Review

Therapeutic siRNA targeting the cancer cell stemness regulator PRDI-BF1 and RIZ domain zinc finger protein 14

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Abstract: PRDI-BF1 and RIZ (PR) domain zinc finger protein 14 (PRDM14), first reported in 2007 to be overexpressed in breast cancer, plays an important role in breast cancer proliferation. Subsequent studies reported that PRDM14 is expressed in embryonic stem cells, primordial germ cells, and various cancers. PRDM14 was reported to confer stemness properties to cancer cells. These properties induce cancer initiation, cancer progression, therapeutic resistance, distant metastasis, and recurrence in refractory tumors. Therefore, PRDM14 may be an ideal therapeutic target for various types of tumors. Silencing PRDM14 expression using *PRDM14*-specific siRNA delivered through an innovative intravenous drug delivery system reduced the size of inoculated tumors, incidence of distant metastases, and increased overall survival in nude mice without causing adverse effects. Therapeutic siRNA targeting *PRDM14* is now being evaluated in a human phase I clinical trial for patients with refractory breast cancer, including triple-negative breast cancer.

Keywords: PRDM14, chimera siRNA, therapeutic oligonucleotides, pancreatic cancer, triple-negative breast cancer, unit polyion complex

Introduction

The cancer stem cell (CSC) hypothesis predicts that CSCs play important roles in cancer initiation, progression, invasion, resistance to cancer therapy, and relapse. CSCs were identified in leukemia in 1994,[1] and in the $CD44^+CD24^-$/low fraction of breast cancer tissue in 2003.[2] Subsequently, CSCs were detected in several tumor types. On the other hand, the CSC hypothesis remains contentious. From a negative perspective, approximately 25% of unselected tumor cells derived from melanoma of 12 different patients, including non-CSCs, were able to initiate a tumor in more highly immunocompromised NOD/SCID interleukin-2 receptor gamma chain null mice.[3] According to another report, Id1 expression is positively related to the high self-renewal capacity of glioma in murine models. However, tumor cells with low Id1 expression indicate higher proliferative potential than those with high expression.[4]

PRDI-BF1 and RIZ (PR) domain zinc finger protein 14 (PRDM14) contains a PR domain, with homology to the Su(var)3-9, enhancer-of-zeste and trithorax (SET) methyltransferase domain, and six zinc finger domains, which are DNA-binding motifs found in transcription factors.[5),6)] PRDM14 is expressed in embryonic stem cells (ESCs) and primordial germ cells (PGCs) and promotes ES cell

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Non-standard abbreviation list: ASO: antisense oligonucleotide; CaP: calcium phosphate; CBFA2T2: core-binding factor, runt domain, alpha subunit 2 translocated to 2; CSC: cancer stem cell; DDS: drug delivery system; ESC: embryonic stem cell; GCT: germ cell tumor; H3K27me3: histone H3 lysine 27; HSC: hematopoietic stem cell; HSP90a: heat shock protein 90-α; miRNA: microRNA; N/P: nitrogen-to-phosphate; NSCLC: non-small cell lung cancer; ON: oligonucleotide; PEG: polyethylene glycol; PGC: primordial germ cell; PLL: poly-l-lysine; PLO: poly-l-ornithine; PMDA: Pharmaceuticals and Medical Devices Agency; PR: PRDI-BF1 and RIZ; PRC2: polycomb repressive complex 2; PRDM: PRDI-BF1 and RIZ domain zinc finger protein; RISC: RNA-induced silencing complex; SET: Su(var)3-9, enhancer-of-zeste and trithorax; siRNA: small interfering RNA; TDG: thymine DNA glycosylase; TET: ten-eleven translocation; TNBC: triple-negative breast cancer; TLR: Toll-like receptor; uPIC: unit polyion complex.

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pluripotency epigenetically.[6)–8)]

Our group has reported that PRDM14 expression is elevated in two-thirds of breast cancer cases, caused by the amplification of the 8q13.3 region containing *PRDM14* in some cases.[9)] We and others have reported that PRDM14 is overexpressed in many types of cancer, such as acute lymphatic leukemia; germ cell tumor (GCT); non-small cell lung cancer (NSCLC); and gastric, pancreatic, ovarian, and renal cancers.[9)–13)] PRDM14 confers phenotypes, including resistance to chemotherapy, tumorigenicity, and distant metastasis, on cancer cells by modifying gene expression epigenetically.[10),12)]

Nuclear localization of PRDM14 poses a challenge regarding targeted delivery of antibodies. To develop small molecules as PRDM14 inhibitors, at least, it needs information on X-ray or *in silico* speculation of crystal structure, or a screening system based on protein–protein interactions, *etc.* On the other hand, small interfering RNAs (siRNAs) can knockdown the expression of target genes using information about the nucleotide sequence along the target mRNA. However, several extracellular and intracellular barriers limit their clinical use. When administered through systemic intravenous injection, siRNAs are degraded by endonucleases or exonucleases in the blood and are filtered through the glomerulus based on particle size. Accumulation of siRNAs in the target regions as passive targeting is challenging. Unmodified siRNAs activate Toll-like receptor (TLR) 3 as an innate immune response.[14)] siRNAs cannot diffuse through cell membranes, and even if they do penetrate cells through endocytosis or pinocytosis, they must escape from endosomes to reach their target mRNAs on the RNA-induced silencing complex (RISC). RISC only degrades target mRNAs when the siRNA antisense/guide strand is completely base-paired to the target mRNA.[15)] However, when only the seed regions of siRNAs (nucleotide positions 2–8) of the antisense strands have complementary sequences with target mRNAs, the regions are tolerated by the RISC, leading to undesired translational arrest of those mRNAs, the so-called off-target effects.[16)] Therefore, chemical modification of siRNAs, design of siRNA sequences, and suitable drug delivery systems (DDSs) are needed to develop clinical applications.

