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Chromosome 1 Open Reading Frame 190 Promotes Activation of NF-κB Canonical Pathway and Resistance of Dendritic Cells to Tumor-Associated Inhibition In Vitro

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Tumor-associated dendritic cells (DCs) often induce T cell anergy or deletion and regulatory T cells instead of antitumor immunity. Although many tumor-associated Ags have been found, there is still no effective vaccine for cancer. Thus, novel rational strategies to enhance the immunogenicity of cancer-specific Ags are needed. Chromosome 1 open reading frame 190 (c1orf190), a gene that encodes a 239-aa hypothetical protein and contains multiple kinase phosphorylation sites, has a wide relationship with multiple signaling pathway molecules and can be regulated by multiple factors, such as TLR ligands. In this study, we demonstrate that c1orf190 can activate NF-κB, drive the production of cytokines, and promote the Ag-presenting function and the priming ability of DCs. Furthermore, c1orf190 can promote resistance of DCs to tumor-associated inhibition not only in the Ag-presenting function but also in the priming ability to induce Ag-specific T lymphocytes. Thus, c1orf190, an NF-κB activator, may be a candidate gene for regulating the function of DCs to resist tumor-associated factor-mediated dysfunction. We also found that c1orf190-mediated cytokine release is achieved by activating the canonical but not the noncanonical NF-κB pathway. The Journal of Immunology, 2010, 185: 6719–6727.
C1orf190 may be a candidate gene for regulating the function of DCs.

Materials and Methods

Preparation of monocyte-derived DCs

Monocyte-derived DCs were prepared from monocytes according to our previously described protocol (17, 18). Briefly, after Ficoll-Hypaque separation, peripheral blood cells were resuspended in RPMI 1640 medium supplemented with 10% FBS, 2 mM l-glutamine, and 1% penicillin-streptomycin. The cells were incubated overnight at 37°C, and the non-adherent cells were removed by gentle pipetting. The adherent cells were cultured in RPMI 1640 and supplemented with 10% FBS containing 1000 U/ml GM-CSF (R&D Systems, Minneapolis, MN) and 1000 U/ml IL-4 (R&D Systems). After 6 d, floating DCs were harvested in PBS and cell morphology was determined by standard microscopic techniques. Phenotypes of DCs were analyzed by PE- or FITC-labeled anti-CD11c (3.9), anti-human MHC class II-DR (L243), anti-human CD86 (IT2.2), anti-human CD80 (2D10.4), anti-human CD40 (HB14), anti-human B7H1 (M1H1), and anti-human CD11b (ICRF44) that were purchased from BD Pharmingen (San Diego, CA).

Gene cloning

C1orf190 cDNA was obtained by the PCR from monocyte-derived DCs with TaqDNA polymerase. The PCR product was cloned into the eukaryotic expression vector pcDNA3.1/V5-His-TOPO as c1orf190 pcDNA (Invitrogen, Carlsbad, CA). C1orf190 pcDNA was used to transfect HEK 293T cells using Lipofectamine 2000 (Invitrogen) and used to transfected DCs using a Nucleofection approach according to the manufacturer’s protocol (Amaxa Biosystems, Cologne, Germany) after sequencing. The forward primer 5’-ATGGAGGGGACCGTGGAATCCAGACG-3’ and reverse primer 5’-CAAGAAGGTCACTACATCTGGGCACGT3’ were used to clone human c1orf190 gene.

RT-PCR analysis

Total cellular RNA was prepared using TRIzol reagent (Invitrogen) as recommended by the manufacturer. RT-PCR was performed by SuperScript one-step RT-PCR with Platinum Taq according to the protocol provided (Invitrogen). The amplification conditions included the following steps: one cycle of CDNA synthesis and pre-denaturation (50°C for 15 min, 94°C for 2 min), 40 cycles of PCR amplification (denaturation at 94°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min/kb), and one cycle of final extension (72°C for 10 min). The primers used in this study are listed in Supplemental Table I.

Reporter assay

For the reporter assay, p-NF-κB–secreted alkaline phosphatase (SEAP), p-AP-1-SEAP, p-NF-AT-SEAP, p-MyC-SEAP, p-serum response element (SRE)-SEAP, p-cAMP response element (CRE)-SEAP, p-hepatocyte response element (HSE)-SEAP, and positive control p-SEAP2 as well as negative control p-TATA-like-SEAP reporter constructs were purchased from BD Clontech (Palo Alto, CA) and used to cotransfect the cells with the plasmids. As described before (19), 293T cells were seeded in 96-well plates and cotransfected with c1orf190 construct combined with reporter plasmids as indicated. Twenty-four hours after transfection with Lipofectamine 2000, according to the manufacturer’s instructions, cells were treated with the indicated reagents or left untreated for 12 h. The samples were harvested and processed as described above for determination of SEAP activity and normalized for the β-galactosidase activity (BD Clontech). The amount of transfected DNA was equalized with empty expression vectors, which were also used in the control, along with either NF-κB or other SEAP reporter constructs.

