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Intranuclear interactomic inhibition of FoxP3 suppresses functions of Treg cells

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ABSTRACT

Regulatory T cells (Treg cells) are crucial for the maintenance of immunological tolerance, and it has been reported that Treg cells are enriched within the tumor micro-environment for immune evasion due to their immunosuppressive functions. To inhibit Treg cells functions, FoxP3, a lineage-specific transcription factor responsible for the differentiation and functions of Treg cells, was functionally targeted by a nucleus-transducible (nt) form of various FoxP3 functional subdomains. These nt modified domains can be delivered into the nucleus effectively and work as interactomic inhibitors via disruption of the endogenous FoxP3-mediated transcription complex. Among these domains, nt-FoxP3-FKH (Forkhead DNA binding domain) is most effective at restoring NFAT activity suppressed by FoxP3, and inhibiting the binding of endogenous FKH-containing proteins to FKH DNA binding sequences without influencing the viability and activation of T cells. The suppressive functions of TGF-β-induced iTreg cells and thymus-derived tTreg cells were substantially blocked by nt-FoxP3-FKH, accompanied with down-regulation of CTLA-4 surface expression and IL-10 secretion of Treg cells. In addition, nt-FoxP3-FKH upregulated the expression of IL-2 and IFN-γ in Treg cells. Therefore, nt-FoxP3-FKH has the potential to be a novel therapeutic agent to modulate the immune-evasive tumor environment created by Treg cells without the need for genetic modifications.

1. Introduction

Treg cells are indispensable for suppressing autoimmunity and preventing exuberant immunologic responses to pathogens [1]. However, such immune regulatory functions can be extended to tumor evasion mechanisms. Indeed, Treg cells capable of suppressing the effector functions of tumor-specific T cells have been found in many types of human tumors [2]. Therefore, as a means of improving the therapeutic efficacy of anti-tumor T cells as well as the prophylactic potential of cancer vaccines, several immunotherapies targeting $FoxP3^{+}CD4^{+}$ Treg cells, including depletion of Treg cells, are currently being tested in clinical trials [3].

FoxP3 is a master transcription factor specific to Treg cells and supports their differentiation and functions [4]. FoxP3 contains four functional domains: a proline-rich N-terminal domain (amino acids 1–193), a single C2H2 zinc finger motif (amino acids 197–222), a leucine zipper-like motif (amino acids 239–259) and a carboxyl-terminal winged-helix/forkhead (FKH) domain (amino acids 337–409) [5]. Associational studies of genetic mutations in the coding region of FoxP3 gene in patients with IPEX (Immunodysregulation Polyendocrinopathy Enteropathy X-linked syndrome) have been most informative for elucidating the structure–function correlation of each FoxP3 domain [6]. These studies have also revealed that each domain has specific binding partners [7].

Recent biochemical studies have indicated that FoxP3 forms a supra-molecular complex of 400–800 kDa or larger with 361 cofactors, and the corresponding FoxP3-mediated transcriptome may determine the functional outcome of Treg cells [8]. Therefore, preventing the formation of a functional FoxP3 interactome presents a new immunotherapeutic approach to specifically suppress the functions of Treg cells. Such suppression would re-balance the tumor microenvironment.

In this study, we have shown that the nt-FoxP3-FKH containing FKH domain of FoxP3 and the Hph-1-PTD (protein transduction

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domain) can be delivered into the nucleus effectively and inhibit FoxP3-mediated transcription in an interactomic manner. Intranuclear delivery of FKH domain of FoxP3 suppressed the functions of Treg cells, leading to down-regulation of CTLA-4 and IL-10 as well as up-regulation of IL-2 and IFN-γ in Treg cells. These results will provide a novel therapeutic strategy for cancer immunotherapy without genetic alterations, where the appropriated pro-inflammatory cytokine milieu needs to be restored by inhibition of Treg cells functions.

2. Materials and methods

2.1. Generation and purification of nucleus-transducible form of FoxP3-functional subdomains

We each of FoxP3-subdomains combined with Hph-1-PTD was cloned into pET28a plasmid, and each DNA was transformed into BL-21 Codon plus(DE3)RIPL *Escherichia coli* strain (Invitrogen). The protein purification was carried out as previously described [9], excluding wash and elution buffer Urea concentration (1 M).

