LRP11 promotes stem-like T cells via MAPK13-mediated TCF1 phosphorylation, enhancing anti-PD1 immunotherapy

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ABSTRACT

Background Tumor-infiltrating T cells enter an exhausted or dysfunctional state, which limits antitumor immunity. Among exhausted T cells, a subset of cells with features of progenitor or stem-like cells has been identified as TCF1+ CD8+ T cells that respond to immunotherapy. In contrast to the finding that TCF1 controls epigenetic and transcriptional reprogramming in tumor-infiltrating stem-like T cells, little is known about the regulation of TCF1. Emerging data show that elevated body mass index is associated with outcomes of immunotherapy. However, the mechanism has not been clarified.

Methods We investigated the proliferation of splenic lymphocytes or CD8+ T cells induced by CD3/CD28 stimulation in vitro. We evaluated the effects of low-density lipoprotein (LDL) and LRP11 inhibitors, as well as MAPK13 inhibitors. Additionally, we used shRNA technology to validate the roles of LRP11 and MAPK13. In an in vivo setting, we employed male C57BL/6J injected with B16 cells or MC38 cells to build a tumor model to assess the effects of LDL and LRP11 inhibitors, LRP11 activators, MAPK13 inhibitors on tumor growth. Flow cytometry was used to measure cell proportions and activation status. Molecular interactions and TCF1 status were examined using Western blotting. Moreover, we employed RNA sequencing to investigate the effects of LDL stimulation and MAPK13 inhibition in CD8+ T cells.

Results By using a tumor-bearing mouse model, we found that LDL-induced tumor-infiltrating TCF1+PD1+CD8+ T cells. Using a cell-based chimeric receptor screening system, we showed that LRP11 interacted with LDL and activated TCF1. LRP11 activation enhanced TCF1+PD1+CD8+ T-cell-mediated antitumor immunity, consistent with LRP11 blocking impaired T-cell function. Mechanistically, LRP11 activation induces MAPK13 activation. Then, MAPK13 phosphorylates TCF1, leading to increase of stem-like T cells.

Conclusions LRP11-MAPK13-TCF1 enhanced antitumor immunity and induced tumor-infiltrating stem-like T cells.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ TCF1 promotes the stemness of T cells, which is beneficial for cells to exert their antitumor effect.

WHAT THIS STUDY ADDS

⇒ LRP11 induces the activation of MAPK13, leading to the phosphorylation of TCF1 and increase of stem-like T cells.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ It provides new research ideas and methods for the combination of PD1 immune checkpoint blockade therapy.

INTRODUCTION

In the tumor microenvironment, immune cells, particularly T cells, enter an exhausted or dysfunctional state with reduced cytokine production and persistently increased expression of inhibitory receptors,1,2 such as programmed cell death protein 1 (PD1) (encoded by Pdcd1) and TIM3 (encoded by Havcr2), limiting immunotherapy, including adoptive cell therapy of autologous T or chimeric antigen receptor (CAR) T cells and blockade of inhibitory receptors, which has shown remarkable success in clinical cancer treatment.3–7 Exhausted T (Tex) cells were reported to be driven by nuclear factor of activated T cells (NFAT), interferon regulatory factor 4 (IRF4), nuclear receptor subfamily 4 group A (NR4A) and thymocyte selection-associated high-mobility group (HMG) box protein (TOX) through transcriptional and epigenetic reprogramming.8–11 Recent advances have indicated that Tex cells show substantial heterogeneity both in patients with cancer and experimental models.12–14 A subset of cells with features of progenitor or stem-like cells plays a critical role in immunotherapy.15,16
deacetylase domain, thereby enforcing chromatin accessibility. TCF1 goes beyond T-cell maturation and T memory formation and has new functions in maintaining the long-term survival and functional responses of T cells. TCF1 is needed for better long-term persistence of tumor-infiltrating T cells and tumor control by its direct interaction with regulators and cooperation with other transcription factors, which is supported by clinical data. On immunotherapy, TCF1+ PD1+ stem-like T cells are higher in patients with tumors and are positively correlated with the response to immunotherapy and overall survival. These stem-like T cells in non-small cell lung cancer are also defined as CXCR5+ TIM3+ progenitor T cells, which share phenotypic and transcriptional features with TCF1+ stem-like T cells. Likewise, SLAMF6 positively correlates with TCF1 levels in stem-like CD8+ T cells in tumor tissues. In addition, these stem-like T cells exhibit a more potent proliferative and functional capacity in response to immunotherapy.