Small interfering RNA experiments

To knock down c1orf190, the small interfering RNA (siRNA) templates used to knock down the c1orf190 were selected and designed using the siRNA target finder (http://www.ambion.com/techlib/misc/siRNA_finder.html) (Supplemental Table II). To maximize knockdown and minimize off-target effects, each gene was given two couples of the siRNA templates. Searches of the Homo sapiens genome database (BLAST) were carried out to ensure that the sequences would not target other genes. Then the siRNA template was cloned into the p-feline immunodeficiency virus (FIV) double-promoter siRNA-expressing vector p-FIV-H1-U6-copGFP, which contains human U6 and mouse H1 double promoters according to the protocol described by the manufacturer (System Biosciences, Mountain View, CA). Irrelevant siRNA was used as a control. siRNA structures were directly used to transfect the 293T cells using Lipofectamine 2000 and used to transfect DCs using a Nucleofection approach. Silencing of the target molecule was demonstrated using RT-PCR.

siRNA for NF-κB–inducing kinase (siNIK), IKKα (siIKKα), or control siRNA (50 nmol/ml) was used to transfect the cells using Lipofectamine 2000 according to the manufacturer’s instruction. The siRNA sequences used to target human NIK and IKKα mRNA were as follows: siNIK, 5’-GCUCCGCUACAGGCUUGATT-3’ (sense) and siIKKα, 5’-GCUCUGUAGUCCUGAATTT-3’ (sense). Nonsilencing control siRNA is an irrelevant siRNA with random nucleotides (5’-ACUATCUAGUACTACTCCCTT-3’). Sequences were synthesized and annealed by Shanghai Sangon Biological Engineering Technology and Services (Shanghai, China). NF-κB essential modulator (NEMO) siRNA (M-003767-02) and non-targeting control (D001201-03) were obtained from Dharmacon (Lafayette, CO) as a pool of four annealed dsRNA oligonucleotides.

NEMO-binding domain peptide-mediated NF-κB inhibition

For NEMO-binding domain (NBD) peptide-mediated inhibition, both the functional wild-type TAT-NBD (YGRKKRRQRRR–G–TTLWSLWQME) and the negative control mutant TAT-NBD (YGRKKRRQRRR–G–TTL-DSALQME) were used to cotransfect the cells using Lipofectamine 2000 along with either p-FIV-H1-U6-copGFP, which contains human U6 and mouse H1 double promoters according to the protocol provided by Tao et al. (20, 21). The c1orf190-transfected DCs were incubated for 2 h with the NF-κB peptide or control mutant NB. The effect on the NF-κB activity

FIGURE 1. Characterization of c1orf190 gene. A. The ideogram of human chromosome 1 with c1orf190. C1orf190 is located in 46669006–46686928pb on chromosome 1p34. B. The schematic diagram of c1orf190 protein showing 26 hits with a high probability of occurrence by ScanProsit (http://us.expasy.org/tools/scanprosite/). C. The expression of c1orf190 as a cytoplasmic protein. 293T cells were transfected with V5-tagged c1orf190 vectors and stained using FITC-labeled anti-V5 Abs. An image was collected by confocal microscopy (original magnification x400) (C2). An image collected using bright light was used as a control (C1). D. The transcriptional levels of c1orf190 in DCs after exposure to different TLR ligands. DCs were cultured with 5 μg/ml bacterium DNA, 1 μg/ml LPS, and 2.5 μg/ml polyinosinic-polycytidylic acid. After 24 h, c1orf190 transcriptional levels were detected using RT-PCR.
and cytokine release were analyzed after 24 h. NBD and control mutant peptides were used at a concentration of 50 μM.

Western blot analysis

For Western blot analyses, the cells were harvested and subjected to SDS-PAGE. Following the transfer to a Hybond-P membrane (Amersham Biosciences, Piscataway, NJ), the samples were analyzed by Western blotting with anti-p-IκBα or anti-active IκBα Ab (Cell Signaling Technology, Beverly, MA). The protein–Ab complexes were detected using the peroxidase-conjugated secondary Ab (Boehringer Mannheim, Mannheim, Germany) and ECL (Amersham Biosciences).

Immunofluorescence and fluorescence microscopy

The cells were washed with PBS, fixed with 3.7% formaldehyde solution for 10 min, permeabilized with 0.1% (v/v) Triton X-100 in BPS for 5 min, and blocked with PBS containing 1% BSA for 30 min. For the transfected cells, plasmid expressing V5 FITC-labeled mAb (Invitrogen) was added at 1 μg/ml to detect V5. For the nuclear localization of RelA/p65, the transfected cells were incubated for 1 h with a 1/1000 dilution of the specific polyclonal antiserum against RelA/p65 (sc-372; Santa Cruz Biotechnology, Santa Cruz, CA). The cells were then washed with PBS and labeled with a Cy3-conjugated secondary Ab (Millipore, Bedford, MA). The nuclei were stained with DAPI (blue). Original magnification ×40. RLU, relative light unit.