2.2. Cell culture

For the isolation of CD4 T cells, splenocytes from 8 to 10 weeks-old FoxP3-IRES-RFP (FIR) mice were purified by CD4 magnetic bead (miltenyi Biotec). And then naïve T (T_N) cells ($CD4^+CD62L^+RFP^-$) or tTreg cells ($CD4^+RFP^+$) were sorted by FACSAria II cell sorter (BD Biosciences). The purity of the sorted cells was typically above 98%. For T cell activation, T_N cells were activated with plate bound α-CD3/CD28 monoclonal antibody (mAb) (2 μg/ml) in 7.5% Fetal Bovine Serum (FBS, Cellgro), 100 U/ml penicillin/streptomycin, 2 mM L-glutamine, 50 μM β-mercaptoethanol (Sigma–Aldrich) in RPMI media (Lonza). For TGF-β-induced iTreg cells differentiation, T_N cells were activated in the presence of IL-2 (100 U/ml) and 5 ng/ml of TGF-β (R&D) for 4 days. For tTreg cells expansion, tTreg cells were activated with plate bound α-CD3 mAb (5 μg/ml) and α-CD28 mAb (1 μg/ml) in the presence of IL-2 (500 U/ml).

2.3. In vitro intranuclear transduction kinetics of nt-FoxP3-functional subdomains

T_N cells were treated with nt-FoxP3-functional subdomains for proper time. Then, cells were washed twice and intracellular staining was performed according to transcription factor staining protocol (eBioscience). Intracellular level of intranuclear transduced nt-FoxP3-functional subdomains were measured by anti-HA mAb (Cell signaling) fluorescence level by FACSCalibur (BD Biosciences) and data were analyzed by FlowJo software (Treestar).

2.4. Immunocytochemistry

Hela cells were incubated with 1 μM of nt-FoxP3-functional subdomains for 15 min or 6 h. An anti-HA-mAb to detect the nt-FoxP3-functional subdomains was incubated with the cells for O/N at 4 °C temperature. Samples were stained with 0.1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) solution to visualize the nucleus and analyzed by confocal microscopy (Carl Zeiss).

2.5. Cell viability assay

Cell survival was determined using a CCK-8 assay kit. Briefly, T_N cells (1×10^5/well) in 96-well plate were incubated with nt-FoxP3-functional subdomains or Hph-1-EGFP control protein (1 μM, 100 nM, and 10 nM concentration) for 24 h. After the incubation, CCK-8 reagents were added to the culture, and 450 nm absorbance values were obtained after 6 h. The absorbance measured in this assay correlates with the number of living cells.

2.6. Luciferase assay

293T cells were transfected (For NFAT activity) with the plasmid encoding NAFT-CA (constitutively active version of NFAT, addgene), FoxP3, NFAT-luciferase reporter (NFAT-Luc) and Renilla reporter or (For FKH activity) with the plasmid encoding 3 × FKH-luciferase reporter, SV40 minimal promoter reporter flanked by multiple FKH binding sites and Renilla reporter by polyethylenimine (PEI, Sigma–Aldrich) for 6 h in 100-mm dish. Cells were harvested and separated (1×10^5/well) in 24-well plate with 1 μM of nt-FoxP3-functional subdomains for additional 24 h. Assays for luciferase activity were performed according to the manufacturer's instructions and the value was normalized by Renilla activity (Promega).

2.7. In vitro functional analysis of nt-FoxP3-functional subdomains

T cells were incubated with each of nt-FoxP3-functional subdomain or Hph-1-EGFP protein in T cell activation or iTreg cells polarization or tTreg cells expansion condition. And then, culture media was analyzed for ELISA performed as described in the manufacturer's protocol (R&D). For proliferation assay, labeled responder T cells (5×10^4/well) through cell proliferation dye 670 (2.5 μM, eBioscience) were stimulated with soluble α-CD3 mAb (0.5 μg/ml) in the presence of ΔCD4-APC (1×10^5/well) treated with mitomycin C (50 μg/ml, Sigma–Aldrich) and were cultured with nt-FoxP3-functional subdomains (100 nM) for 72 h in 96-well round bottom plates. For Treg cell suppression assay, 5×10^4, labeled responder cells were stimulated for 3 days as described above, and culture with 10^5 (2:1), 5×10^4 (1:1), 2.5×10^4 (0.5:1) iTreg cells or tTreg cells cultured with nt-FoxP3-functional subdomains or EGFP control protein. Proliferation of responder cells were measured on FACSCalibur (BD Biosciences).

2.8. Statistical analysis

The results are expressed as a mean ± the standard error of the mean. ($n = 3$ or more) Statistical analysis of group differences was examined using an unpaired Student's t-test. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ were considered to be significant.

3. Results

3.1. Generation of nucleus-transducible forms of FoxP3-functional subdomains

It has been established that FoxP3 plays a major role in forming a Treg cell-specific transcription complex and establishes Treg cell-integrity. Our previous study demonstrated that T cells functionally similar to Treg cells can be successfully generated by the intranuclear delivery of full-length FoxP3 protein into primary T cells using Hph-1-PTD, resulting in the effective suppression of autoimmunity in vitro and in vivo [9]. To inhibit the immunosuppressive functions of Treg cells without using genetic methods, we developed a novel strategy to disrupt the formation of the FoxP3-mediated transcriptome by intranuclear delivery of a functional subdomain of FoxP3. This functional subdomain of FoxP3 delivered into the nucleus of Treg cells works as a competitive and interactomic inhibitor preventing endogenous FoxP3 from interacting with either transcriptional cofactors or DNA. Disintegration of FoxP3-mediated transcriptome formation leads to the prevention of the gene expression induced by FoxP3 (Fig. 1).