In this review, we first describe the physiological functions of PRDM14 and the relationship between PRDM14 molecules and cancer. Next, we introduce RNA interference strategies against *PRDM14*, using double-stranded DNA/RNA chimeras, combined with innovative nanocarriers of siRNA drugs, which are highly effective in suppressing malignant features of solid cancers, do not cause severe toxicity, and are undergoing phase I clinical trials for the treatment of refractory breast cancer.

1. Molecular structure and physiological function of PRDM14

All members of the PRDM family share a conserved structure, containing an N-terminal PR domain, which is a subtype of the SET domain, as the catalytic active site of histone methyltransferases; however, PRDM14 lacks histone methyltransferase activity.[17)] The PR domain is followed by six C2H2 zinc finger motifs, which mediate sequence-specific DNA binding and protein–protein interactions (Fig. 1A).

PRDM14 is expressed in mouse and human ESCs and murine PGCs and is required for the maintenance of ESC pluripotency and early differentiation in PGCs.[6)–8)] PRDM14 suppresses the transcription of *DNA methyltransferase 3A/B/L* and recruits ten-eleven translocation (TET) 1/2 DNA dioxygenase to target genes. Thus, PRDM14 ensures global hypomethylation of DNA, including pluripotency-associated and germline-specific genes, in ground-state ESCs and PGCs in mice. TET 1/2 can catalyze the sequential oxidation of 5-methylcytosine to 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine, to be able to generate substrates for thymine DNA glycosylase (TDG)-dependent base excision repair by TDG, GADD45A, and various other proteins.[18)] However, it is unclear how 5mC oxidation is regulated and whether TDG is involved in the last step of demethylation. PRDM14 enhances the reprogramming efficiency of fibroblasts to induced pluripotent stem cells and can replace KLF4, but not POU5F1/OCT4, SOX2, or c-MYC, as Yamanaka's factors.[19)] PRDM14 interacts directly with polycomb repressive complex 2 (PRC2) and binds to silenced developmental genes and *ZEB1* enhancer of mesenchymal-to-epithelial transition in human ESCs with trimethylation of histone H3 lysine 27 (H3K27me3) as a transcriptional repressive histone mark.[20)]

PRDM14 maintains pluripotency in ESCs and early differentiation of PGCs by interacting with core-binding factor, runt domain, alpha subunit 2 translocated to 2 (CBFA2T2), which is involved in both transcriptional activation and repression depending on target genes.[21)] The MTG family con-

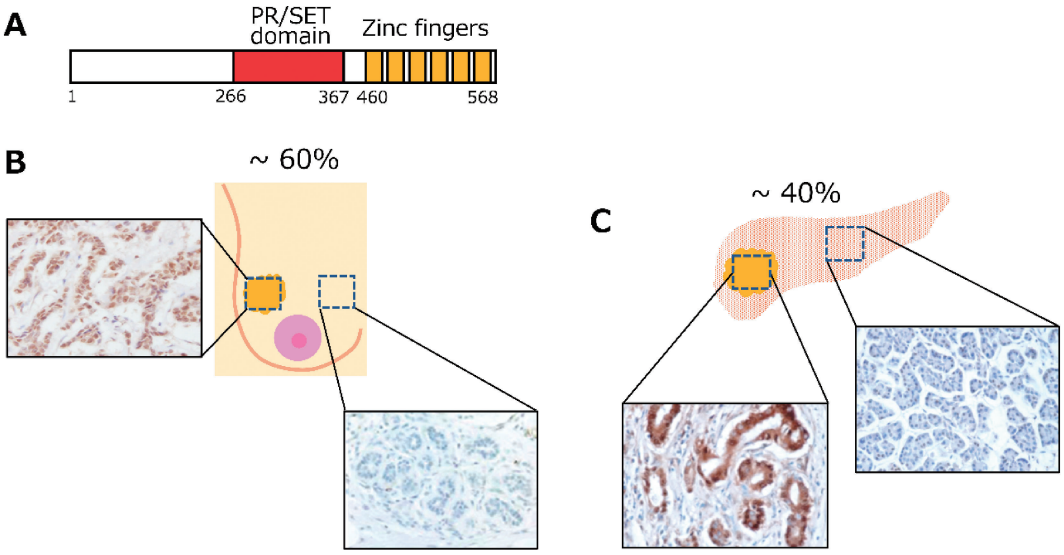

Fig. 1. Structure and expression of PRDI-BF1 and RIZ (PR) domain zinc finger protein 14 (PRDM14) in breast and pancreatic cancer. (A) PRDM14 structure. PRDM14 contains a PR domain that shares homology with Su(var)3-9, enhancer-of-zeste and trithorax (SET) methyltransferase domain, and six zinc-finger motifs. (B, C) PRDM14 expression in breast and pancreatic cancer. PRDM14 is not expressed in normal and differentiated tissues. PRDM14 expression is markedly increased in approximately (B) 60% of breast and (C) 40% of pancreatic cancers. The figures are modified from Refs. 10 and 12.