FIGURE 2. C1orf190 activates NF-κB. A, The expression of c1orf190 in the transfected 293T cells and DCs. A1, The transcriptional levels of c1orf190 in 293T cells (A1,1) and DCs (A1,2) transfected with c1orf190-targeted siRNA (siRNA) or control siRNA (mocksiRNA); A2, The protein levels of V5-fused c1orf190 in 293T cells (A2,1) and DCs (A2,2) transfected by V5-tagged c1orf190 vectors (C1orf190/V5) or control vectors (Vector.ctr). 293T cells and DCs were transfected using the protocol described in Materials and Methods. The transcriptional levels of c1orf190 were detected using RT-PCR. C1orf190/V5 fusion protein was detected by anti-V5 Abs (Invitrogen) using Western blotting. B, Effect of c1orf190 siRNAs and ectopic c1orf190 on the activity of transcriptional factors NF-κB, AP-1, and NF-AT. C1orf190-specific siRNAs downregulated the activity of NF-κB and NF-AT, whereas ectopic c1orf190 upregulated the activity of NF-κB and NF-AT in both 293T cells (B1) and DCs (B2). MocksiRNA, siRNA, Vector.ctr, and c1orf190 are 293T cells (B1) or DCs (B2) transfected by control siRNA, c1orf190 siRNA, control vectors, and c1orf190 vectors, respectively. C, The effect of c1orf190 siRNA and ectopic c1orf190 on NF-κB activity is dose-dependent. Increasing amounts of c1orf190 siRNA or c1orf190 vectors were cotransfected with p-SEAP reporter constructs (250 ng) into 293T cells. The supernatants were harvested and tested using a SEAP reporter assay after 24 h. D, Ectopic c1orf190 promotes the nuclear translocation of p65. 293T cells (D1) or DCs (D2) transfected by c1orf190 vector (C1orf190) or control vector (Vector.ctr) were plated on gelatin-coated coverslips and the subcellular localization of NF-κB p65 was analyzed by immunofluorescence with a specific polyclonal antiserum against p65. The nuclei were stained with DAPI (blue). Original magnification ×40. RLU, relative light unit.

Functional analysis

To determine the function of DCs transfected by c1orf190, we first observe the effect of c1orf190 on the ability of DCs to stimulate the Ag-specific T cells.

We prepared influenza peptide-specific HLA-A0201–restricted CD8+ CTLs using our previous protocol (18). In brief, isolated CD8+ T lymphocytes with a purity of >95% were seeded into 48-well plates (PolySorb; Nunc, Roskilde, Denmark) at a concentration of 5 × 10^5 cells/well in 10% human AB serum-RPMI 1640 medium. As APCs, autologous DCs were incubated with 20 μg/ml peptides in serum-free RPMI 1640 medium for 2 h at 37°C in 5% CO2. After washing, DCs were added to the plates at a concentration of 1 × 10^5 cells/well. IL-7 (10 ng/ml) was added at the initiation of the cultures. After incubation for 48 h, IL-2 (10 U/ml) was added to the cultures. As the controls, CD8 T cells were cultured with cytokines alone at the same time. In some cases, at day 14 and day 21, CD8 T cells were stimulated with peptide-loaded, irradiated autologous DCs. Influenza virus-specific CTLs were then cocultured with HLA-A2–restricted peptide-pulsed DCs transfected with c1orf190 or c1orf190 siRNA. After incubation for 48 h, supernatants were collected and IFN-γ was measured by ELISA. To load DCs with peptides, DCs were pulsed with influenza-restricted peptide (20 μg/ml) in RPMI 1640 medium for 3 h.
To investigate whether c1orf190-transfected DCs could resist the effect of tumor-associated factors on the priming ability of DCs, we initially generated synthetic Melan-A/MART-1 mRNA, which can induce MHC class I-restricted cytotoxic T cells in vivo and in vitro (22, 23). A full-length cDNA fragment from the plasmid (American Type Culture Collection, Manassas, VA) was subcloned into the plasmid pSP-64 (poly(A)) at Hinc1 and Xmn1 (Promega, Madison, WI) in front of a synthetic poly(A) tail, which allows in vitro transcription under the control of an SP6 promoter. The plasmids were linearized behind the poly(A) tail by restriction enzyme digestion at Fsp1 site and in vitro transcribed with the SP6 mMESSAGE mMACHINE kit (Ambion, Austin, TX) according to the protocol provided by the manufacturer. Purification of in vitro transcripts was performed with RNeasy Mini anion-exchange spin columns (Qiagen, Valencia, CA) according to the RNA cleanup protocol provided by the manufacturer. These synthetic Melan-A/MART-1 mRNAs were used to cotransfect DCs with c1orf190 vectors, c1orf190 siRNA, or control vectors using Nucleofector technology (Amazax Biosystems, Gaithersburg, MD). The transfected DCs, after having been cocultured with ovarian carcinoma SK-OV-3, cervical carcinoma HeLa cells, and SiHa cells in a 24-Transwell plate, TGF-β1 (10 ng/ml), or IL-10 (10 ng/ml) for 18 h. These treated DCs, after having been loaded with HLA-A2–restricted peptides, were cocultured with influenza virus-specific CTLs. After 48 h, supernatants were collected and IFN-γ was measured by ELISA.