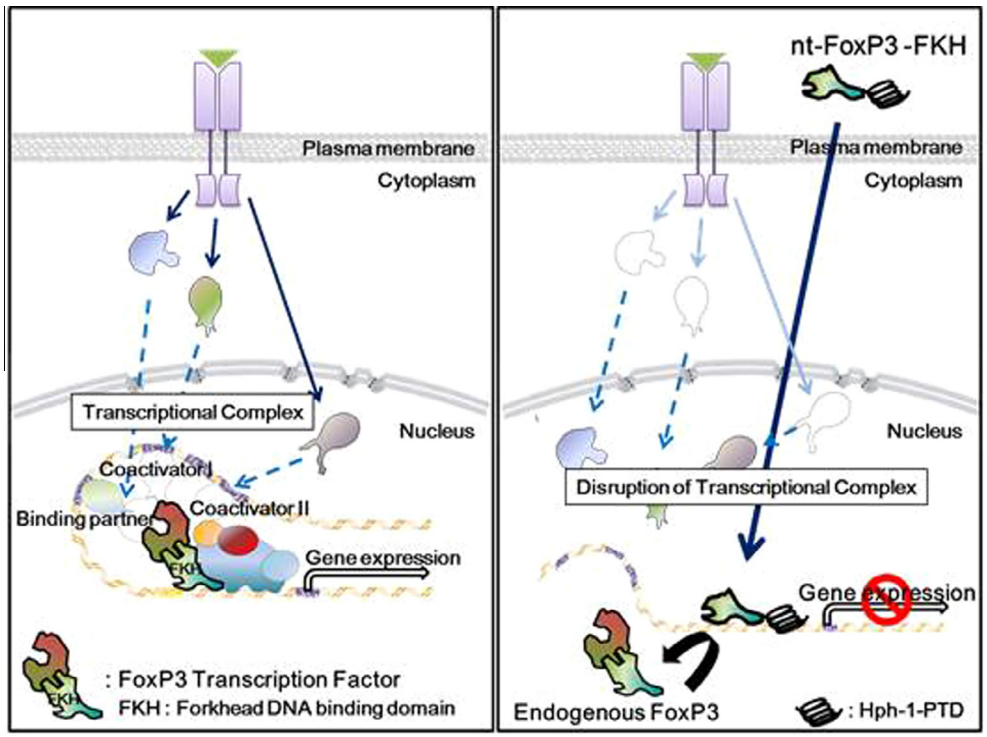

Fig. 1. Novel strategy to modulate the functions of FoxP3. Intra-nucleus delivery of functional subdomains of FoxP3 can disrupt a transcription complex either by interactomic competition with endogenous FoxP3 for DNA or cofactor binding.

DNA sequences encoding each functional subdomain of FoxP3 were inserted into the pET plasmid vector with double Hph-1-PTD (YARVRRRGPRR). These domains include the proline-rich N-terminal domain (nt-FoxP3-PR), the zinc finger and leucine-zipper-like motif (nt-FoxP3-ZL), the highly conserved carboxy terminal FKH domain (nt-FoxP3-FKH) and the region located in between the leucine zipper and the FKH domain (nt-FoxP3-AR) known to play important roles in FoxP3 functions by binding AML1/RUNX1 [10]. Each recombinant protein was expressed in *E. coli*, purified under denaturing conditions, and characterized by western blot with anti-HA or an anti-FoxP3 mAb that detects exon 2 in the N-terminal domain (Fig. 2A and B).

3.2. Effective intranuclear delivery of nt-FoxP3-functional subdomains without cellular toxicity

To examine the intracellular transduction efficiency of nt-FoxP3-functional subdomains in primary CD4 T cells, $CD4^{+}CD25^{-}$ T cells were incubated with the nt-FoxP3-functional subdomains followed by measurements of the intracellular level of each subdomain protein by intracellular staining. All nt-FoxP3-functional subdomains have similar intra-cellular transduction capacity (Fig. 2C), these domains were detectable in cells at 2 h post transduction, reached the maximal intracellular level at 48 h, and started to decrease in concentration until 96 h post transduction (data not shown). Since it is critical for nt-FoxP3-functional subdomains to be transduced into the nucleus to modulate FoxP3-mediated transcription, we also measured the intranuclear transduction kinetics of nt-FoxP3-functional subdomains. The subdomains were all detectable in the cytoplasm as well as in the nucleus within 15 min after transduction, and most proteins were localized completely to the nucleus at 6 h after transduction (Fig. 2D). The transduced nt-FoxP3-functional subdomains did not cause any adverse effects on cell viability of CD4 T cells even at high concentrations (Fig. 2E).