sists of CBFA2T1/MTG8/RUNX1T1, CBFA2T2/MTGR1, and CBFA2T3/MTG16/ETO2, which participate in oncogenic translocations with RUNX1 in acute myeloid leukemia.[22] PRDM14 is not expressed in mouse and human adult normal tissues, except for a weak expression in the testes; this status is a favorable condition for avoiding side effects in normal tissues. Aberrant PRDM14 expression has been reported in many types of cancers, described in detail in the next chapter.

2. Role of PRDM14 in human cancer

PRDM14 expression is higher in certain types of tumors, including breast cancer (Fig. 1B), NSCLC, gastric cancer, pancreatic cancer (Fig. 1C), ovarian tumors, testicular GCTs, renal cancer, and T-/B-cell acute lymphoblastic leukemia, than in normal tissues.[9)–13),23),24)] Gene amplification or copy number gain of *PRDM14* on chromosome 8q13.3 has been reported in breast cancer, NSCLC, head and neck cancer, and intracranial GCT.[9),10),23)–26)]

PRDM14 is required for stemness phenotypes in breast and pancreatic cancer cells, such as sphere formation, side population identified via dye efflux, chemotherapy resistance, increased proliferation, and distant metastasis (Fig. 2). PRDM14 induces epigenetic changes that regulate the expression of genes involved in cancer stemness, metastasis, and chemoresistance.[10),12)] Moreover, enrichment of DNA regions occupied by PRDM14 in breast cancer cells coincides with promoters containing both H3K4me3 and H3K27me3 histone marks, which are called "bivalent marks" observed in undifferentiated cells.[10)] PRDM14 overexpression was observed in pancreatic cancer tissues from patients with chronic pancreatitis as a pre-cancerous region and pancreatic intraepithelial neoplasia: PanIN as early-stage intraductal cancer, compared with normal pancreatic tissues.[12),27)]

In pancreatic cancer, PRDM14 downregulates microRNAs (miRNAs), including miR-125a-3p, which regulates Fyn expression and metastasis.[12)] The downregulation of miR-424 in response to hyperglycemia enhances invasion and hyperactivation of the CSC pool in triple-negative breast cancer (TNBC) cells through PRDM14 expression. CDC*42*, a target of miR-424, phosphorylates p21-activated kinase 1, which activates STAT5. The binding of phosphorylated STAT5 to the *PRDM14* promoter leads to PRDM14 activation, which might induce breast cancer progression in patients with diabetes.[28)]

PRDM14 overexpression could be a potential biomarker for predicting unfavorable prognosis in NSCLC, and PRDM14 promotes cell migration through extracellular matrix degradation in a human lung cancer cell line through MMP/TIMP.[29),30)] In