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### Materials and Methods

Supernatants from culture medium were collected and subjected to an ELISA assay using ELISA kits for the cytokines IL-1β, IL-6, and IL-12p70 (Pierce/Endogen, Rockford, IL). The OD value of each of the samples was measured at 450 nm using a SpectraMax 190 ELISA plate reader. Cytokine levels were quantified by two to three titrations using standard curves and expressed in picograms per milliliter.

### Statistical analysis

For statistical analysis, we used the Student t test, and a 95% confidence limit was taken to be significant (defined as p < 0.05)

### Results

C1orf190 activates NF-κB

C1orf190, a hypothetical protein, which can be detected in human DCs (GEO DataSets [http://www.ncbi.nlm.nih.gov/sites/entrez]: GDS2750/235214_at/C1orf190/Homo sapiens, GDS2453/235214_at/C1orf190/Homo sapiens, and GDS1249/235214_at/C1orf190/Homo sapiens), includes a 720-bp open reading frame that encodes a 239-aa protein (LOC541468) (Fig. 1). A ScanProsite analysis (http://us.expasy.org/tools/scanprosite/) showed that c1orf190 contained N-myristoylation sites (MYRSTYL), an amidation site (AMIDATION), CKII phosphorylation sites (CK2_PHOPHO_SITES), a leucine zipper pattern (LEUCINE_ZIPPER), a cAMP- and cGMP-dependent protein kinase phosphorylation site (CAMP_PHOPHO_SITE), and a protein kinase C phosphorylation site (PKC_PHOPHO_SITE). A fusion protein of c1orf190 tagged with VS could be detected in the cytoplasm of transfected 293T cells (Fig. 1C). Importantly, c1orf190 expression in DCs could be regulated by multiple factors, including TLR ligands (GEO DataSets: GDS2750/235214_at/C1orf190/Homo sapiens; Fig. 1D), hypoxia (GEO DataSets: GDS2750/235214_at/C1orf190/Homo sapiens), and peroxisome proliferator-activated receptor-γ ligand or the retinoic acid receptor-α antagonist (GEO DataSets: GDS2453/235214_at/C1orf190/Homo sapiens). CKII (24), PKC (25, 26), cAMP, and cGMP-dependent protein kinase (27), as protein serine/threonine kinase.

### FIGURE 3

C1orf190 drives the production of proinflammatory cytokines and promotes the Ag-presenting function of DCs. A. The transcriptional levels of IL-1β, IL-6, and IL-12 in DCs transfected by c1orf190 siRNA or ectopic c1orf190. Ctr., C1orf190, MocksiRNA, and siRNA are DCs transfected by control vectors, c1orf190, control siRNA, and c1orf190 siRNA, respectively. B. Cytokine secretion by DCs transfected by c1orf190 or c1orf190 siRNA. Supernatants from culture medium were collected and subjected to an ELISA assay using ELISA kits for the cytokines IL-1β, IL-6, and IL-12 as described in Materials and Methods. Ctr., C1orf190, MocksiRNA, and siRNA are, respectively, DCs transfected by control vectors, c1orf190 vectors, control siRNA, and c1orf190 siRNA. C, C1orf190 promotes the Ag-presenting function of DCs. Influenza virus-specific CTLs were cocultured with different numbers of HLA-A2–restricted peptide-pulsed DCs, which were transfected with control vectors, c1orf190 vectors (C1orf190), control siRNA (MocksiRNA), or c1orf190 siRNA (siRNA). The supernatants were collected and IFN-γ was measured by ELISA after incubation for 48 h.
kinases, may phosphorylate many different proteins. Furthermore, because TLRs and hypoxia-associated genes play a critical role in controlling differentiation of DCs, C1orf190 might have an important role in regulating the differentiation and function of DCs.

To explore the biological function of C1orf190, we investigated whether C1orf190 could regulate the activity of transcription factors NF-κB, AP-1, NF-AT, glucocorticoid response element (GRE), HSE, CRE, MyC, and SRE. Because HEK 293T cells and DCs could express endogenous C1orf190 (Fig. 2A), we first knocked down the C1orf190 to observe the effect of C1orf190 degradation on the activity of transcription factors. siRNA templates of C1orf190 were designed, synthesized, and cloned into p-FIV-H1-U6-copGFP. Whereas NF-κB-SEAP, NFAT-SEAP, GRE-SEAP, CRE-SEAP, MYC-SEAP, and HSE-SEAP were cotransfected into 293T cells or DCs with C1orf190-targeted siRNA with demonstrated transfection (Fig. 2A), NF-κB and NF-AT activity was significantly reduced, whereas other transcription factors such as AP-1, CRE, SRE, GRE, HSE, and MYC were not affected or were only slightly affected (Fig. 2B and Supplemental Fig. 1). Moreover, downregulation of NF-κB activity mediated by C1orf190-targeted siRNAs was dose-dependent (Fig. 2C). When the dose of C1orf190-targeted siRNA structures was >500 ng, suppression of the NF-κB activity was >50%.