Despite its role in stem-like T cells, the regulation of TCF1 is less understood. Exhausted T cells are rescued by CD28/B7 pathway during tumor immunotherapy. Recent studies have shown that dendritic cells provide an essential niche to promote stem-like T cell maintenance. Therefore, environmental cues can regulate the generation and maintenance of stem-like TCF1+ CD8+ T cells needed for durable cancer remission. However, the cellular signaling pathways that drive the conversion to a stem-like TCF1+ CD8+ T-cell phenotype remain unexplored.

Interestingly, clinical and experimental data show that elevated body mass index (BMI) correlates with favorable outcomes in patients receiving immunotherapy. Although a high level of PD1 expression is considered to contribute to these outcomes, the mechanisms that enhance antitumor immunity in people with elevated BMI are still unknown. Here, we found that low-density lipoprotein (LDL), which correlates with high-fat diet-induced obesity, promoted CD8+ T-cell proliferation and TCF1 expression through LRP11. Similarly, LRP11 agonistic antibodies showed a powerful antitumor capacity. LRP11 blockade limited TCF1+ CD8+ T cells during PD1 blockade immunotherapy. Of note, LRP11 activation induced MAPK13 phosphorylation and transport into the nucleus, where MAPK13 functioned as a kinase and promoted TCF1 phosphorylation, leading to transcriptional reprogramming in CD8+ T cells and enhancing PD1 blockade immunotherapy. Therefore, the LRP11-MAPK13 axis promotes stem-like TCF1+ CD8+ T cells with transcriptional reprogramming, enhancing anti-PD1 immunotherapy.

METHODS

Cell lines

HEK293T cells were obtained from ATCC (CRL-11268), and B16-F10 and MC38 cells were obtained from CCTCC (IM-M002, IM-M006). DMEM containing 10% fetal bovine serum (FBS) (Yeasen Biotechnology, Shanghai, China) and 1% penicillin/streptomycin (Thermo Fisher Scientific, Warrington, USA) was used. All cells were cultured at 37°C and 5% CO2. All cell lines were routinely tested for mycoplasma contamination with the GMyc-PCR Mycoplasma Test Kit (Yeasen Biotecnology) according to the manufacturer’s instructions.

Mice

Male C57BL/6J mice aged 6–8 weeks and high-fat diet C57BL/6J mice aged 10–11 weeks were purchased from Beijing Huafukang Bioscience. All mice were housed in the Experimental Animal Center of Tongji Hospital, Huazhong University of Science and Technology, at a temperature of 20°C–26°C, 40%–70% humidity, and a 12-hour daytime and 12-hour dark cycle in a specific pathogen-free environment.

In vitro T-cell isolation and stimulation

Splenic murine T cells were isolated from C57BL/6J mice aged 6–8 weeks by Mouse 1x Lymphocyte Separation Medium (Dakewe Biotech, Shenzhen, China) according to the manufacturer’s instructions. T-cell proliferation, purified T cells were stained with CellTrace Violet (C34557, Thermo Fisher Scientific) according to the manufacturer’s instructions. T-cell activation was initiated using 5 µg/mL anti-CD3 (BE0001-IFAB; BioXCell, New Hampshire, USA) plus 2 µg/mL anti-CD28 (BE0015; BioXCell). After 72 hours (day 3), cells were collected for flow cytometry. Cells were cultured with RPMI-1640 complete medium with 10% FBS (Yeasen Biotechnology), 1% penicillin/streptomycin (Thermo Fisher Scientific), and 50 µM β-mercaptoethanol (Solarbio, Beijing, China). In selected experiments, cells were stimulated in the presence of stimulants or inhibitors, namely, LDL (YB001; Yiyuan Biotech; 80 µg/mL, Guangzhou, China), OXLDL (YB002; Yiyuan Biotech; 80 µg/mL), HDL (YB003; Yiyuan Biotech; 80 µg/mL), LRP11 antibody (sc-514698; Santa Cruz; 3 µg/mL, Biotech, Dallas, Texas, USA) and MAPK13 IN (HY-18850; MedChemExpress; 25 µM, China).