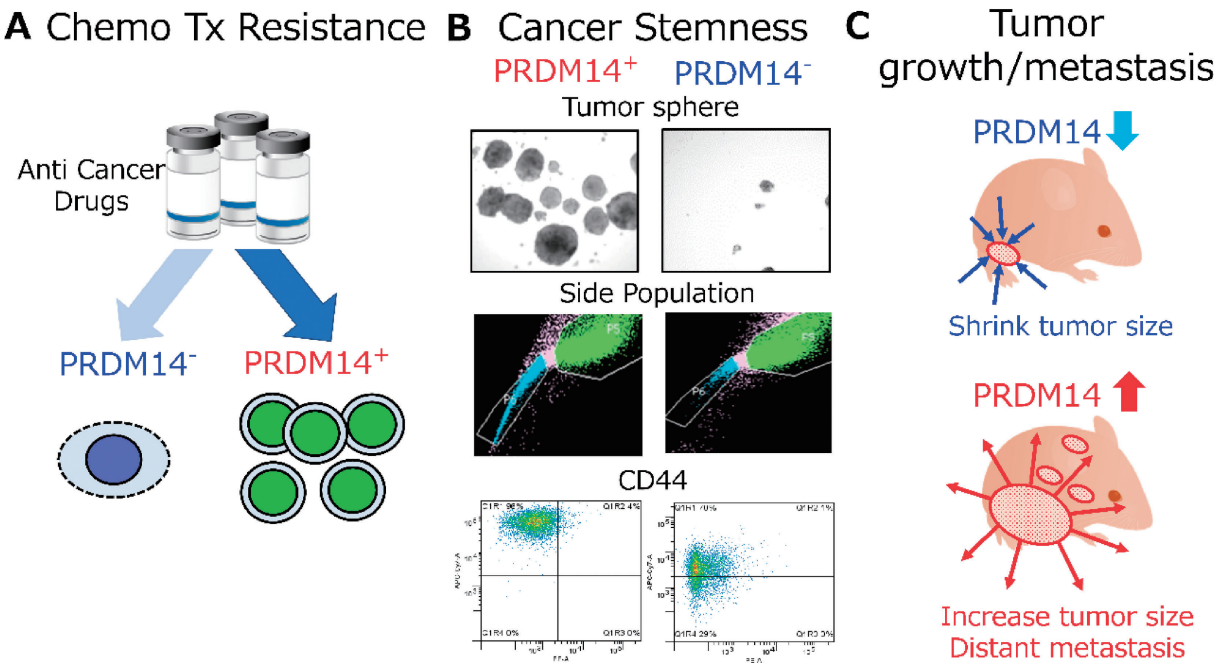

Fig. 2. PRDM14 confers malignant phenotypes on cancer cells, such as (A) resistance to chemotherapy, (B) cancer stemness, and (C) tumor growth and distant metastasis *in vivo*. Therefore, it is a suitable target for new cancer therapies. The figures are modified from Refs. 10 and 12. PRDM14: PRDI-BF1 and RIZ (PR) domain zinc finger protein 14.

human GCTs, PRDM14 is expressed in embryonal carcinomas, seminomas, intracranial germinomas, and yolk sac tumors but not in differentiated teratomas.[24] Constitutive overexpression of PRDM14 in PGC-like cells from pluripotent stem cells leads to delayed differentiation and increased proliferation.

Hematopoietic stem cells (HSCs) with the overexpression of Prdm14 indicate expansion of a cell population with a phenotype of common lymphoid progenitors and can initiate mouse T cell acute lymphoblastic leukemia with activated Notch1 signaling. Prdm14 can bind Cbfa2t3 expressed in HSCs and progenitor cells, instead of Cbfa2t2 as a Prdm14 original partner in ESCs and PGCs, to establish CSCs that initiate leukemia.[31] Moreover, preleukemic cells induced Prdm14 show decreased expression of genes involved in chromosomal stability and DNA repair.[32] Prdm14 binding to intron IV of Notch1 gene induces ligand-independent Notch1 production through recombination activating gene recombinase deletion of the Notch 1 promoter.[33]

PRDM14 has been reported to interact with components of PRC2 and CBFA2T2 in ESCs and TET1/2 in epiblast-like cells. In TNBC, PRDM14 directly binds to glucose-regulated protein 78 and heat shock protein 90-α (HSP90a). HSP90 inhibitors significantly decreased the breast cancer stem-like phenotype in HCC1937 cells but not in PRDM14 knockdown HCC1937 cells.[34]

By contrast, aberrant promoter methylation of PRDM14 has been reported in human papillomavirus-induced cervical cancers and high-grade non-muscle invasive bladder cancer.[35] Ectopic expression of PRDM14 in human papillomavirus 16-positive cancer cell lines induced apoptosis through direct transcriptional activation of the pro-apoptotic genes NOXA and PUMA.[36]

3. Oligonucleotide therapeutics targeting *PRDM14*

Oligonucleotides (ONs) are short synthetic nucleic acids, and ON-based therapeutics, such as antisense ONs (ASO), siRNAs, miRNAs, decoys, aptamers, and CpG oligodeoxynucleotides (DNA), have been under clinical development. ASOs, siRNAs, and miRNAs are complementary to target mRNAs and down-regulate post-transcriptional gene expression. Decoys are designed to inhibit the activity of the target protein. Aptamers recognize target proteins by their molecular structure and interact directly with the target proteins. CpG DNA is recognized by cells of the innate immune system through TLR9.

In addition to ASOs and siRNAs, PRDM14, as a transcriptional factor, binds to the DNA consensus sequence,[37] so that its transcriptional decoy ONs are also therapeutic drug candidates, which mimic the consensus DNA binding site of a specific transcription factor in the promoter region of its target genes, are therapeutic drug candidates. We selected siRNA drugs as ON-based therapeutics, because the design methods of siRNA sequences are better established compared with other ONs; however, there are several technical difficulties associated with siRNAs and their delivery. Unlike ASOs, which are single-stranded, cell-permeable oligodeoxynucleotides that degrade target mRNA through RNase H activity (gapmers), siRNAs are 19–25 bp long double-stranded RNAs that cannot readily diffuse across cell membranes. siRNAs are exogenously transfected into cells with carriers, escape the endosome, and are incorporated into the RNAi machinery. siRNAs are recognized by Argonaute 2, which is a component of the RISC. The sense/passenger strand is degraded and its antisense/guide strand binds with perfect complementarity to the target mRNA sequence, which is cleaved through the exonuclease activity of Argonaute 2.[38] Off-target effects refer to the sequence-dependent downregulation of non-target transcripts due to complete or partial complementarity, especially in the seed region (positions 2–8 from the 5′ end of the antisense strand).[39] To prevent off-target silencing, siRNA sequences should have low thermodynamic stability in this region and lack similarity to any non-targeted mRNA by two or more mismatches.[40] Selection of the siRNA sequence is critical for target gene repression without off-target effects. The use of a double-stranded chimeric siRNA (chimera siRNA) in place of DNA in the seed region can reduce off-target effects, stabilize the siRNA in the blood, and reduce its immunogenicity caused by binding to TLRs *in vivo*.[41),42] We selected siRNA sequences using siDirect (http://sidirect2.rnai.jp/) to eliminate any similarity with non-target transcripts up to at least five mismatches *in silico*.[43] Several chimeric siRNAs against *PRDM14* were generated, and two sequences were selected after estimating the RNAi effect and phenotypes *in vitro* using cancer cells expressing PRDM14 (Fig. 3A).