To further confirm the effect of C1orf190 on transcription factors NF-κB, AP-1, NF-AT, GRE, HSE, CRE, MyC, and SRE, ectopic C1orf190 was used to cotransfect 293T cells or DCs with each of the reporter plasmids. The overexpression of ectopic C1orf190 in 293T cells and DCs was demonstrated with RT-PCR and Western blotting (Fig. 2A). The results show that ectopic C1orf190 remarkably upregulated the activity of NF-κB and NF-AT, whereas the activity of other transcription factors GRE, AP-1, HSE, CRE, MyC, and SRE was only slightly upregulated or stayed constant (Fig. 2B and Supplemental Fig. 1). Ectopic C1orf190-mediated NF-κB activity was also dose-dependent (Fig. 2C). C1orf190 not only affected NF-κB activity but also impacted nuclear localization of p65 proteins. As shown in Fig. 2D, the ectopic C1orf190 increased the nuclear localization of p65 in both 293T cells and DCs. Thus, these data suggest that C1orf190 may regulate the activity of transcription factors, especially NF-κB.

C1orf190 promotes the production of proinflammatory cytokines and the Ag-presenting function of DCs

NF-κB plays a critical role in DC activation and the expression of proinflammatory cytokines and chemokines (21). Thus, we examined the effect of C1orf190 on the production of proinflammatory cytokines and on the Ag-presenting function of DCs. C1orf190, but not the control vector, resulted in the upregulation of IL-1β, IL-6, and IL-12. As illustrated in Fig. 3A and 3B, the levels of IL-1β, IL-6, and IL-12 in the C1orf190-transfected DCs were significantly higher than those in control vector-transfected DCs (p < 0.05). Conversely, C1orf190 siRNA but not control siRNAs downregulated the mRNA and protein expression of IL-1β, IL-6, and IL-12 (p < 0.05; Fig. 3). Importantly, C1orf190 promoted the Ag-presenting function of DCs. As shown in Fig. 3C, peptide-pulsed DCs transfected with C1orf190 could stimulate influenza peptide-specific CD8 T cells to produce higher levels of IFN-γ than could peptide-pulsed control vector-transfected DCs (p < 0.05), whereas peptide-specific CD8 T cells cocultured with peptide-pulsed DCs transfected by C1orf190 siRNA only produced lower levels of IFN-γ as compared with control siRNA (p < 0.05). Thus, C1orf190 as an NF-κB activator is capable of regulating inflammatory cytokine production and Ag-presenting function of DCs.

C1orf190 promotes resistance of DCs to tumor-associated inhibition

Tumor-associated factors, especially IL-10 and TGF-β, can inhibit the production of cytokines and decrease the functional capacity of DCs (28–31). Because C1orf190 could activate NF-κB and promote the Ag-presenting and priming function of DCs, we next investigated whether C1orf190-transfected DCs could resist the effect of tumor-associated factors on DCs. To test this idea, C1orf190-transfected DCs were exposed to TGF-β, IL-10, or different kinds of tumor supernatants. Morphological analysis showed that while C1orf190-transfected DCs were exposed to tumor supernatants, DCs still maintained their morphological structure with higher levels of expression of MHC class II-DR, CD40, CD86, and CD80.