CD8+ T cells were sorted from spleen or tumor using APC-labeled anti-CD8 antibody (100712; BioLegend, USA) and anti-APC magnetic beads (5220210488; Miltenyi Biotec, Shanghai, China). CD8+ T cells were stained with CellTrace Violet (C34557, Thermo Fisher Scientific). CD8+ T-cell activation was initiated using 1 µg/mL anti-CD3 (BE0001-IFAB; BioXCell) plus 0.5 µg/mL anti-CD28 (BE0015; BioXCell). 25 U/mL IL-2 and 10 ng/mL IL-12. Cells were split in half, with fresh medium IL-2 used to replace the old medium every 2 days. Cells were cultured with RPMI-1640 complete medium with 10% FBS (Yeasen Biotecnology), 1% penicillin/streptomycin (Thermo Fisher Scientific), and 50 µM β-mercaptoethanol (Solarbio). In selected experiments, cells were stimulated in the presence of stimulants or inhibitors, namely, LDL (YB001; Yiyuan Biotech; 80 µg/
mL, LRP11 antibody (sc-514698; Santa Cruz; 3 µg/mL) and MAPK13 IN (HV-18850; MedChemExpress; 25 µM).

SLAMF6+PD1+CD8+ T cells were sorted from TILs using FITC-labeled anti-CD8+ antibody (553031; BD Biosciences, USA), PE-labeled anti-SLAMF6 antibody (134606; BioLegend, USA) and APC-labeled anti-PD1 antibody (562671; BD Biosciences, USA) by SONY MA900 multi-functional fully automated flow cytometer. SLAMF6+PD1+CD8+ T cells were stained with CellTrace Violet (C34557, Thermo Fisher Scientific). SLAMF6+PD1+CD8+ T cells activation was initiated using 1 µg/mL anti-CD3 (BE0001-1FAB; BioXCell) plus 0.5 µg/mL anti-CD28 (BE0015-1; BioXCell). After 72 hours (day 3), cells were collected for flow cytometry. In selected experiments, cells were stimulated in the presence of stimulants or inhibitors, namely, LDL (YB001; Yiyuan Biotech; 80 µg/mL, Guangzhou, China).

Human peripheral blood mononuclear cell (PBMC) were collected by Lymphocytes Separation Medium (LTS1077; TBD, China). Cell culture methods and stimulation proliferation methods were same as above. In selected experiments, cells were stimulated in the presence of stimulants or inhibitors, namely, LDL (YB001; Yiyuan Biotech; 80 µg/mL, Guangzhou, China), LRP11 antibody (sc-514698; Santa Cruz; 3 µg/mL, Biotech) and MAPK13 IN (HV-18850; MedChemExpress; 25 µM, China).

**Lentiviral vector experiments**

TCF1 cDNA was obtained from the thymus gland of C57BL/6J mice, and TCF1 D1, TCF1 D2 and TCF1 D3 cDNA clones were extended from TCF1 cDNA using PCR. Each cDNA was cloned and inserted into the pCDH plasmid and lentivirus were produced in 293T cells. Lentiviral vector eGFP (Lenti-X) and TCF1 shRNA were cloned and inserted into the pCMV pCDH plasmid and TCF1 D1, TCF1 D2 and TCF1 D3 cDNA clones were extended from TCF1 cDNA using PCR. Each cDNA was cloned and inserted into the pCMV pCDH plasmid and lentivirus were produced in 293T cells. Lentiviral vector eGFP (Lenti-X) and TCF1 shRNA were cloned and inserted into the pCMV pCDH plasmid and lentivirus were produced in 293T cells. TCF1 shRNA with PSPX and PMD2G plasmids using Lipo8000. CD8+ T cells were transduced with shRNA lentivirus in the presence of polybrene (2 µg/mL) and MAPK13 IN (HY18850) by SONY MA900 multi-functional fully automated flow cytometer. SLAMF6+PD1+CD8+ T cells were stained with CellTrace Violet (C34557, Thermo Fisher Scientific). SLAMF6+PD1+CD8+ T cells activation was initiated using 1 µg/mL anti-CD3 (BE0001-1FAB; BioXCell) plus 0.5 µg/mL anti-CD28 (BE0015-1; BioXCell). After 72 hours (day 3), cells were collected for flow cytometry. In selected experiments, cells were stimulated in the presence of stimulants or inhibitors, namely, LDL (YB001; Yiyuan Biotech; 80 µg/mL, Guangzhou, China).

