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PII-SKIN SCI Journal

2017. 05. 10

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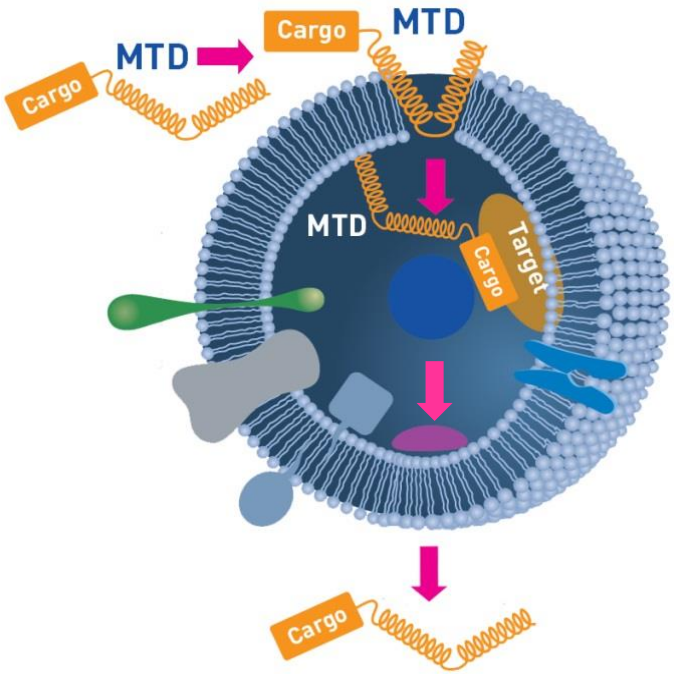
Platform Technology : MITT™



Cell-to-Cell delivery

Cargo (Functional Material)

Therapeutic antibodies

Recombinant Proteins

Peptides

siRNA and DNAs

Low MW molecule(chemical)

Nano-particle or liposome

◆ **MTD (Macromolecule Transduction Domain)**

Consist of 5~15 amino acids

Intracellular Cargo delivery

- **MITT - Macromolecule Intracellular Transduction Technology**
- **MTD - Macromolecule Transduction Domain**
- **PII-SKIN - "Penetrating to Skin"**

PII-SKIN Platform technology

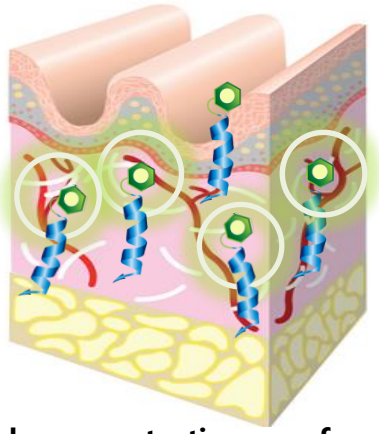
Cargo : Active Ingredient

Growth factor/Peptide/ Functional materials

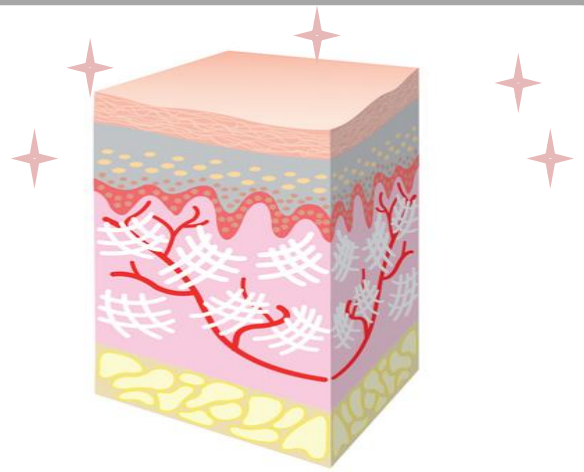
MTD : Transdermal Peptide

5~15 Amino acid
(peptide with alpha-helical structure)

- **PII-SKIN Platform technology to improve transdermal delivery of functional materials**
  : Anti-aging/wrinkle, Hair growth, Brightening, etc.

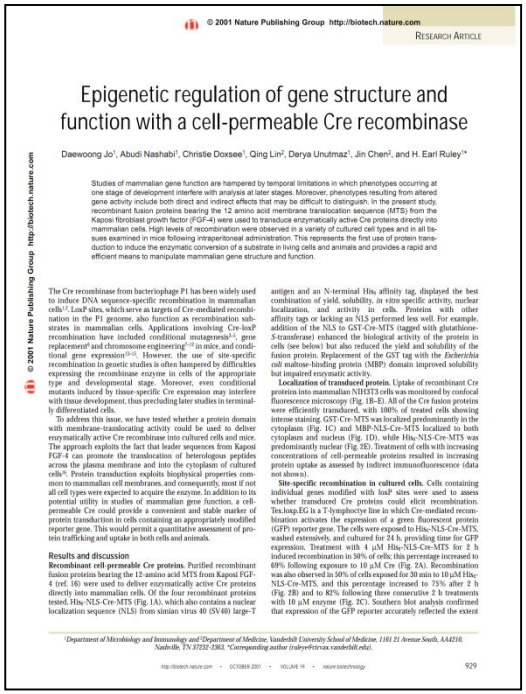


Improved penetration of functional materials into stratum corneum and other skin layers by MTD



Maximized efficacy through the delivery of functional materials into specific targets