3.3. Characterization of nt-FoxP3-functional subdomains as inhibitors of FoxP3

Previous study demonstrated that FoxP3 interacts with NFAT to repress the trans-activation of transcription of inflammatory cytokine genes independent of the nuclear localization of NFAT [11]. To determine which nt-FoxP3-functional subdomains can inhibit the suppressive activity of FoxP3 on NFAT-mediated gene expression, we transduced each nt-FoxP3-functional subdomain into 293T cells, which were co-transfected with the plasmids expressing NFAT-CA (constitutively active form of NFAT), full-length FoxP3, and luciferase driven by NFAT binding sites. Interestingly, nt-FoxP3-FKH is most effective in restoration of NFAT-CA activity when compared with the activities of the other nt-FoxP3-functional subdomains or EGFP as a control (Fig. 3A).

Next, we investigated whether the effects of nt-FoxP3-FKH require direct DNA binding. The DNA binding ability of nt-FoxP3-FKH and subsequent transcription inhibition were measured in 293T cells, which were transfected with the plasmid expressing the luciferase gene driven by the SV40 minimal promoter flanked by multiple FKH binding sites ($3 \times$ FKH-luc). As shown in previous studies, the FKH domain of many endogenous transcription factors present in 293T cells can induce luciferase expression through the binding to FKH binding sites [12]. Consistent with the result in Fig. 3A, nt-FoxP3-FKH treatment resulted in substantial reduction of luciferase expression compared to that of the control protein or other nt-FoxP3-functional subdomains (Fig. 3B). This result demonstrated that nt-FoxP3-FKH directly binds to FKH binding sites via interactomic competition with other FKH domain-containing proteins.

As ectopic expression of FoxP3 or transduction of cell-permeable FoxP3 protein in T cells induced a hypo-responsive state in CD4 T cells [13], we investigated the effects of nt-FoxP3-functional subdomains on T cell proliferation or apoptosis. None of the nt-FoxP3-functional subdomains influenced apoptosis or proliferation