**Tumor models**

B16-F10 (2×10^5) or MC38 (5×10^5) cells were injected subcutaneously into the right flank of mice. Tumor sizes were measured at the indicated time after transplantation, and tumor volume was quantified as width×length. Mice were sacrificed on day 14, and the weight of the excised tumor mass was determined.

**In vivo treatment of mice**

Mice were intraperitoneally (i.p.) treated with 12 mg/kg anti-PD1 (BE0273; BioXCell), 40 mg/kg LDL (YB001; Yiyuan Biotech), 20 mg/kg LRP11 agonist (agLRP11), 20 mg/kg MAPK13-IN (HV-18850; MedChemExpress) or control immunoglobulin (Rat IgG2b; BP0090; BioXCell) at the indicated time points.

**Mouse lymphadenectomy and tumor resection**

Before surgical operations, mice were anesthetized with 10 mg/mL sodium amobarbital (150 µL/mouse) for full muscle relaxation. The anesthetized mice were placed onto the sterilized surgery table, and the peritumoral skin was incised to expose the tumor.

The draining lymph nodes (DLNs) were carefully removed, and the tumor was excised intact.

**Preparation of single-cell suspensions from mouse samples**

Mouse spleens, DLNs and tumors were mashed through a 70 µm cell strainer (BD Biosciences). Tumor microenvironment-infiltrating lymphocytes were further purified using a discontinuous Percoll gradient (170891109; Cytiva, USA). Cells at the interface were harvested and washed twice before further use. Splenocytes and DLN cells were washed twice before flow cytometry.

**Flow cytometric analyses**

Immune cells extracted from mice were used for flow cytometric analyses. For surface staining, cells were incubated with antibodies for 30 min at 4°C and subsequently washed with PBS containing 0.2% BSA. Cell surface staining was performed with anti-mouse CD45-BV785 (103149), CD4-BV510 (300546), CD8-FITC (100705), CTLA4-PE (106306), OX40-PerCP/Cy5.5 (119424), PD1-BV421 (135221) (all from Biolegend) and CD44-FITC (553133), CD62L-APC/Cy7 (560514) (all from BD Biosciences).

For Perforin, TNFα and Granzyme B staining, after surface labeling as described above, cells were fixed and permeabilized by Fixation and Permeabilization Solution (554722; BD Biosciences) at 4°C for 20 min, incubated in saponin-containing buffer (0554723; BD Biosciences) with antibodies (Perforin-PE, Granzyme B-BV421) at a dilution of 1:100 for 30 min at 4°C, subsequently washed with PBS containing 0.2% BSA, and resuspended in PBS for detection. TNFα-PE/Cy7 (506324), Perforin-PE (506324) and Granzyme B-BV421 (396413) were all from Biolegend.

For TCF1 staining, after staining of the surface marker, cells were fixed and permeabilized by Fixation and Permeabilization Solution (554722; BD Biosciences) at 4°C for 20 min, incubated in saponin-containing buffer (0554723; BD Biosciences) with antibodies (Perforin-PE, Granzyme B-BV421) at a dilution of 1:100 for 30 min at 4°C, subsequently washed with PBS containing 0.2% BSA, and resuspended in PBS for detection. TNFα-PE/Cy7 (506324), Perforin-PE (506324) and Granzyme B-BV421 (396413) were all from Biolegend.

For TCF1 staining, after staining of the surface marker, cells were fixed and permeabilized by Fixation and Permeabilization Solution (554722; BD Biosciences) at 4°C for 20 min, incubated in saponin-containing buffer (0554723; BD Biosciences) with antibodies (Perforin-PE, Granzyme B-BV421) at a dilution of 1:100 for 30 min at 4°C, subsequently washed with PBS containing 0.2% BSA, and resuspended in PBS for detection. TNFα-PE/Cy7 (506324), Perforin-PE (506324) and Granzyme B-BV421 (396413) were all from Biolegend.