Innovative siRNA delivery strategies are needed for *in vivo* assays and clinical usage, because naked

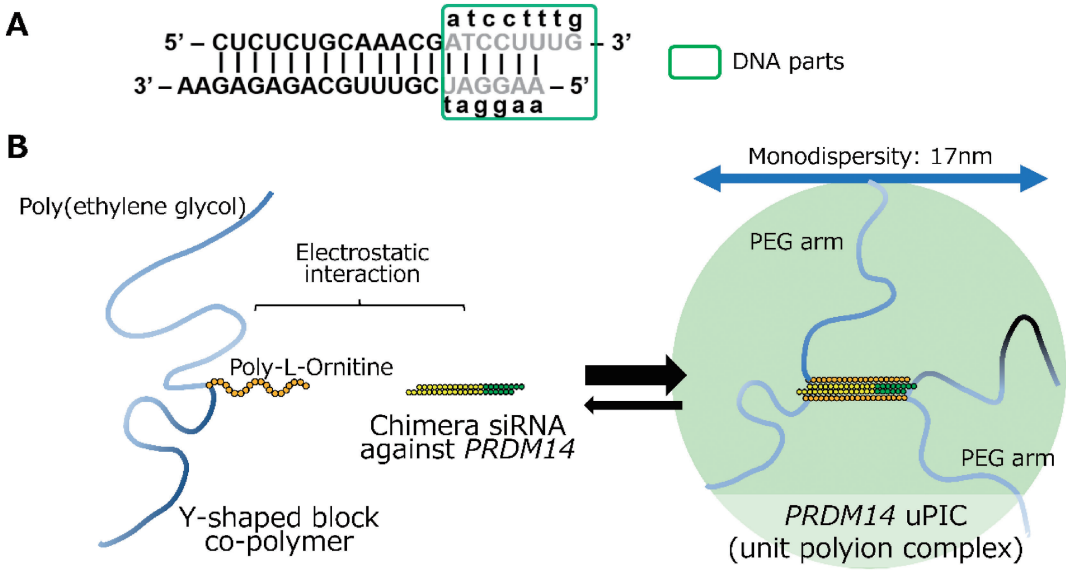

Fig. 3. Double-stranded RNA/DNA chimera (chimera siRNA) against *PRDM14* and unit polyion complex (uPIC). (A) Chimera siRNA is a type of siRNA with an RNA–DNA chimera modification that avoids off-target effects owing to the formation of an RNA-induced silencing complex by the sense strand. It exhibits excellent stability in the bloodstream and low immunogenicity through Toll-like receptors *in vivo*. (B) uPIC consisting of Y-shaped block copolymers (PEG-poly-l-lysine or PEG-poly-l-ornithine) and chimera siRNA. The cationic segment of the block copolymer binds to the negatively charged phosphates of the siRNA. An equilibrium state exists between block copolymers and siRNA. uPIC enables a more efficient delivery of siRNAs to tumors through enhanced permeability and retention (EPR) effects, as a result of their narrow and monodispersed diameter distribution. The figures are modified from Ref. 46. PEG: polyethylene glycol, PRDM14: PRDI-BF1 and RIZ (PR) domain zinc finger protein 14, siRNAs: small interfering RNAs.

siRNAs are degraded by RNases in the blood and filtered through the glomerulus without accumulation at target lesions, stimulating the innate immune system. siRNAs must penetrate target cell membranes and escape from endosomes. We reported that the use of calcium phosphate (CaP) hybrid micelles,[44] polyethylene glycol (PEG)–poly-l-lysine (PLL) block copolymers,[45] and branched PEGylated poly-l-ornithine (PLO)[46] because DDSs for siRNA may overcome several difficulties encountered by conventional systemic delivery systems. The complex composed of these carriers with siRNA accumulates in solid tumors through enhanced permeability and retention effects because of their small particle size.

CaP hybrid micelles are stealth nanoparticles comprising a CaP-siRNA core surrounded by enveloping PEG-polyanion block copolymers, which reduce non-specific interactions in the blood, such as protein corona formation and phagocytosis. Furthermore, PEG-polyanion-coated CaP micelles enabled acidic pH-selective membrane disruption based on the distinctive change in the protonation state of the side chain unit, and CaP hybrid micelles induce endosomal escape after endocytic internalization.[44] Chimeric *PRDM14* siRNA combined with CaP hybrid micelles reduced the size of inoculated tumors and suppressed the formation of distant lung metastasis formed by TNBC in nude mice. However, treatment of a xenografted cancer model with CaP hybrid micelles was discontinued because contamination with free calcium ions induced convulsions in the *in vivo* model.[10]

PEG-PLL and PEG-PLO form the unit polyion complex (uPIC), and not micelles, with siRNA because PEG has a high molecular weight (Fig. 3B).[45),47] PEG-PLO exhibits higher retention in the blood at a lower nitrogen-to-phosphate (N/P) ratio (*i.e.*, positively charged -NH_2 of polycationic DDS to negatively charged phosphates of siRNA) than PEG-PLL; in other words, PEG-PLO can carry siRNAs with smaller amounts of polymer than PEG-PLL.[46] uPIC can accumulate siRNA in target cancer tissues, and not in the liver and spleen, through enhanced permeability and retention effects resulting from narrow and monodisperse size distribution (approximately 17 nm) compared with typical siRNA DDSs, such as lipid nanoparticles (Fig. 4).[45]