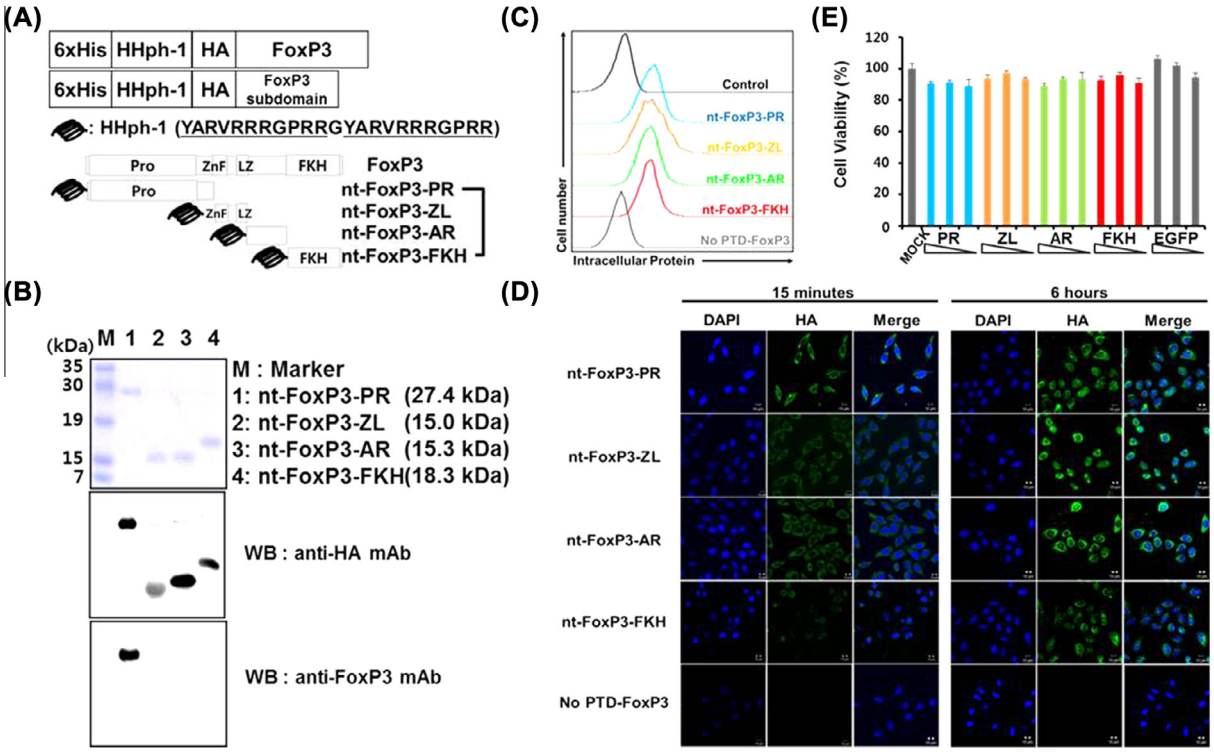

Fig. 2. Generation of nucleus-transducible forms of FoxP3-functional subdomains. (A) Construct of four types of nt-FoxP3-functional subdomains with Hph-1-PTD (nt-FoxP3-PR: FoxP3-proline rich domain, nt-FoxP3-ZL: FoxP3-zinc finger and leucine zipper domain, nt-FoxP3-AR: FoxP3-AML1/RUNX1 binding region, nt-FoxP3-FKH: FoxP3-Forkhead DNA binding domain). (B) The proteins purified under denaturing conditions were confirmed by Western blot with anti-HA or anti-FoxP3 mAb. (C) Intracellular transduction efficiency of each nt-FoxP3-functional subdomain was examined by intracellular staining with anti-HA mAb after mouse $CD4^+CD25^-$ T cells were treated with 1 μM of the protein for 2 h. (D) Nuclear localization of each nt-FoxP3-functional subdomain was analyzed by intracellular staining with anti-HA mAb at 15 min or 6 h of post-transduction followed by confocal microscopic analysis. (E) CD4 T cells were treated with decreasing amounts of nt-FoxP3-functional subdomains (1 μM, 100 nM, or 10 nM), and cell viability was measured by CCK-8 assay. Error bars represent S.D.

of effector T cells which were generated from T_N cells by TcR stimulation with anti-CD3/28 mAb (Fig. 3C). The induced expression of CD69 on the surface of activated T cells was not affected by treatment with any of nt-FoxP3-functional subdomains. In the presence of TCR stimulation, CD69 expression was similar among all treatment conditions. Unlike transduction of full-length FoxP3 protein or ectopic expression of FoxP3 which can induce the expression CD25, a marker for T cell activation as well as for Treg cells, intranuclear delivery of the nt-FoxP3-functional-subdomains did not increase CD25 expression (Fig. 3D). Thus, nt-FoxP3-FKH has the capacity to inhibit the transcriptional activity of endogenous FoxP3 in interactomic and competitive manner without affecting T cell activation and viability.

3.4. The nt-FoxP3-FKH overcomes the inhibitory effect of FoxP3 in iTreg cells and tTreg cells

A variety of tumor-derived factors including IL-10, TGF-β, VEGF, PGE2, and soluble phosphatidylserines contribute to the formation of an extensive immunosuppressive microenvironment. It is also known that TGF-β secreted from tumor cell-stimulated immature myeloid DCs induces the conversion of T_N cells to $FoxP3^+$ Treg cells [14]. To examine if nt-FoxP3-FKH or other nt-FoxP3-functional subdomains alter immunosuppressive activity of TGF-β-induced Treg cells (iTreg cells), we conducted a mixed lymphocyte suppression assay with iTreg cells treated with the nt-FoxP3-functional subdomains.

Differentiation of T_N cells ($CD4^+FoxP3^-CD62L^+$) from FIR mice were induced under iTreg cells polarizing conditions in the presence of each of the nt-FoxP3-functional subdomains for 96 h. In the next step, iTreg cells generated under different conditions were co-cultured with CFSE-labeled responder cells at varying ratios. We found that nt-FoxP3-FKH-treated iTreg cells demonstrated a substantially reduced immunosuppressive activity compared to that of iTreg cells treated with EGFP or the other nt-FoxP3-functional subdomains (Fig. 4A).

To test whether the inhibition of FoxP3 by nt-FoxP3-functional subdomains during iTreg cells differentiation affected the expression of suppressor–effector genes, we evaluated CTLA4 protein expression on iTreg cells by flow cytometry. While FoxP3 protein levels were comparable among all conditions, CTLA4 expression, which is known to be dependent on NFAT activity, was significantly decreased by nt-FoxP3-FKH treatment compared to the control (Fig. 4B).

Next, we investigated changes in the cytokine secretion pattern of iTreg cells upon treatment with nt-FoxP3-functional subdomains. Previous studies have shown that FoxP3 is a repressor of IL-2 and IFN-γ gene expression through direct interaction with NFAT [11]. Consistent with the results in Fig. 3A and B, nt-FoxP3-FKH treated iTreg cells showed a significant increase in IL-2 and IFN-γ expression in a dose-dependent manner when compared with iTreg cells treated with EGFP. In addition, iTreg cells treated with other nt-FoxP3-functional subdomains showed a slight increase in IL-2 expression (Fig. 4C–E).