**Protein assays**

For the phosphorylated TCF1 assay, purified CD8+ T cells were stimulated with LDL (80 µg/mL), LRP11 blocking antibody (3 µg/mL) and MAPK13 IN (25 µM) for the
indicated times. For nuclear fraction isolation, cells were lysed using a Nuclear and Cytoplasmic Protein Extraction Kit (P0028; Beyotime) with protease/phosphatase inhibitor cocktail (Cell Signaling). Nuclear extracts were precleared with protein A/G beads (16-663; Miltenyi) before incubation with anti-TCF1 (C63D9 or C46G7) antibodies at 4°C overnight. Protein A/G beads were added and further incubated for 4 hours. The beads were washed in buffer (50 mM Tris-HCl (pH 7.4), 200 mM NaCl, 1 mM EDTA, 0.5% NP40) five times, 5x SDS loading buffer was added, and the samples were boiled at 100°C for 10 min.

For immunoprecipitation (IP), purified CD8+ T cells were processed as described above. CD8+ T cells and transfected 293T cells were lysed using 0.5% NP40 ice-cold PBS (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 0.5% NP40) with protease/phosphatase inhibitor cocktail (Cell Signaling Technology). Protein A/G beads (16-663; Miltenyi) were used for preclearing protein before incubation with anti-MAPK13 (sc-271292; Santa Cruz) or anti-DDDDK tag (Ab205606; Abcam, Shanghai, China) antibodies at 4°C overnight. Protein A/G beads were added and further incubated for 4 hours. The beads were washed in buffer (50 mM Tris-HCl (pH 7.4), 200 mM NaCl, 1 mM EDTA, 0.5% NP40) five times, 5x SDS loading buffer was added, and the samples were boiled at 100°C for 10 min.

Immunoblotting was performed using the Bio-Rad Western blot workflow. Membranes were blocked in 5% BSA for primary antibodies and secondary antibodies. The antibodies used were anti-TCF1 (C63D9 or C46G7; Cell Signaling Technology), anti-MAPK13 (sc-271292; Santa Cruz), anti-DDDDK tag (Ab205606; Abcam), anti-phospho-p38 MAPK (sc-17168; Proteintech), and anti-pan phospho-serine/threonine (Ab205606; Abclonal, Wuhan, China). The signal was detected with enhanced chemiluminescence (P10300; New Cell & Molecular Biotech, Suzhou, China). For all representative immunoblots shown, n=3 separate experiments were carried out.

ELISAs
Culture supernatant from splenocytes treated with the indicated stimulants or inhibitors was collected and detected by an ELISA kit (MultiScience Technology, Hangzhou, China) according to the manufacturer’s instructions. ELISA kit information is as follows: Mouse TNFα ELISA Kit (EK282/4-96) and Mouse IFNγ ELISA Kit (EK280/3-96).

Quantitative real-time RT-PCR
Total RNA was extracted from cultured cells using RNAsafe200 (220010; Fastagen) according to the manufacturer’s instructions. RNA was reverse-transcribed with Hifair II first Strand cDNA Synthesis SuperMix (11120ES06; Yeasen Biotechnology) for qPCR to generate cDNA. Real-time PCR was performed on a Veriti 96-Well Thermal Cycler (Applied Biosystems, USA) using Hieff qPCR SYBR Green Master Mix (11203ES50; Yeasen Biotechnology). Real-time PCR primers used for detecting expression are listed in the KEY RESOURCES TABLE.

Chimeric reporter gene construction
The sequence encoding the extracellular portion of the mouse-indicated genes (Lrp1, Lrp5, Lrp6, Lrp11, Lrp12 or Ldlr) and CD3ε intracellular portion were amplified by PCR from cDNA isolated from C57BL/6j mice. The reporter fusions were constructed by overlapping PCR using NEBuilder HiFi DNA Assembly Master Mix (New England Biolabs). The resulting fusion DNA fragment was cloned and inserted into pMXsIG and transfected into BWZ.36 reporter cells.

β-galactosidase assay
A β-galactosidase enzyme assay system (Promega, Madison, Wisconsin, USA) was used to quantify β-galactosidase activity in each chimeric reporter. Briefly, chimeric reporters were stimulated with LDL or vehicle control for 24 hours. Then, the cells were harvested, and the β-galactosidase activity assay was performed using the standard method following the manufacturer’s recommended protocol.