In an *in vivo* assay, silencing *PRDM14* in cancer cells using a chimeric siRNA combined with PEG-PLL or PEG-PLO reduced the size of xenograft (including patient-derived xenografts) or orthograft tumors and suppressed the formation of distant metastatic lesions in the lung, liver, or peritoneum formed by TNBC or pancreatic cancer cells (Fig. 5).[12),46] A higher number of PEG-PLLs is

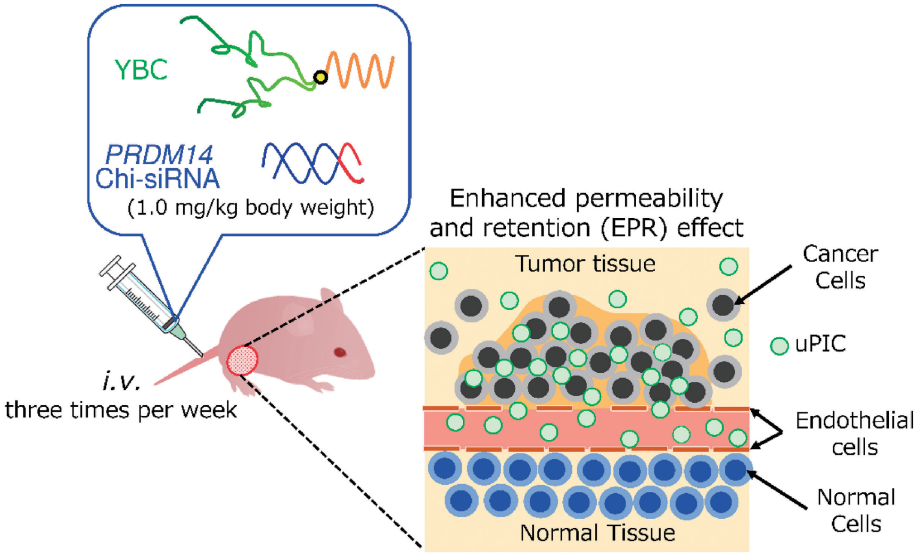

Fig. 4. Scheme describing methods used to induce an orthografted tumor model of PRDM14$^+$ breast cancer cells. Chimera siRNA (1.0 mg/kg body weight) mixed with Y-shaped block copolymer (YBC) at a determined N/P ratio is injected into mice through the tail vein three times per week. uPIC accumulates the siRNA in target cancer tissues, rather than in the liver and spleen, due to the enhanced permeability and retention effects. N/P: nitrogen-to-phosphate, PRDM14: PRDI-BF1 and RIZ (PR) domain zinc finger protein 14, siRNAs: small interfering RNAs, uPIC: unit polyion complex.