SCI Journal of ProCell Therapeutics



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RESEARCH ARTICLE

Epigenetic regulation of gene structure and function with a cell-permeable Cre recombinase

Daewoong Jo[1], Abudi Nashabi[1], Christie Doxsee[1], Qing Lin[2], Derya Unutmaz[1], Jin Chen[2], and H. Earl Ruley[1]*

Studies of mammalian gene function are hampered by temporal limitations in which phenotypes occurring at one stage of development interfere with analysis at later stages. Moreover, phenotypes resulting from altered gene activity include both direct and indirect effects that may be difficult to distinguish. In the present study, recombinant fusion proteins bearing the 12 amino acid membrane translocation sequence (MTS) from the Kaposi fibroblast growth factor (FGF-4) were used to transduce enzymatically active Cre proteins directly into mammalian cells. High levels of recombination were observed in a variety of cultured cell types and in all tissues examined in mice following intraperitoneal administration. This represents the first use of protein transduction to induce the enzymatic conversion of a substrate in living cells and animals and provides a rapid and efficient means to manipulate mammalian gene structure and function.

The Cre recombinase from bacteriophage P1 has been widely used to induce DNA sequence-specific recombination in mammalian cells[1,2]. LoxP sites, which serve as targets of Cre-mediated recombination in the P1 genome, also function as recombination substrates in mammalian cells. Applications involving Cre-loxP recombination have included conditional mutagenesis[3–5], gene replacement[6] and chromosome engineering[7–12] in mice, and conditional gene expression[13–15]. However, the use of site-specific recombination in genetic studies is often hampered by difficulties expressing the recombinase enzyme in cells of the appropriate type and developmental stage. Moreover, even conditional mutants induced by tissue-specific Cre expression may interfere with tissue development, thus precluding later studies in terminally differentiated cells.

To address this issue, we have tested whether a protein domain with membrane-translocating activity could be used to deliver enzymatically active Cre recombinase into cultured cells and mice. The approach exploits the fact that leader sequences from Kaposi FGF-4 can promote the translocation of heterologous peptides across the plasma membrane and into the cytoplasm of cultured cells[16]. Protein transduction exploits biophysical properties common to mammalian cell membranes, and consequently, most if not all cell types were expected to acquire the enzyme. In addition to its potential utility in studies of mammalian gene function, a cell-permeable Cre could provide a convenient and stable marker of protein transduction in cells containing an appropriately modified reporter gene. This would permit a quantitative assessment of protein trafficking and uptake in both cells and animals.

Results and discussion

Recombinant cell-permeable Cre proteins. Purified recombinant fusion proteins bearing the 12-amino acid MTS from Kaposi FGF-4 (ref. 16) were used to deliver enzymatically active Cre proteins directly into mammalian cells. Of the four recombinant proteins tested, His_6-NLS-Cre-MTS (Fig. 1A), which also contains a nuclear localization sequence (NLS) from simian virus 40 (SV40) large-T antigen and an N-terminal His_6 affinity tag, displayed the best combination of yield, solubility, in vitro specific activity, nuclear localization, and activity in cells. Proteins with other affinity tags or lacking an NLS performed less well. For example, addition of the NLS to GST-Cre-MTS (tagged with glutathione-*S*-transferase) enhanced the biological activity of the protein in cells (see below) but also reduced the yield and solubility of the fusion protein. Replacement of the GST tag with the *Escherichia coli* maltose-binding protein (MBP) domain improved solubility but impaired enzymatic activity.

Localization of transduced protein. Uptake of recombinant Cre proteins into mammalian NIH3T3 cells was monitored by confocal fluorescence microscopy (Fig. 1B–E). All of the Cre fusion proteins were efficiently transduced, with 100% of treated cells showing intense staining. GST-Cre-MTS was localized predominantly in the cytoplasm (Fig. 1C) and MBP-NLS-Cre-MTS localized to both cytoplasm and nucleus (Fig. 1D), while His_6-NLS-Cre-MTS was predominantly nuclear (Fig. 2E). Treatment of cells with increasing concentrations of cell-permeable proteins resulted in increasing protein uptake as assessed by indirect immunofluorescence (data not shown).

Site-specific recombination in cultured cells. Cells containing individual genes modified with loxP sites were used to assess whether transduced Cre proteins could elicit recombination. Tet.lox.pEG is a T-lymphocyte line in which Cre-mediated recombination activates the expression of a green fluorescent protein (GFP) reporter gene. The cells were exposed to His_6-NLS-Cre-MTS, washed extensively, and cultured for 24 h, providing time for GFP expression. Treatment with 4 μM His_6-NLS-Cre-MTS for 2 h induced recombination in 50% of cells; this percentage increased to 69% following exposure to 10 μM Cre (Fig. 2A). Recombination was also observed in 50% of cells exposed for 30 min to 10 μM His_6-NLS-Cre-MTS, and this percentage increased to 75% after 2 h (Fig. 2B) and to 82% following three consecutive 2 h treatments with 10 μM enzyme (Fig. 2C). Southern blot analysis confirmed that expression of the GFP reporter accurately reflected the extent