The functional capacity of nt-FoxP3-FKH to inhibit FoxP3-mediated transcription was also confirmed with thymic-derived Treg (tTreg) cells. tTreg cells ($CD4^+FoxP3^+$) isolated from FIR mice were incubated with different nt-FoxP3-functional subdomains under Treg cells expansion conditions. After 48 h when most proteins were localized in the nucleus, tTreg cells were co-cultured with

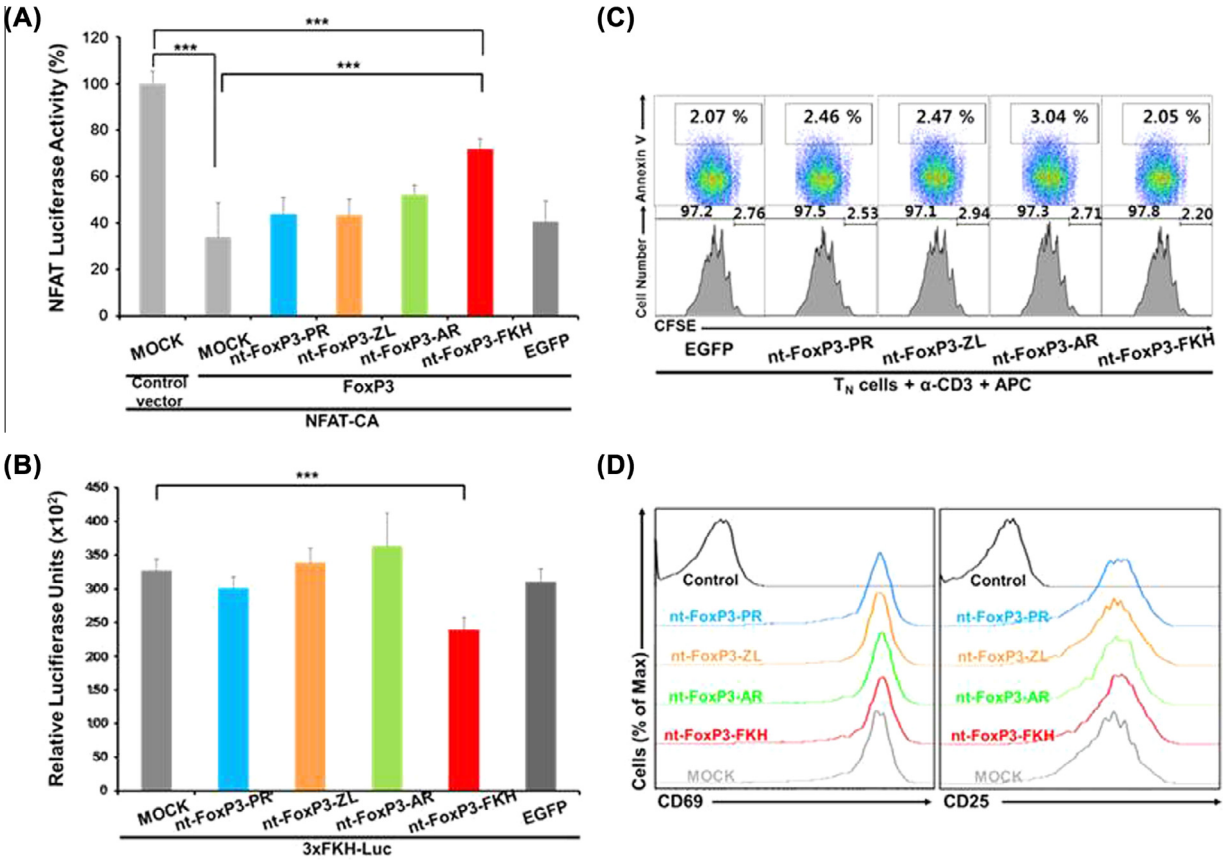

Fig. 3. nt-FoxP3-FKH restored transcription activities suppressed by FoxP3 without influencing cell proliferation and viability. (A, B) 293T cells were transiently transfected with (A) plasmids encoding NFAT-CA, FoxP3 and NFAT-luciferase reporters or (B) 3 × FKH-luciferase (Luc) reporter constructs which include 3 repeats of forkhead binding DNA sequence. Cells were incubated for 24 h in the presence of each nt-FoxP3-functional subdomain or EGFP control protein (1 μM). Relative luciferase unit was normalized by *Renilla* activity. (C) CFSE-labelled T_N cells were stimulated with soluble α-CD3 mAb in the presence of ΔCD4-APC treated with mitomycin C and cultured with each nt-FoxP3-functional subdomain (100 nM) for 72 h. (D) T_N cells were stimulated with plate bound α-CD3/CD28 mAb with each nt-FoxP3-functional subdomain (100 nM) and the level of CD69 or CD25 expression was analyzed by flow cytometry. Error bars represent S.D. $^{***}P < 0.001$ was considered to be significant.