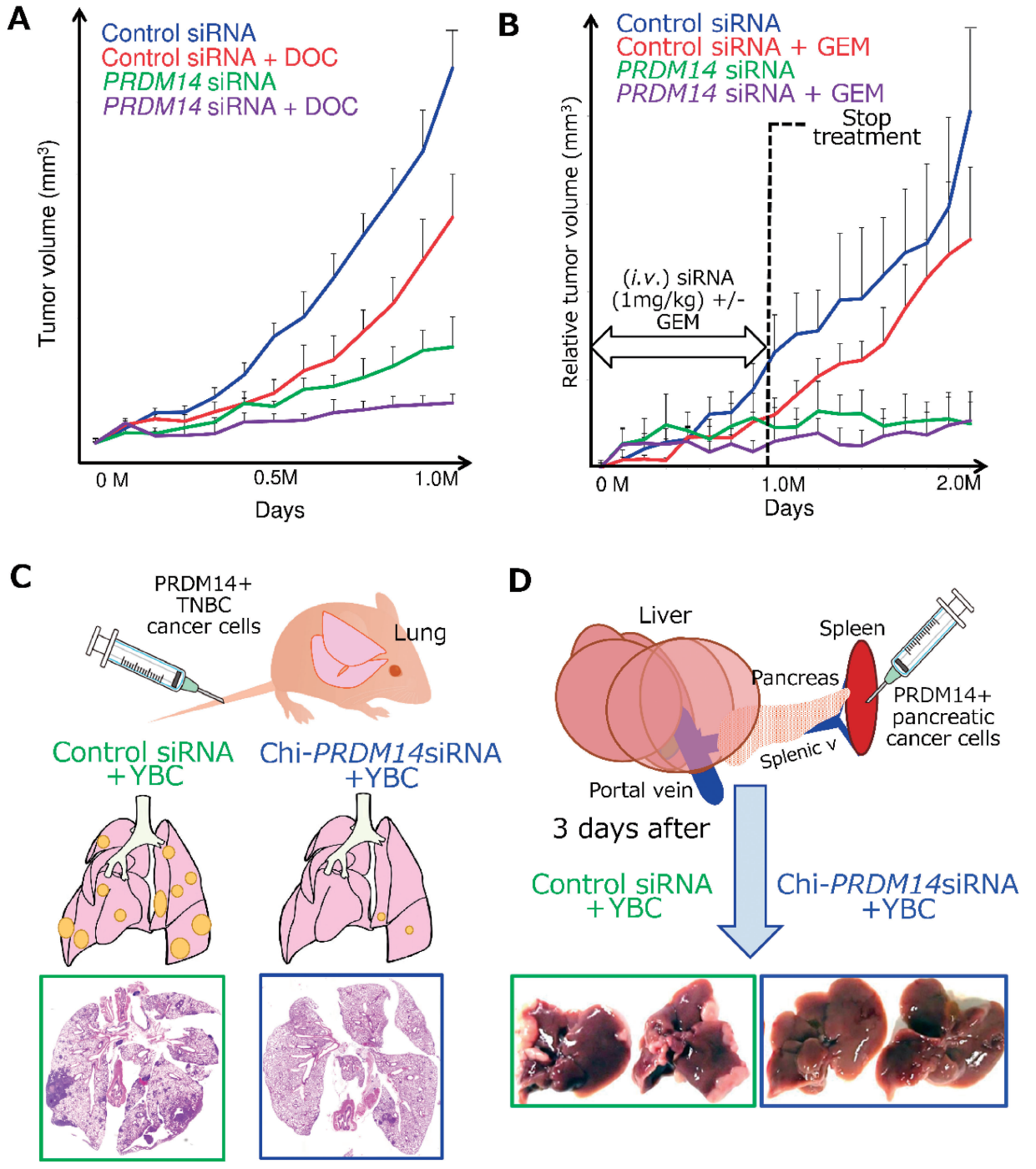

Fig. 5. *PRDM14* siRNA reduces the tumorigenicity of breast and pancreatic cancer cells *in vivo*. (A) The volume of orthograft tumors derived from PRDM14$^+$ breast cancer cells in mice administered uPIC by intravenous administration three times a week, with or without docetaxel treatment, through intraperitoneal injection once a week. (B) Volume of xenograft tumors derived from PRDM14$^+$ pancreatic cancer cells in mice administered uPIC by intravenous administration three times a week with or without gemcitabine treatment through intraperitoneal injection once a week. Mice were left untreated for 2 months and then euthanized. The figures are modified from Ref. 46. (C, D) Suppression of (C) lung or (D) liver metastasis in mice injected with PRDM14$^+$ breast or pancreatic cancer cells and treated with *PRDM14* uPIC. The figures are modified from Refs. 12 and 46. PRDM14: PRDI-BF1 and RIZ (PR) domain zinc finger protein 14, uPIC: unit polyion complex.

required, at an N/P ratio of 10, compared with PEG-PLO molecules, at an N/P ratio of 5, to carry one siRNA molecule. Thus, a uPIC composed of PEG-PLL has a higher viscosity than one with PEG-PLO, which raises the possibility of infusion-related reactions and vein embolization.

Based on these results, we proceeded to preclinical tests on animals, including rats and monkeys, using a chimera siRNA against *PRDM14* and PEG-PLO. The plasma concentration of *PRDM14* uPIC was higher than that expected from the administered dose ratio, which was speculated to be caused by high-level retention and protection of siRNA through PEG-PLO in blood. We observed no serious adverse reactions in animals, but infusion-related reactions, abnormal serum lipid levels, and liver weight gain were observed in some animals. Only a high dose of uPICs induced hepatocyte vacuolation, which was irreversible within the 14-d recovery period, without tissue damage and elevated hepatic enzyme levels.[46)]

After determining the formulation of the siRNA drug, containing specific chimera siRNA against *PRDM14* and PEG-PLO, we manufactured it according to Good Manufacturing Practices for Investigational New Drugs (Fig. 6). Investigator-initiated phase I clinical trials are ongoing in female patients with refractory or recurrent metastatic breast cancer, including TNBC, at the Cancer Institute Hospital of the Japanese Foundation for Cancer Research from September 2020. This study was designed to assess the safety and recommended dose of the siRNA drug targeting *PRDM14* administered intravenously every week to patients, with a standard $3 + 3$ dose-escalation design. Detailed information was obtained from the website of the Japan Registry of Clinical Trials (https://jrct.niph.go.jp/en-latest-detail/jRCT2031190181).

Concluding remarks

Genes that are overexpressed in cancers and not normal tissues, such as *PRDM14*, are potential targets for anti-tumor therapies using ASOs or siRNAs that specifically suppress the expression of these genes. PRDM14 interacts with heat shock proteins or CBFA2T3 in cancer cells, which enhance the stemness phenotype. PRDM14 also regulates cancer cell migration and metastasis through miRNAs, or extracellular matrix degradation in cancers.