![FIGURE 4. C1orf190 promotes resistance of DCs to the tumor-associated inhibition in presenting Ag(s). A. Ag-presenting function of DCs transfected by C1orf190 is not affected by TGF-β1 or IL-10. Influenza virus-specific CTLs were cocultured with HLA-A2-restricted peptide-pulsed DCs (CTLs/DCs, 10:1), which were transfected with C1orf190 (C1orf190) or Ctr. and then exposed to TGF-β1 or IL-10 for 3 d. Ctr. in A1 and A2, DCs transfected by control vectors without TGF-β1 or IL-10 treatment; TGFβCtr. in A1, DCs transfected by control vectors with TGF-β1 (10 ng/ml) treatment; IL10Ctr. in A2, DCs transfected by control vectors with IL-10 (10 ng/ml) treatment; C1orf190 in A1 and A2, DCs transfected by C1orf190 vectors without TGF-β1 or IL-10 treatment; TGFβ1C1orf190 in A1, DCs transfected by C1orf190 vectors with TGF-β1 (10 ng/ml) treatment; IL10C1orf190 in A2, DCs transfected by C1orf190 vectors with IL-10 (10 ng/ml) treatment. B, The Ag-presenting function of DCs transfected by C1orf190 is not affected by tumor-associated factors. Vector ctr, C1orf190, MocksRNA, or siRNA A, respectively, the relative secretion of peptide-specific CTLs after coculture with DCs transfected by control vectors, C1orf190 vectors, control siRNA, or C1orf190 siRNA upon exposure to different treatments, including control medium (Med.), ovarian carcinoma SK-OV3 (Tu1; American Type Culture Collection), cervical carcinoma HeLa cells (Tu2; American Type Culture Collection), SiHa cell (Tu3; American Type Culture Collection), TGF-β1 (TGFβeta; 10 ng/ml) or IL-10 (10 ng/ml). Relative secretion is the cytokine secretion (pg/ml) in CTLs stimulated with the treated DCs divided by the cytokine secretion (pg/ml) in CTLs stimulated with the control-treated DCs.](http://www.jimmunol.org/Downloadedfrom)
CD80, CD86, and B7-H1 (Supplemental Fig. 2), whereas DCs transfected with control vectors lost typical structures and attachment ability with a lower expression of MHC class II-DR, CD40, CD80, CD86m and B7-H1 (Supplemental Fig. 2), implying that the c1orf190-tranfected DCs have an ability to resist the effect of tumor-associated factors on DCs. Importantly, unlike control vector-transfected DCs that had the reduced Ag-presenting functions upon exposure to tumor-associated factor TGF-β or IL-10, c1orf190-transfected DCs still had a strong Ag-presenting function even in the presence of tumor-associated factor TGF-β or IL-10 as compared with the control (p < 0.05; Fig. 4A). To further determine the resistance of c1orf190-transfected DCs against tumor-associated factors, c1orf190-transfected DCs were exposed to different kinds of tumors, including ovarian carcinomas and cervical carcinomas, in a Transwell plate. C1orf190-transfected DCs indeed exhibited strong resistance to the tumor-associated factors and produced higher levels of IFN-γ as compared with controls (p < 0.05), whereas c1orf190 siRNA-transfected DCs had reduced Ag-presenting function and produced lower levels of IFN-γ compared with controls (p < 0.05), especially after exposure to tumor supernatants (Fig. 4B).

We also investigated the effect of c1orf190 on the priming ability of DCs in the presence of tumor-associated factors. Toward this end, we employed the tumor-associated Ag Melan-A/MART-1, which has been demonstrated to induce Ag-specific HLA-A0201–restricted cytotoxic CD8+ lymphocytes (22, 23). We first investigated whether c1orf190 could promote DCs to induce Ag-specific T lymphocytes. As shown in Fig. 5B, indeed, Ag-specific T lymphocytes induced by DCs transfected by c1orf190 released higher levels of IFN-γ than did those induced by DCs transfected by the control plasmids in response to Melan-A/MART-1–restricted peptide-loaded DCs, whereas c1orf190-specific siRNA reduced the priming ability of DCs. Next, we sought to determine whether DCs transfected by c1orf190 could resist the effect of tumor-associated factors on the priming ability of DCs. As shown in Fig. 5C, the ability of DCs untransfected or transfected by control empty plasmid or control siRNA in inducing Melan-A/MART-1–specific T lymphocytes could be significantly reduced by IL-10, TGF-β, or tumor supernatants (p < 0.05). Especially, DCs transfected with c1orf190 siRNA had the more remarkably decrease in inducing Melan-A/MART-1–specific T lymphocytes in the presence of IL-10, TGF-β, or tumor supernatants (p < 0.001).