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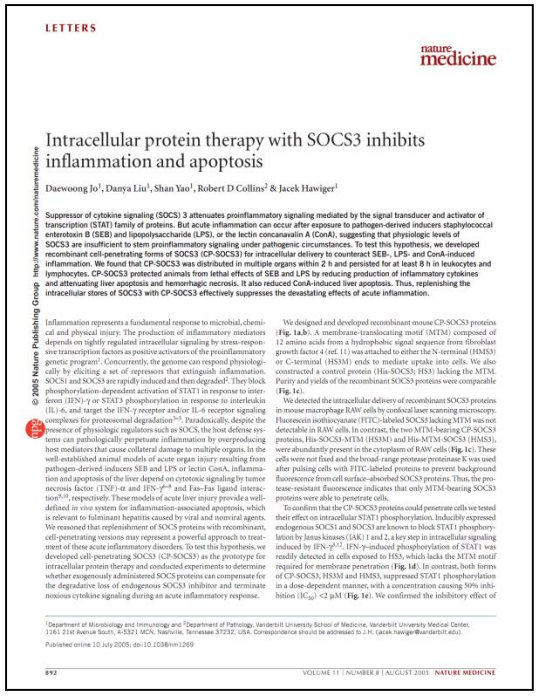

LETTERS

nature medicine

Intracellular protein therapy with SOCS3 inhibits inflammation and apoptosis

Daewoong Jo[1], Danya Liu[1], Shan Yao[1], Robert D Collins[2] & Jacek Hawiger[1]

Suppressor of cytokine signaling (SOCS) 3 attenuates proinflammatory signaling mediated by the signal transducer and activator of transcription (STAT) family of proteins. But acute inflammation can occur after exposure to pathogen-derived inducers staphylococcal enterotoxin B (SEB) and lipopolysaccharide (LPS), or the lectin concanavalin A (ConA), suggesting that physiologic levels of SOCS3 are insufficient to stem proinflammatory signaling under pathogenic circumstances. To test this hypothesis, we developed recombinant cell-penetrating forms of SOCS3 (CP-SOCS3) for intracellular delivery to counteract SEB-, LPS- and ConA-induced inflammation. We found that CP-SOCS3 was distributed in multiple organs within 2 h and persisted for at least 8 h in leukocytes and lymphocytes. CP-SOCS3 protected animals from lethal effects of SEB and LPS by reducing production of inflammatory cytokines and attenuating liver apoptosis and hemorrhagic necrosis. It also reduced ConA-induced liver apoptosis. Thus, replenishing the intracellular stores of SOCS3 with CP-SOCS3 effectively suppresses the devastating effects of acute inflammation.

Inflammation represents a fundamental response to microbial, chemical and physical injury. The production of inflammatory mediators depends on tightly regulated intracellular signaling by stress-responsive transcription factors as positive activators of the proinflammatory genetic program[1]. Concurrently, the genome can respond physiologically by eliciting a set of repressors that extinguish inflammation. SOCS1 and SOCS3 are rapidly induced and then degraded[2]. They block phosphorylation-dependent activation of STAT1 in response to interferon (IFN)-γ or STAT3 phosphorylation in response to interleukin (IL)-6, and target the IFN-γ receptor and/or IL-6 receptor signaling complexes for proteosomal degradation[3–5]. Paradoxically, despite the presence of physiologic regulators such as SOCS, the host defense systems can pathologically perpetuate inflammation by overproducing host mediators that cause collateral damage to multiple organs. In the well-established animal models of acute organ injury resulting from pathogen-derived inducers SEB and LPS or lectin ConA, inflammation and apoptosis of the liver depend on cytotoxic signaling by tumor necrosis factor (TNF)-α and IFN-γ[6–8] and Fas–Fas ligand interaction[9,10], respectively. These models of acute liver injury provide a well-defined *in vivo* system for inflammation-associated apoptosis, which is relevant to fulminant hepatitis caused by viral and nonviral agents. We reasoned that replenishment of SOCS proteins with recombinant, cell-penetrating versions may represent a powerful approach to treatment of these acute inflammatory disorders. To test this hypothesis, we developed cell-penetrating SOCS3 (CP-SOCS3) as the prototype for intracellular protein therapy and conducted experiments to determine whether exogenously administered SOCS proteins can compensate for the degradative loss of endogenous SOCS3 inhibitor and terminate noxious cytokine signaling during an acute inflammatory response.

We designed and developed recombinant mouse CP-SOCS3 proteins (**Fig. 1a,b**). A membrane-translocating motif (MTM) composed of 12 amino acids from a hydrophobic signal sequence from fibroblast growth factor 4 (ref. 11) was attached to either the N-terminal (HM53) or C-terminal (HS3M) ends to mediate uptake into cells. We also constructed a control protein (His-SOCS3; HS3) lacking the MTM. Purity and yields of the recombinant SOCS3 proteins were comparable (**Fig. 1c**).

We detected the intracellular delivery of recombinant SOCS3 proteins in mouse macrophage RAW cells by confocal laser scanning microscopy. Fluorescein isothiocyanate (FITC)–labeled SOCS3 lacking MTM was not detectable in RAW cells. In contrast, the two MTM-bearing CP-SOCS3 proteins, His-SOCS3-MTM (HS3M) and His-MTM-SOCS3 (HM53), were abundantly present in the cytoplasm of RAW cells (**Fig. 1c**). These cells were not fixed and the broad-range protease proteinase K was used after pulsing cells with FITC-labeled proteins to prevent background fluorescence from cell surface–absorbed SOCS3 proteins. Thus, the protease-resistant fluorescence indicates that only MTM-bearing SOCS3 proteins were able to penetrate cells.