CFSE-labeled responder cells at varying ratios. In tTreg cells treated with nt-FoxP3-FKH, the percentage of CFSE-negative T cells was substantially increased in a concentration dependent manner, and an increase in IL-2 (data not shown) and a decrease in IL-10 secretion was observed (Fig. 4F and G). Taken together these results strongly suggest that nt-FoxP3-FKH debilitates the immunosuppressive functions of Treg cells by inhibition of FoxP3-mediated gene expression.

4. Discussion

The pivotal role of FoxP3$^+$ Treg cells in mediating immune homeostasis and maintaining peripheral tolerance has become widely appreciated [15]. However, the immunoregulatory function of Treg cells may hinder the activity of the tumor-specific T effector cells, and therefore Treg cells represent one of the main obstacles to effective anti-tumor responses [16]. Several cancer immunotherapies targeting FoxP3$^+$ Treg cells such as reducing Treg cells or attenuating their suppressive activity in tumor tissues have been attempted [17]. However, the caveat in using such drugs lies in the lack of specificity, which leads to inconsistent clinical outcomes in different groups. Therefore, a novel strategy without genetic alteration, cellular toxicity, or the usage of viral vectors needs to be developed.

In this study, we demonstrated that intranuclear delivery of nt-FoxP3-functional subdomains into Treg cells suppresses the transcriptional activities of endogenous FoxP3 in an interactomic inhibitory manner, leading to the inhibition of the immune-suppressive potential of Treg cells. Among nt-FoxP3-functional subdomains, the nt-FoxP3-FKH is the most effective in functional suppression of Treg cells. This result is consistent with the previous finding that missense mutations in IPEX patients have been mostly localized to the FKH domain of the FoxP3 gene, which was responsible for DNA binding and consequently for its activator/repressor functions. As expected, treatment of Treg cells with nt-FoxP3-FKH significantly increases IL-2 and IFN-γ expression, but decreases CTLA-4 and IL-10 expression in Treg cells.

The effectiveness of functional modulation of a transcription factor heavily relies on its accessibility to the nucleus where most transcription factors function to regulate gene expression. It has been reported by our group and others that Hph-1-PTD can be used as stable and controllable transducing machinery [18]. In particular, our previous study and this one demonstrated that Hph-1-PTD-conjugated full-length FoxP3 protein as well as nt-FoxP3-functional subdomains can be delivered into the nucleus within a short time frame and exert their functions effectively.

Among different nt-FoxP3-functional subdomains the nt-FoxP3-FKH domain alone can directly bind the FKH binding sequence and regulate the transcriptional activity of the FoxP3 protein most effectively by interactomic competitive inhibition. Structural analysis of the FKH domain of the FoxP3 protein indicates that the FKH domain contains the DNA-binding region, the NFAT1 binding region, and a domain-swapped FoxP3 dimer region [19].