**FIGURE 5.** C1orf190 promotes resistance of DCs to the tumor-associated inhibition in inducing Ag-specific T cells. A. The expression of Melan-A/MART-1 and c1orf190 in DCs cotransfected with Melan-A/MART-1 (Mart-1) and c1orf190 siRNA (C1orf190 siRNA, DC2), siRNA control (Ctr.siRNA, DC3), control vector (Ctr. Vector, DC4), c1orf190 (C1orf190, DC5), or c1orf190 plus c1orf190 siRNA (C1orf190 siRNA, DC6). DC1, DCs cotransfected with control vector (Ctr. Vector) and control siRNA (Ctr.siRNA). The transcriptional levels of Melan-A/MART-1 and c1orf190 in cotransfected DCs were detected using RT-PCR. The transcriptionsal levels of Melan-A/MART-1 and c1orf190 in cotransfected DCs were detected using RT-PCR. B. DCs transfected with Melan-A/MART-1 induce Melan-A/MART-1–specific CD8+ T lymphocytes. Melan-A/MART-1–specific CD8+ T cells were induced according to the protocol described in Materials and Methods. DC1T, DC2T, DC3T, DC4T, DC5T, and DC6T are Melan-A/MART-1–specific CD8+ T lymphocytes induced by DC1, DC2, DC3, DC4, DC5, and DC6, respectively. The release of IFN-γ by DC1T, DC2T, DC3T, DC4T, DC5T, and DC6T were detected in response to HLA-A2010–restricted MART-1 peptide-loaded autologous DCs (Mart-1 DC) or control peptide-loaded autologous DCs (Ctr.DC). C. C1orf190 promotes resistance of DCs to tumor-associated factors in inducing Ag-specific CD8+ T lymphocytes. MocksiRNA, siRNA, Vector,ctr, and c1orf190 are, respectively, the relative secretion of Melan-A/MART-1–specific CD8+ T lymphocytes, which were induced by the treated DC2, DC3, DC4, and DC5 in response to HLA-A2010–restricted Melan-A/MART-1 peptide-loaded autologous DCs. The treated DC2, DC3, DC4, and DC5 were generated after exposed to medium (Ctr.), ovarian carcinoma SK-OV3 (Tu1), cervical carcinoma HeLa cells (Tu2), and SiHa cells (Tu3), TGF-β1 (TGFbeta; 10 ng/ml), or IL-10 (10 ng/ml) according to the protocol described in Materials and Methods. As described above, DC2, DC3, DC4, and DC5 are DCs cotransfected with Melan-A/MART-1 mRNA and c1orf190 siRNA, c1orf190 siRNA control, control vector, or c1orf190. Relative secretion is the cytokine secretion (pg/ml) in Melan-A/MART-1–specific T cells induced by the treated DCs divided by the cytokine secretion (pg/ml) in Melan-A/MART-1–specific T cells induced by the control-treated DCs.
However, the priming ability of DCs transfected with c1orf190 to induce Ag-specific T lymphocytes was not remarkably affected by IL-10, TGF-β, or tumor supernatants. Thus, our results clearly reveal that c1orf190 may promote the resistance of DCs to tumor-associated inhibition.

C1orf190-driven proinflammatory cytokine introduction occurs via the canonical NF-κB pathway

NF-κB is an inducible transcription factor that is controlled by two principal signaling cascades, the classical/canonical NF-κB activation pathway and the alternative/noncanonical pathway (32). Next, we examined whether c1orf190 drives proinflammatory cytokine introduction via the canonical NF-κB pathway or the noncanonical pathway using the NBD peptide, which is a highly selective inhibitor of the canonical NF-κB pathway and IKKα-targetted and/or NIK-targetted siRNA, which acts to silence the noncanonical pathway as described by others (20, 21, 33). In MDCs cotransfected with NBD peptide and c1orf190, NBD peptide not only blocked c1orf190-mediated IKKα phosphorylation but also decreased IL-1β, IL-6, and IL-12 production mediated by c1orf190 (p < 0.05; Fig. 6B). As a control, mutant NBD peptide had no effect (Fig. 6B). We also detected the effect of NEMO siRNA on the canonical NF-κB pathway. NEMO siRNA also inhibited c1orf190-mediated IKKα phosphorylation and reduced IL-1β, IL-6, and IL-12 production mediated by c1orf190 (p < 0.05; Fig. 6). However, siRNA-mediated knockdown of the noncanonical pathway did not remarkably affect the production of IL-1β, IL-6, or IL-12 in the c1orf190-transfected DCs as compared with controls (Supplemental Fig. 3). The degraded IKKα and NIK by siRNA for the noncanonical NF-κB pathway-associated kinases IKKα (siIKKα) and NIK (siNIK) could be observed (Supplemental Fig. 3). Thus, our results suggest that c1orf190-driven production of proinflammatory cytokines and improved Ag-presenting function are mediated by activating the canonical NF-κB pathway, not the noncanonical pathway.

Discussion

In the studies presented, we have demonstrated that c1orf190 can activate the activity of NF-κB, drive the production of proinflammatory cytokines, and promote the Ag-presenting and priming function of DCs via the canonical NF-κB pathway. Thus, c1orf190, as an NF-κB activator, may be involved in the regulation of DC function and play an important role in the induction of innate and adaptive immune responses. Meanwhile, we have also examined the ability of c1orf190-transfected DCs to resist the effect of tumor-associated factors on the Ag-presenting and priming function of DCs. This might suggest an approach for enhancing vaccine immunogenicity.

Examination of circulating and tumor-infiltrating DCs in tumor-bearing animals and in cancer patients has revealed that DCs are functionally impaired in their ability to induce T cell responses. Tumor-associated factors, such as IL-10 (28) and TGF-β (29, 34),

![FIGURE 6.](http://www.jimmunol.org/)