To confirm that the CP-SOCS3 proteins could penetrate cells we tested their effect on intracellular STAT1 phosphorylation. Inducibly expressed endogenous SOCS1 and SOCS3 are known to block STAT1 phosphorylation by Janus kinases (JAK) 1 and 2, a key step in intracellular signaling induced by IFN-γ[3,12]. IFN-γ–induced phosphorylation of STAT1 was readily detected in cells exposed to HS5, which lacks the MTM motif required for membrane penetration (**Fig. 1d**). In contrast, both forms of CP-SOCS3, HS3M and HM53, suppressed STAT1 phosphorylation in a dose-dependent manner, with a concentration causing 50% inhibition (IC_{50}) <2 μM (**Fig. 1e**). We confirmed the inhibitory effect of

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Published online 10 July 2005; doi:10.1038/nm1269

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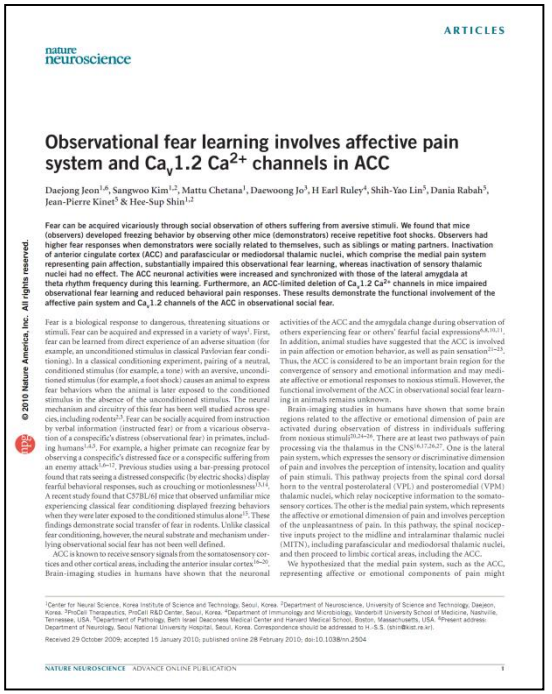
Nature Medicine



ARTICLES

nature neuroscience

Observational fear learning involves affective pain system and $Ca_v1.2$ Ca^{2+} channels in ACC

Daejong Jeon[1,6], Sangwoo Kim[1,2], Mattu Chetana[1], Daewoong Jo[3], H Earl Ruley[4], Shih-Yao Lin[5], Dania Rabah[5], Jean-Pierre Kinet[5] & Hee-Sup Shin[1,2]

Fear can be acquired vicariously through social observation of others suffering from aversive stimuli. We found that mice (observers) developed freezing behavior by observing other mice (demonstrators) receive repetitive foot shocks. Observers had higher fear responses when demonstrators were socially related to themselves, such as siblings or mating partners. Inactivation of anterior cingulate cortex (ACC) and parafascicular or mediodorsal thalamic nuclei, which comprise the medial pain system representing pain affection, substantially impaired this observational fear learning, whereas inactivation of sensory thalamic nuclei had no effect. The ACC neuronal activities were increased and synchronized with those of the lateral amygdala at theta rhythm frequency during this learning. Furthermore, an ACC-limited deletion of $Ca_v1.2$ Ca^{2+} channels in mice impaired observational fear learning and reduced behavioral pain responses. These results demonstrate the functional involvement of the affective pain system and $Ca_v1.2$ channels of the ACC in observational social fear.

Fear is a biological response to dangerous, threatening situations or stimuli. Fear can be acquired and expressed in a variety of ways[1]. First, fear can be learned from direct experience of an adverse situation (for example, an unconditioned stimulus in classical Pavlovian fear conditioning). In a classical conditioning experiment, pairing of a neutral, conditioned stimulus (for example, a tone) with an aversive, unconditioned stimulus (for example, a foot shock) causes an animal to express fear behaviors when the animal is later exposed to the conditioned stimulus in the absence of the unconditioned stimulus. The neural mechanism and circuitry of this fear has been well studied across species, including rodents[2,3]. Fear can be socially acquired from instruction by verbal information (instructed fear) or from a vicarious observation of a conspecific's distress (observational fear) in primates, including humans[1,4,5]. For example, a higher primate can recognize fear by observing a conspecific's distressed face or a conspecific suffering from an enemy attack[5,6–12]. Previous studies using a bar-pressing protocol found that rats seeing a distressed conspecific (by electric shocks) display fearful behavioral responses, such as crouching or motionlessness[9,13]. A recent study found that C57BL/6J mice that observed unfamiliar mice experiencing classical fear conditioning displayed freezing behaviors when they were later exposed to the conditioned stimulus alone[12]. These findings demonstrate social transfer of fear in rodents. Unlike classical fear conditioning, however, the neural substrate and mechanism underlying observational social fear has not been well defined.

ACC is known to receive sensory signals from the somatosensory cortices and other cortical areas, including the anterior insular cortex[14–20]. Brain-imaging studies in humans have shown that the neuronal activities of the ACC and the amygdala change during observation of others experiencing fear or others' fearful facial expressions[5,8,10,11]. In addition, animal studies have suggested that the ACC is involved in pain affection or emotion behavior, as well as pain sensation[21–23]. Thus, the ACC is considered to be an important brain region for the convergence of sensory and emotional information and may mediate affective or emotional responses to noxious stimuli. However, the functional involvement of the ACC in observational social fear learning in animals remains unknown.