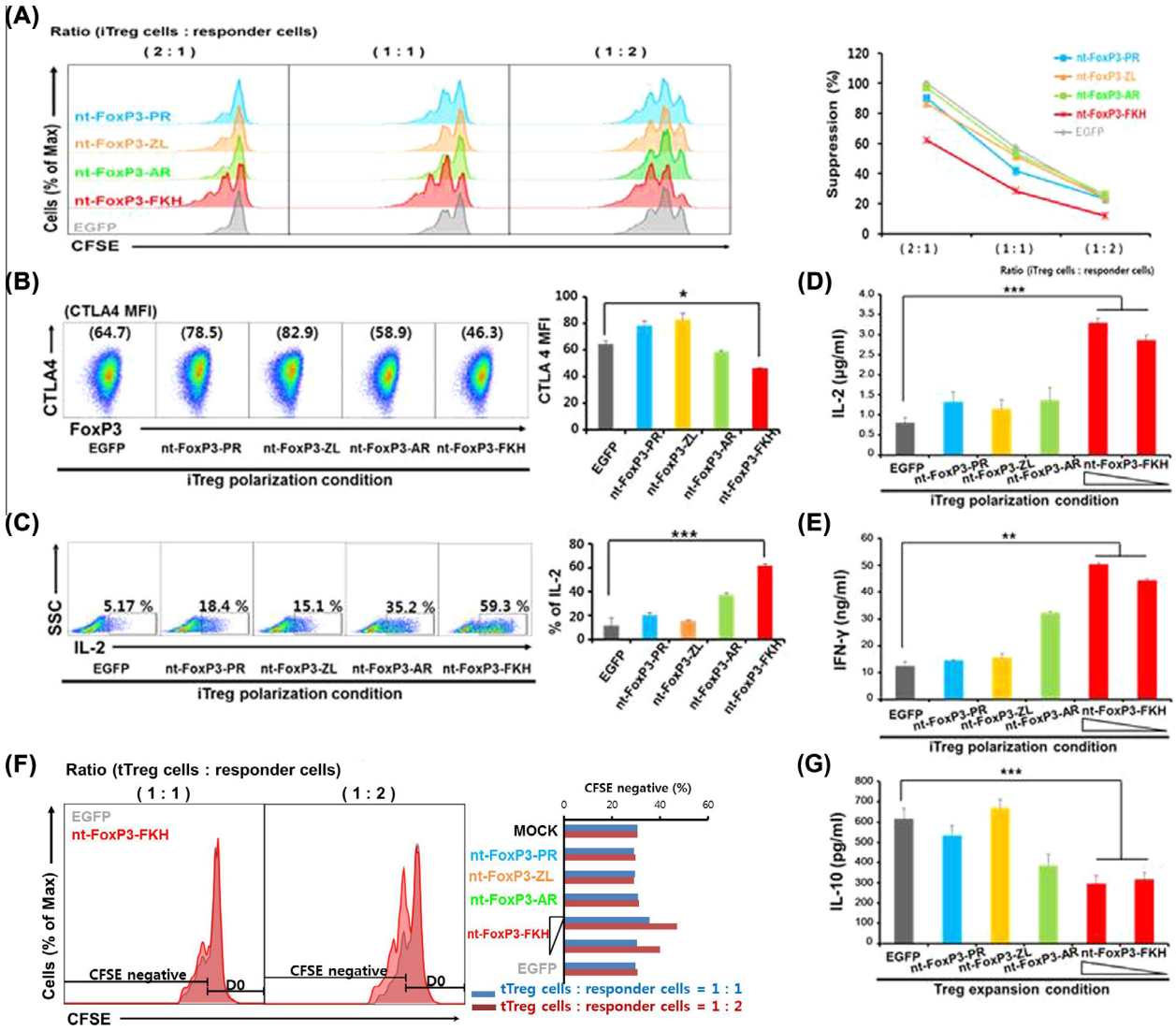

Fig. 4. nt-FoxP3-FKH significantly inhibited the functions of Treg cells. (A–E) T_N cells from FIR mice were incubated in iTreg polarization conditions with each nt-FoxP3-functional subdomain for 96 h. (A) To elucidate iTreg cells suppression activity, CFSE-labeled responder cells were cultured with these iTreg cells at different ratios. The suppression activity was calculated by the percentage of cells staying at division 0 (D0), compared to that with the EGFP-treated control group. (B, C) iTreg cells were harvested and stained for intracellular antigens CTLA4, FoxP3 or IL-2. (D, E) The level of IL-2 or IFN-γ production in the culture media was analyzed by ELISA. (F, G) tTreg cells were cultured in Treg cells expansion condition with each nt-FoxP3-functional subdomain (100 nM) for 48 h. (F) tTreg cells were harvested for suppression assay (right panel: percentage of CFSE negative population after treatment with each nt-FoxP3-functional subdomain). (G) The level of IL-10 level in culture media was analyzed by ELISA. Error bars represent S.D. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ were considered to be significant.

Therefore, the modulatory effects of the FKH domain on FoxP3-mediated gene expression could be due to the loss of FoxP3 binding to the promoter regions with NFAT or the loss of dimerization of FoxP3 via the FKH domain. Although the proline-rich N-terminal region is known to be involved in the suppressive activity of Treg cells, in this study, nt-FoxP3-PR did not show significant inhibitory effects on the Treg cells suppressive activity. This is probably because its suppressive capacity necessitates DNA binding to inhibit endogenous FoxP3 functions. Surprisingly, the nt-FoxP3-AR-containing Runx1 binding site demonstrated substantial inhibition of FoxP3 functions as shown in Fig. 4. Therefore, the nucleus-transducible form of the FKH domain and proline-rich N-terminal domain or AR domain together may exert a stronger inhibitory effect on FoxP3 functions than that of each subdomain.

A recent study by Rudra et al. has identified several FoxP3 binding partners, some of which are already known to interact with FoxP3 [8]. Interestingly, the authors did not observe other FoxP3-associated proteins such as Irf4 or Hif1α in their system, suggesting that the FoxP3 interactome may change in need of particular functionality of Treg cells in different environments. That is, the binding partners participating in forming FoxP3 complexes may determine not only the molecular functions of FoxP3 but also the relevant phenotype of Treg cells in order to effectively control the immune system. Therefore, specific disruption of physical interactions of FoxP3 with its binding partners through nt-FoxP3-functional subdomains can be used for regulation of Treg cells functions in different disease settings.

In summary, our study presents a novel therapeutic strategy to directly modulate the functions of FoxP3 protein via interactomic competition without genetic manipulation. Furthermore, our strategy of directly targeting FoxP3 transcription complexes may provide more effective cancer immunotherapeutic method as it can modulate Treg cells activity rather than completely eliminating the cells from the immune system. Nucleus-transducible forms of various FoxP3 functional subdomains in this study can be used in vaccine and tumor therapy.

Acknowledgments

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