To target *PRDM14*, ASOs, transcription factor decoys, and siRNAs are adaptable for anti-tumor ONs therapeutics. However, several issues, especially involving "off-target effects" and "delivery into the

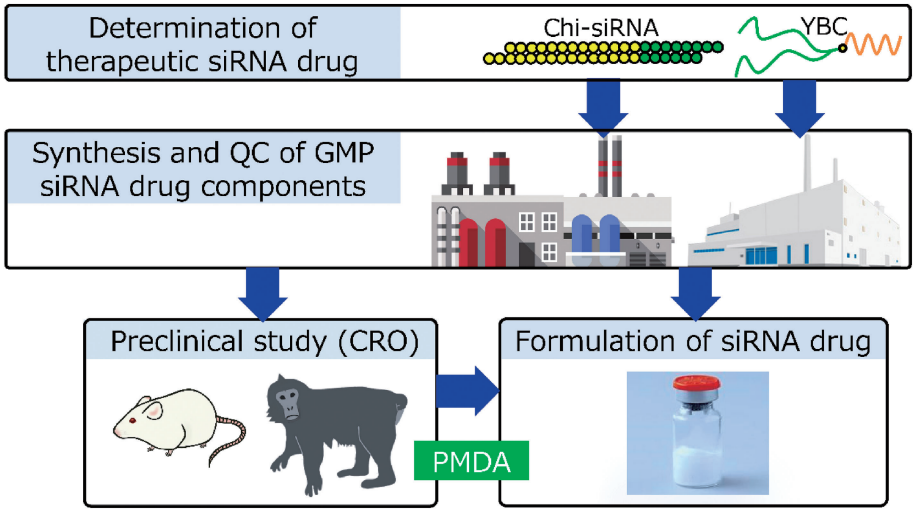

Fig. 6. From basic studies to clinical studies. First, identifying the best combination of the components of the siRNA drug. *PRDM14* uPIC is an ideal oligonucleotide-based therapeutic agent against cancer. Next, synthesis and quality check of siRNA drug components in accordance with Good Manufacturing Practices (GMP) for Investigational New Drugs: characterization of the pharmacokinetics, safety, and antitumor activities of the drug in mice, rats, and monkeys as a preclinical study. To produce a medicinal product for clinical trials, determining a suitable pharmaceutical formulation is necessary. After optimizing the *PRDM14* uPIC formulation, phase I studies are being carried out in humans in accordance with Pharmaceuticals and Medical Devices Agency (PMDA) regulations. PRDM14: PRDI-BF1 and RIZ (PR) domain zinc finger protein 14, siRNAs: small interfering RNAs, uPIC: unit polyion complex.

lesion site", remain to be addressed. siRNAs can knockdown the expression of target genes. Unlike ASOs and transcription factor decoys, siRNA sequences can be selected using the siDirect program, based on genome information biology, to avoid sequence-dependent off-target effects.[43] To avoid "sequence-dependent off-target effects" or to achieve "delivery into the lesion site", chemical modification of siRNAs and DDSs have been developed for clinical applications. Chimera siRNA reduces sequence-dependent and non-sequence-dependent off-target effects, without using chemical modifications.[41] Suitable DDSs are important for the stability of functional siRNAs in the bloodstream, delivered into the cancerous region without entrapping the reticuloendothelial system, and penetrating cancer cell membranes and endosomes. CaP hybrid micelles fulfill these requirements; however, micelles caused convulsions in a subset of tumor-bearing mice. PEG-PLL or PEG-PLO also met this condition, and *PRDM14* uPIC demonstrates antitumor effects.[12),46] A higher number of PEG-PLL molecules is required, compared with PEG-PLO molecules, to carry one molecule of siRNA; therefore, a complex of siRNA with PEG-PLL causes adverse effects, and is expensive to use for treatment, compared with PEG-PLO. A complex of siRNA with PEG-PLO as DDS has been selected and a phase I clinical trial started.

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Ethics statement

All animal experiments were performed with the approval of the Institutional Animal Care and Use Committee of The University of Tokyo (approval number: PA17-104, PA15-20, PA13-02). Ina Research Inc. obtained ethics approval from the Association for Assessment and Accreditation of Laboratory Animal Care International.

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Profile

Kohzoh Imai was born in Hokkaido Prefecture in 1948 and graduated from Sapporo Medical University in 1972. He majored in internal medicine, especially oncology and gastroenterology. He received his PhD degree in 1976 and worked as a postdoctoral fellow funded by an NIH Fogarty International Fellowship at Scripps Clinic and Research Foundation between 1978 and 1981. He became Professor of Medicine at Sapporo Medical University in 1994. He was elected President of Sapporo Medical University in 2004 and served a 6-year term. Then, he was appointed as Director of IMSUT Hospital at the Institute of Medical Science, The University of Tokyo from 2010 to 2014. He was then appointed as Head of the Medical Research Platform Office at the same university between 2015 and 2019, and the Director of Kanagawa Cancer Center Research Institute from 2014 to 2016. He was then invited to become a Guest Professor of Hokkaido University. He is currently a core member of the Japan Agency for Medical Research and Development (AMED). He has developed the diagnostic method for digestive tract cancer utilizing the methylation of genes expressed in cancer cells. He has also dedicated himself to translational research on treatment with siRNAs targeting PRDM14 in cancer cells. This nucleic acid-based drug is a novel approach for cancer and is expected soon to be applied clinically in patients with cancer following a promising proof of concept in mouse models and other animal experiments. For his accomplishments, he received the Medal with Purple Ribbon, the ISOBM Award from International Society of Oncology and Biomarkers (ISOBM), and further awards in Japan.