Brain-imaging studies in humans have shown that some brain regions related to the affective or emotional dimension of pain are activated during observation of distress in individuals suffering from noxious stimuli[10,24–26]. There are at least two pathways of pain processing via the thalamus in the CNS[6,17,26,27]. One is the lateral pain system, which expresses the sensory or discriminative dimension of pain and involves the perception of intensity, location and quality of pain stimuli. This pathway projects from the spinal cord dorsal horn to the ventral posterolateral (VPL) and posteromedial (VPM) thalamic nuclei, which relay nociceptive information to the somatosensory cortices. The other is the medial pain system, which represents the affective or emotional dimension of pain and involves perception of the unpleasantness of pain. In this pathway, the spinal nociceptive inputs project to the midline and intralaminar thalamic nuclei (MITN), including parafascicular and mediodorsal thalamic nuclei, and then proceed to limbic cortical areas, including the ACC.

We hypothesized that the medial pain system, such as the ACC, representing affective or emotional components of pain might

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Received 29 October 2009; accepted 15 January 2010; published online 28 February 2010; doi:10.1038/nn.2504

NATURE NEUROSCIENCE ADVANCE ONLINE PUBLICATION 1

Nature Neuroscience

- Nature Biotechnology : MTD development history - Personal paper of founder
- Nature Medicine: Study of MTD applied to immunotherapy proteins - Personal paper of founder
- Nature Neuroscience: Study of MTD applied to brain therapeutic proteins
  - KIST & Procell jointly published paper.
  - Provided MTD for KIST research.
  - An example of proven MTD technology from other organizations.

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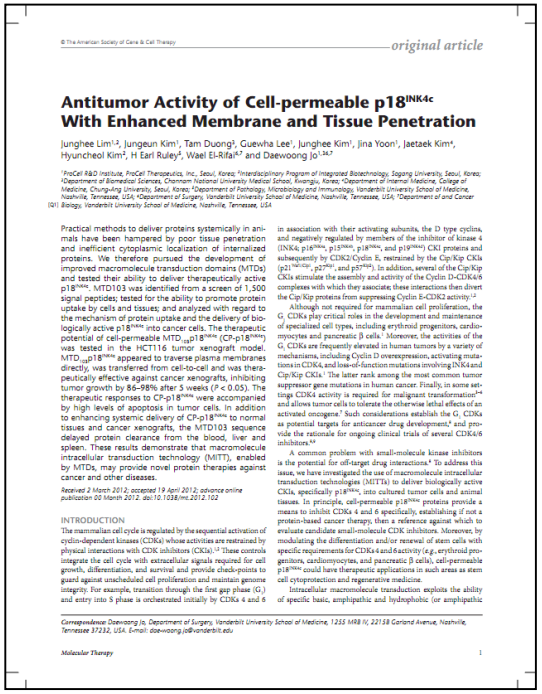

original article

Antitumor Activity of Cell-permeable p18^{INK4c} With Enhanced Membrane and Tissue Penetration

Junghee Lim[1,2], Jungeun Kim[1], Tam Duong[2], Guewha Lee[1], Junghee Kim[1], Jina Yoon[1], Jaetaek Kim[4], Hyuncheol Kim[2], H Earl Ruley[3], Wael El-Rifai[2] and Daewoong Jo[1,2,4,7]

Practical methods to deliver proteins systemically in animals have been hampered by poor tissue penetration and inefficient cytoplasmic localization of internalized proteins. We therefore pursued the development of improved macromolecule transduction domains (MTDs) and tested their ability to deliver therapeutically active p18^{INK4c}. MTD103 was identified from a screen of 1,500 signal peptides; tested for the ability to promote protein uptake by cells and tissues; and analyzed with regard to the mechanism of protein uptake and the delivery of biologically active p18^{INK4c} into cancer cells. The therapeutic potential of cell-permeable MTD$_{103}$p18^{INK4c} (CP-p18^{INK4c}) was tested in the HCT116 tumor xenograft model. MTD$_{103}$p18^{INK4c} appeared to traverse plasma membranes directly, was transferred from cell-to-cell and was therapeutically effective against cancer xenografts, inhibiting tumor growth by 86–98% after 5 weeks ($P < 0.05$). The therapeutic responses to CP-p18^{INK4c} were accompanied by high levels of apoptosis in tumor cells. In addition to enhancing systemic delivery of CP-p18^{INK4c} to normal tissues and cancer xenografts, the MTD103 sequence delayed protein clearance from the blood, liver and spleen. These results demonstrate that macromolecule intracellular transduction technology (MITT), enabled by MTDs, may provide novel protein therapies against cancer and other diseases.

