kumon H: A novel gene expression system strongly enhances the anticancer effects of a REIC/Dkk-3-encoding adenoviral vector. Oncol Rep (2014) 31: 1089–1095.
15. Sakaguchi M, Watanabe M, Kinoshita R, Kaku H, Ueki H, Futami J, Murata H, Inoue Y, Li SA, Huang P, Putranto EW, Ruma IM, Nasu Y, Kumon H and Huh NH: Dramatic increase in expression of a transgene by insertion of promoters downstream of the cargo gene. Mol Biotechnol (2014) 56: 621–630.
16. Hagemann T, Wilson J, Kulbe H, Li NF, Leinster DA, Charles K, Klemm F, Pukrop T, Binder C and Balkwill FR: Macrophages induce invasiveness of epithelial cancer cells via NF-kappa B and JNK. J Immunol (2005) 175: 1197–1205.
17. Huang Z, Wang C, Wei L, Wang J, Fan Y, Wang L, Wang Y and Chen T: Resveratrol inhibits EMMPRIN expression via P38 and ERK1/2 pathways in PMA-induced THP-1 cells. Biochem Biophys Res Commun (2008) 374: 517–521.
18. Liu H, Xu XF, Zhao Y, Tang MC, Zhou YQ and Gao FH: NS-398 promotes pancreatic cancer cell invasion by CD147 and MMP-2 via the activation of P38. Mol Med Rep (2016) 13: 2208–2214.
19. Kong LM, Liao CG, Zhang Y, Xu J, Li Y, Huang W, Zhang Y, Bian H and Chen ZN: A regulatory loop involving miR-22, Sp1, and c-Myc modulates CD147 expression in breast cancer invasion and metastasis. Cancer Res (2014) 74: 3764–3778.

20. Baba M, Inoue M, Itoh K and Nishizawa Y: Blocking CD147 induces cell death in cancer cells through impairment of glycolytic energy metabolism. Biochem Biophys Res Commun (2008) 374: 111–116.
21. Le Floch R, Chiche J, Marchiq I, Naiken T, Ilc K, Murray CM, Critchlow SE, Roux D, Simon MP and Pouysségur J: CD147 subunit of lactate/H+ symporters MCT1 and hypoxia-inducible MCT4 is critical for energetics and growth of glycolytic tumors. Proc Natl Acad Sci U S A (2011) 108: 16663–16668.
22. Huang Q, Li J, Xing J, Li W, Li H, Ke X, Zhang J, Ren T, Shang Y, Yang H, Jiang J and Chen Z: CD147 promotes reprogramming of glucose metabolism and cell proliferation in HCC cells by inhibiting the p53-dependent signaling pathway. J Hepatol (2014) 61: 859–866.
23. Marchiq I, Le Floch R, Roux D, Simon MP and Pouyssegur J: Genetic disruption of lactate/H+ symporters (MCTs) and their subunit CD147/BASIGIN sensitizes glycolytic tumor cells to phenformin. Cancer Res (2015) 75: 171–180.
24. Aït-Ali N, Fridlich R, Millet-Puel G, Clérin E, Delalande F, Jaillard C, Blond F, Perrocheau L, Reichman S, Byrne LC, Olivier-Bandini A, Bellalou J, Moyse E, Bouillaud F, Nicol X, Dalkara D, van Dorsselaer A, Sahel JA and Léveillard T: Rod-derived cone viability factor promotes cone survival by stimulating aerobic glycolysis. Cell (2015) 161: 817–832.
25. Muramatsu T: Basigin (CD147), a multifunctional transmembrane glycoprotein with various binding partners. J Biochem (2016) 159: 481–490.
26. Fu ZG, Wang L, Cui HY, Peng JL, Wang SJ, Geng JJ, Liu JD, Feng F, Song F, Li L, Zhu P, Jiang JL and Chen ZN: A novel small-molecule compound targeting CD147 inhibits the motility and invasion of hepatocellular carcinoma cells. Oncotarget (2016) 7: 9429–9447.
27. Walter M, Simanovich E, Brod V, Lahat N, Bitterman H and Rahat MA: An epitope-specific novel anti-EMMPRIN polyclonal antibody inhibits tumor progression. Oncoimmunology (2015) 5: e1078056.
28. Veeck J and Dahl E: Targeting the Wnt pathway in cancer: the emerging role of Dickkopf-3. Biochim Biophys Acta (2012) 1825: 18–28.
29. Kumon H, Sasaki K, Ariyoshi Y, Sadahira T, Araki M, Ebara S, Yanai H, Watanabe M and Nasu Y: Feasibility of Neoadjuvant Ad-REIC Gene Therapy in Patients with High-Risk Localized Prostate Cancer Undergoing Radical Prostatectomy. Clin Transl Sci (2015) 8: 837–840.