C1orf190 drives proinflammatory cytokine production via the canonical but not the noncanonical NF-κB pathway. A, NBD peptide selectively inhibits c1orf190-mediated phosphorylation of IKKα. DCs were transfected with c1orf190 (C1orf190) or control vectors (Ctr. Vector) and then preincubated with either NBD peptide (NBD) or control mutant NBD peptide (NBDMut) for 2 h. Cell lysates were analyzed by Western blotting. B, NBD peptide decreases IL-1β, IL-6, and IL-12p70 production by c1orf190-transfected DCs. MDCs were transfected by c1orf190 (C1orf190) or control vectors (Ctr. Vector) and then incubated with either NBD peptide (NBD) or control peptide (NBDMut) for 2 h. Supernatants were harvested after 24 h, and secreted IL-1β, IL-6, and IL-12p70 (pg/ml) were measured by ELISA. Results are expressed as means ± SD from one representative experiment of three performed in triplicate. p < 0.05. C, NEMO siRNAs inhibit c1orf190-mediated IKKα phosphorylation and IL-1β, IL-6, and IL-12 production. DCs were transfected with control vector (Ctr. Vector), c1orf190 plasmid (C1orf190), or cotransfected using c1orf190 plasmids (C1orf190) with NEMO-specific siRNA (NEMOsirNA; 100 nM) or control siRNA (Ctr. siRNA; 100 nM). The transcriptional levels of NEMO and GAPDH were analyzed using RT-PCR (C1). Phosphorylated IKKα was analyzed by Western blotting (C2). Supernatants were harvested after 24 h, and secreted IL-1β, IL-6, and IL-12p70 (pg/ml) were measured by ELISA (C3). Results are expressed as means ± SD from one representative experiment of three performed in triplicate.
potentially inhibit the function of Ag-presenting cells, such as DCs, through a repression of inflammatory cytokine production and MHC class II and costimulatory molecule expression. Tumor-associated DCs often induce T cell anergy or deletion and regulatory T cells instead of antitumor immunity. As a result, although there are many tumor-associated Ags found, there is still no effective vaccine for cancer. Thus, novel rational strategies to enhance the immunogenicity of pathogen-specific Ags and cancer-specific Ags are needed. Some immunological adjuvants have been shown to activate NF-κB among their multiple actions, but they are, however, limited in use by their lack of specific, localized, and coordinated effects and consequently by toxicity (35). C1orf190, as an NF-κB-activator, results in the upregulation of cytokines that contain NF-κB sites on the promoters of their genes and promote the Ag-presenting function and priming ability of DCs. Importantly, c1orf190 might repress the resistance of tumor-associated factors. Thus, c1orf190 might be a candidate gene for improving the immunogenicity of tumor vaccine.

C1orf190 contains multiple CKII phosphorylation sites, PKC phosphorylation sites, and cAMP- and cGMP-dependent protein kinase phosphorylation sites, and it has a wide relationship with multiple signaling pathway molecules (Supplemental Fig. 5). These multiple sites may play an important role in mediating NF-κB activity and proinflammatory cytokine production. Indeed, a substantial amount of evidence shows that multiple kinases, such as PKC, CKII, protein kinase A, glycogen synthase kinase-3b, T2K (T6K, NAK), PI3K, AKT, p38, NIK, and even IKK, can induce phosphorylation of NF-κB (9, 10, 16). The distribution of NF-κB1, NF-κB2, RelA, RelB, and c-Rel is widespread and is receptive to many extracellular and intracellular signals. Knockdown of c1orf190 partners affects the activity of NF-κB and the production of proinflammatory cytokines, suggesting that c1orf190-mediated NF-κB activation and proinflammatory cytokine production may be dependent on multiple signaling pathways. Further studies are required to elucidate the biological function of these interactions.

C1orf190-driven proinflammatory cytokine production is mediated by activating the canonical NF-κB pathway but not the noncanonical pathway. C1orf190-mediated IKKα phosphorylation and IL-1β, IL-6, and IL-12 production by DCs may be blocked by NBD peptides or NEMO siRNA. The NBD peptide can block the association of NEMO with the IKK complex and inhibit cytokine-induced NF-κB activation and NF-κB–dependent gene expression (21). NF-κB inhibition by NBD peptide results in blockade of IKK-mediated IkBα phosphorylation and subsequent nuclear translocation and DNA binding of NF-κB p65 in DCs (20). Specific inhibition of the canonical pathway in DCs has been demonstrated to cause immune regulation not only in vitro (36, 37) but also in vivo (37). Others have also shown that the production of proinflammatory cytokine IL-12p70 and IL-6 can be blocked by inhibiting the canonical pathway using NBD peptide (20, 33). The noncanonical pathway is strictly dependent on IKKα homodimers and requires neither IKKβ, nor NEMO/IKKγ (15, 38). Knockdown of IKKα or NIK may result in the increased production of proinflammatory IL-12p70 and IL-6 production in DCs (20, 33). Recent studies in macrophages have also suggested a role for IKKα in the negative regulation of inflammation (39, 40). However, siRNA-mediated knockdown of the noncanonical pathway IKKα and NIK did not remarkably affect the production of c1orf190-mediated IL-1β, IL-6, and IL-12.

Additionally, IL-12, a heterodimeric proinflammatory cytokine that induces the production of IFN-γ, favors differentiation of Th1 cells and forms a link between innate resistance and adaptive immunity (41). Because IL-12 plays a critical role in inducing Th1 responses, it will be interesting to study whether c1orf190 induces Th1 immune responses in vivo.

Disclosures

The authors have no financial conflicts of interest.

References