Received 2 March 2012; accepted 19 April 2012; advance online publication 00 Month 2012. doi:10.1038/mt.2012.102

INTRODUCTION

The mammalian cell cycle is regulated by the sequential activation of cyclin-dependent kinases (CDKs) whose activities are restrained by physical interactions with CDK inhibitors (CKIs).[1] These controls integrate the cell cycle with extracellular signals required for cell growth, differentiation, and survival and provide check-points to guard against unscheduled cell proliferation and maintain genome integrity. For example, transition through the first gap phase (G$_1$) and entry into S phase is orchestrated initially by CDKs 4 and 6 in association with their activating subunits, the D type cyclins, and negatively regulated by members of the inhibitor of kinase 4 (INK4; p16^{INK4a}, p15^{INK4b}, p18^{INK4c}, and p19^{INK4d}) CKI proteins and subsequently by CDK2/Cyclin E, restrained by the Cip/Kip CKIs (p21$^{WAF1/Cip1}$, p27^{Kip1}, and p57^{Kip2}). In addition, several of the Cip/Kip CKIs stimulate the assembly and activity of the Cyclin D-CDK4/6 complexes with which they associate; these interactions then divert the Cip/Kip proteins from supressing Cyclin E-CDK2 activity.[1,2]

Although not required for mammalian cell proliferation, the G$_1$ CDKs play critical roles in the development and maintenance of specialized cell types, including erythroid progenitors, cardiomyocytes and pancreatic β cells.[3] Moreover, the activities of the G$_1$ CDKs are frequently elevated in human tumors by a variety of mechanisms, including Cyclin D overexpression, activating mutations in CDK4, and loss-of-function mutations involving INK4 and Cip/Kip CKIs.[4] The latter rank among the most common tumor suppressor gene mutations in human cancer. Finally, in some settings CDK4 activity is required for malignant transformation[5,6] and allows tumor cells to tolerate the otherwise lethal effects of an activated oncogene.[7] Such considerations establish the G$_1$ CDKs as potential targets for anticancer drug development,[8] and provide the rationale for ongoing clinical trials of several CDK4/6 inhibitors.[8,9]

A common problem with small-molecule kinase inhibitors is the potential for off-target drug interactions.[8] To address this issue, we have investigated the use of macromolecule intracellular transduction technologies (MITTs) to deliver biologically active CKIs, specifically p18^{INK4c}, into cultured tumor cells and animal tissues. In principle, cell-permeable p18^{INK4c} proteins provide a means to inhibit CDKs 4 and 6 specifically, establishing if not a protein-based cancer therapy, than a reference against which to evaluate candidate small-molecule CDK inhibitors. Moreover, by modulating the differentiation and/or renewal of stem cells with specific requirements for CDKs 4 and 6 activity (*e.g.*, erythroid progenitors, cardiomyocytes, and pancreatic β cells), cell-permeable p18^{INK4c} could have therapeutic applications in such areas as stem cell cytoprotection and regenerative medicine.

Intracellular macromolecule transduction exploits the ability of specific basic, amphipathic and hydrophobic (or amphipathic

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Published OnlineFirst October 10, 2011; DOI:10.1158/0008-5472.CAN-11-2015

Therapeutics, Targets, and Chemical Biology

Cancer Research

Cell-Permeable NM23 Blocks the Maintenance and Progression of Established Pulmonary Metastasis

Junghee Lim[1,3], Gyong Jang[1], Seeun Kang[1], Guewha Lee[1], Do Thi Thuy Nga[2], Do Thi Lan Phuong[2], Hyuncheol Kim[3], Wael El-Rifai[4], H. Earl Ruley[5], and Daewoong Jo[1,2,6]

Abstract

Occult metastases are a major cause of cancer mortality, even among patients undergoing curative resection. Therefore, practical strategies to target the growth and persistence of already established metastases would provide an important advance in cancer treatment. Here, we assessed the potential of protein therapy using a cell permeable NM23-H1 metastasis suppressor protein. Hydrophobic transduction domains developed from a screen of 1,500 signaling peptide sequences enhanced the uptake of the NM23 protein by cultured cells and systemic delivery to animal tissues. The cell-permeable (CP)-NM23 inhibited metastasis-associated phenotypes in tumor cell lines, blocked the establishment of lung metastases, and cleared already established pulmonary metastases, significantly prolonging the survival of tumor-bearing animals. Therefore, these results establish the potential use of cell-permeable metastasis suppressors as adjuvant therapy against disseminated cancers. *Cancer Res; 71(23): 7216–25. ©2011 AACR.*

Introduction

Metastasis is an acquired and separately evolving phenotype that enables cancer cells to disseminate and grow at locations distant from the primary tumor site. For many tumors, the molecular changes responsible for initiating metastatic spread have already occurred by the time of initial diagnosis, and are ultimately responsible for most cancer deaths (1, 2). Effective strategies to target disseminated tumors are therefore expected to have tremendous therapeutic benefit.

In principle, antimetastasis therapies could either block activities required for the growth or survival of disseminated cancer cells or restore the expression and/or activity of proteins that function to suppress metastasis. The latter includes more than 20 metastasis suppressors—proteins that selectively inhibit the seeding, growth, or persistence of metastatic foci while having only limited effects on primary tumors (3). NME1, the first reported metastasis suppressor gene, was initially characterized as nucleoside diphosphate kinase (NDK), an enzyme required to maintain cellular pools of nucleoside triphosphates. Interest in NDK as a metastasis suppressor (alternatively named NM23-H1 or NM23) was prompted by studies describing inverse correlations between NM23 expression and metastatic potential, first in melanoma cells (4) and later in other types of tumors (5). Subsequent gene transfer experiments documented the ability of NM23 to suppress metastasis-associated phenotypes both in cultured cells and in animal metastasis models (6–10). The precise mechanism by which NM23 influences metastasis is not understood, in part, because the protein possesses multiple enzymatic activities that directly or indirectly suppress mitogen-activated protein kinase (MAPK) signaling (11, 12); regulate small G-protein functions important in cell motility, cytoskeletal reorganization, and cell adhesion (13–15); and influence genome maintenance (16, 17). Nevertheless, clinical trials based on hormonal activation of endogenous NM23 expression are currently in progress (4).

In the present study, we describe an antimetastasis therapy based on the systemic delivery of a cell penetrating NM23-H1 protein. For this experiment, we developed novel macromolecule transduction domains (MTD) modeled after hydrophobic signal peptides previously shown to promote protein uptake by cultured cells and animal tissues (18). The MTD-NM23 inhibited metastasis-associated phenotypes in tumor cell lines and not only suppressed the establishment of lung metastases but also cleared previously established metastases, significantly prolonging the survival of animals harboring disseminated tumor cells.

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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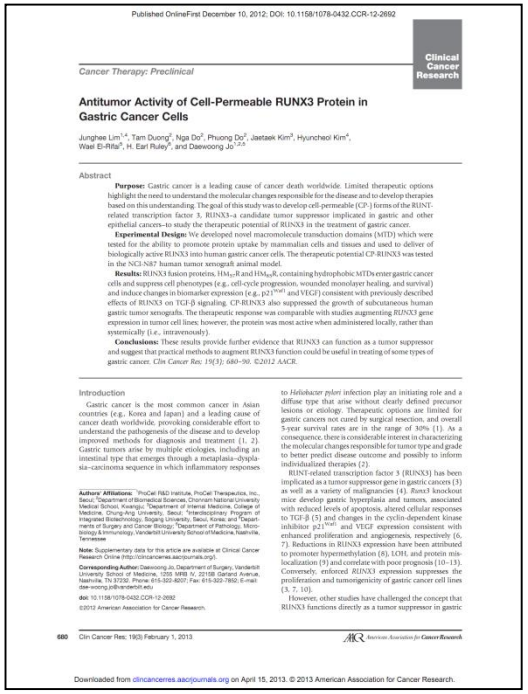
doi: 10.1158/0008-5472.CAN-11-2015



Published OnlineFirst December 10, 2012; DOI: 10.1158/1078-0432.CCR-12-2692

Cancer Therapy: Preclinical

Clinical Cancer Research

Antitumor Activity of Cell-Permeable RUNX3 Protein in Gastric Cancer Cells

Junghee Lim[1,4], Tam Duong[2], Nga Do[2], Phuong Do[2], Jaetaek Kim[3], Hyuncheol Kim[4], Wael El-Rifai[5], H. Earl Ruley[5], and Daewoong Jo[1,2,6,7]

Abstract

Purpose: Gastric cancer is a leading cause of cancer death worldwide. Limited therapeutic options highlight the need to understand the molecular changes responsible for the disease and to develop therapies based on this understanding. The goal of this study was to develop cell-permeable (CP-) forms of the RUNT-related transcription factor 3, RUNX3–a candidate tumor suppressor implicated in gastric and other epithelial cancers–to study the therapeutic potential of RUNX3 in the treatment of gastric cancer.

Experimental Design: We developed novel macromolecule transduction domains (MTD) which were tested for the ability to promote protein uptake by mammalian cells and tissues and used to deliver of biologically active RUNX3 into human gastric cancer cells. The therapeutic potential CP-RUNX3 was tested in the NCI-N87 human tumor xenograft animal model.

Results: RUNX3 fusion proteins, $HM_{35}R$ and $HM_{36}R$, containing hydrophobic MTDs enter gastric cancer cells and suppress cell phenotypes (e.g., cell-cycle progression, wounded monolayer healing and survival) and induce changes in biomarker expression (e.g., p21^{WAF1} and VEGF) consistent with previously described effects of RUNX3 on TGF-β signaling. CP-RUNX3 also suppressed the growth of subcutaneous human gastric tumor xenografts. The therapeutic response was comparable with studies augmenting *RUNX3* gene expression in tumor cell lines; however, the protein was most active when administered locally, rather than systemically (i.e., intravenously).

Conclusions: These results provide further evidence that RUNX3 can function as a tumor suppressor and suggest that practical methods to augment RUNX3 function could be useful in treating of some types of gastric cancer. *Clin Cancer Res; 19(3): 680–90. ©2012 AACR.*

Introduction

Gastric cancer is the most common cancer in Asian countries (e.g., Korea and Japan) and a leading cause of cancer death worldwide, provoking considerable effort to understand the pathogenesis of the disease and to develop improved methods for diagnosis and treatment (1, 2). Gastric tumors arise by multiple etiologies, including an intestinal type that emerges through a metaplasia–dysplasia–carcinoma sequence in which inflammatory responses to *Helicobacter pylori* infection play an initiating role and a diffuse type that arise without clearly defined precursor lesions or etiology. Therapeutic options are limited for gastric cancers not cured by surgical resection, and overall 5-year survival rates are in the range of 30% (1). As a consequence, there is considerable interest in characterizing the molecular changes responsible for tumor type and grade to better predict disease outcome and possibly to inform individualized therapies (2).

RUNT-related transcription factor 3 (RUNX3) has been implicated as a tumor suppressor gene in gastric cancers (3) as well as a variety of malignancies (4). *Runx3* knockout mice develop gastric hyperplasia and tumors, associated with reduced levels of apoptosis, altered cellular responses to TGF-β (5) and changes in the cyclin-dependent kinase inhibitor p21^{WAF1} and VEGF expression consistent with enhanced proliferation and angiogenesis, respectively (6, 7). Reductions in RUNX3 expression have been attributed to promoter hypermethylation (8), LOH, and protein mislocalization (9) and correlate with poor prognosis (10–13). Conversely, enforced *RUNX3* expression suppresses the proliferation and tumorigenicity of gastric cancer cell lines (3, 7, 10).

However, other studies have challenged the concept that RUNX3 functions directly as a tumor suppressor in gastric

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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doi: 10.1158/1078-0432.CCR-12-2692

Molecular Biology **Cancer Research** **Clinical Cancer Research**

- Molecular Biology & Cancer Research & Clinical Cancer Research & Biomaterials

  : Study of MITT applied to development of anticancer drug

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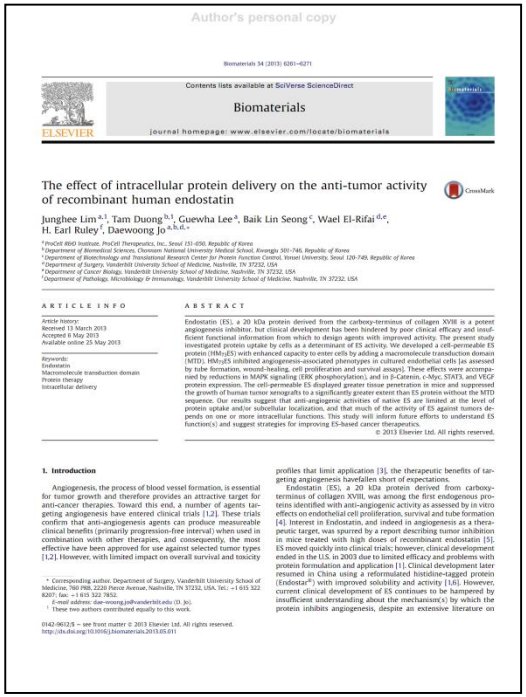

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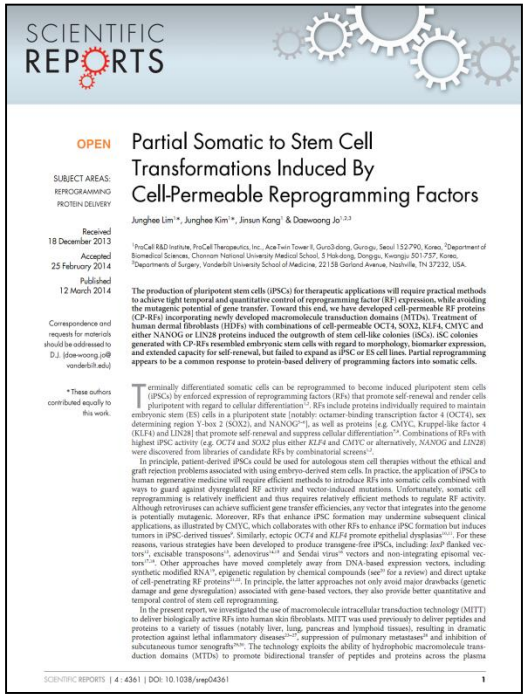

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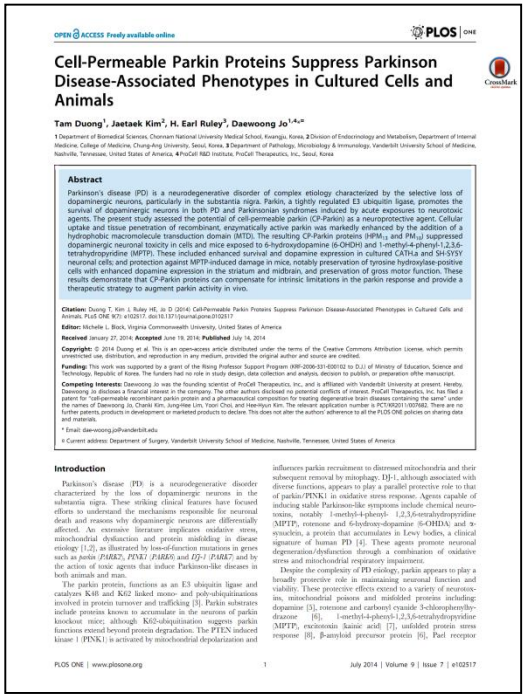

PLOS One

- Scientific Report: Study of MITT applied to development of induced differentiated stem cells
  - A paper published by Procell
- PLOS One: Study of MITT applied to development of Parkinson's remedy
  - A paper published by Procell

Summary

- **Journals of Procell is focused on the study of "Development of Cell-permeable Protein Therapeutics for New Drug Development" applying MITT, the Source technology, and MTD (Macromolecule transduction domain), the basis of technology.**

- **PII-SKIN is a technique of MITT applied to skin, as one branch of MITT, and works by MTD, permeating skin cells, is applied to various substances (eg, growth factor, peptide).**

- **This is included in the technology of MITT & MTD, which allows substances to function by imparting cell permeability. And each study can be used as a resource to confirm the technology.**