RNA-seq
Activated CD8+ T cells were cultured with LDL (80 μg/mL), MAPK13 IN (25 µM) for the indicated times. On days 0, 1, 3, 5, and 7, total RNA was extracted using TRIzol following the instructions. The samples were stored at −80°C until library preparation. The RNA-seq libraries were prepared at Biotechnology (Wuhan, China). All reads were aligned to the mouse genome reference (GRCm38.p6) sequence using HISAT2 with default parameters. The raw reads were sequenced from the Illumina platform and subsequently subjected to quality control (QC) to determine whether the sequencing data were suitable for subsequent analysis. After QC, clean reads were obtained by filtering and then compared with the reference sequence. After the alignment, the distribution and coverage of reads on the reference sequence were determined to judge whether the alignment results pass the second QC of alignment. If it passed, the differentially expressed genes between samples were screened from the gene expression results using DESeq2 software, and based on the differentially expressed genes, GO functional significance enrichment analysis and KEGG pathway significance enrichment analysis were performed by clusterProfiler software. We used the gene expression of each sample as the basis, identified the first principal component (PC1) and the second principal component (PC2) of the gene expression difference between the samples by principal component analysis, and plotted the position of each sample (points of different shapes) with it as the coordinate axis. The clustering relationship of the samples could be seen from the distance between the samples in terms of distance. Data have been deposited in SRA under the accession number PRJNA842919.
Statistical analyses were performed using GraphPad Prism V.9.3.1. Data are presented as the mean±SEM. Multiple groups were compared by two-way ANOVA, followed by pairwise testing corrected according to Sidak or Dunnett’s test when comparisons were made to a single control or between multiple pairs. Two-tailed unpaired Student’s t-test was used for comparisons between two groups. Survival curve statistical analysis was performed using the log-rank test. The p values were considered not significantly different when they were greater than 0.05.

RESULTS

LDL promoted CD8+ T-cell proliferation and activation

Elevated BMI is associated with a higher cancer risk but also correlates with improved survival and favorable outcomes in patients receiving immunotherapy, termed the “obesity paradox”.32–34 We found that tumor growth in mice fed a high-fat diet was slower compared with the control group (online supplemental figure S1A–C). There was an increase in tumor-infiltrating lymphocytes, including CD4+ T cells and CD8+ T cells (online supplemental figure S1D,E), although the naïve, TCM, and TEM CD8+ T cells did not change (online supplemental figure S1F). The most significant change was the increase in TCF1+CD8+ T cells infiltrating the tumor in high-fat diet mice (online supplemental figure S1G), with elevated expression of OX40 (online supplemental figure S1H) and increased production of TNFα and Perforin (online supplemental figure S1I). Additionally, we examined the differences in DLNs and found that, apart from an increase in the proportion of CD8+ T cells (online supplemental figure S1J) and increased production of TNFα and Perforin (online supplemental figure S1N), other indicators remained unchanged (online supplemental figure S1K–M).

However, what contributes to the obesity paradox is unclear. Since LDL correlates with high-fat diet-induced obesity, we screened the effect of different types of lipoproteins on T cells in vitro. Consistent with other studies,35 oxidized LDL (OxLDL) dampened T-cell proliferation (online supplemental figure S2A,B). In contrast, LDL and HDL promoted T-cell proliferation (figure 1A,B, online supplemental figure S2C and online supplemental figure S2D). LDL promoted T-cell function and cytokine secretion (figure 1C,E), although Pdcd1 and Havcr2 expression was induced (figure 1D). The frequency of CD8+ T cells was significantly increased and was almost fourfold greater than that in the no LDL stimulation group (figure 1F). We next assessed expression of costimulatory molecular OX40 and coinhibitory molecular CTLA4 in CD8+ T cells with or without LDL stimulation. LDL did not significantly change OX40 or CTLA4 expression (online supplemental figure S2E). To determine whether LDL directly promoted T-cell proliferation, we sorted CD8+ T cells. The number of CD8+ T cells stimulated by LDL was higher than that of the control (figure 1G,H).

Thus, LDL could directly promote CD8+ T-cell proliferation and function.

To examine the stimulatory effect of LDL on CD8+ T cell proliferation in tumor infiltration, we sorted tumor-infiltrating CD8+ T cells and found that LDL increased both the proportion and quantity of tumor-infiltrating CD8+ T cells (figure 1I,J). To observe the effect of LDL at different stages after CD8+ T cell activation, we collected CD8+ T cells treated with LDL at different time points. PCR results showed that LDL promoted the expression of Tcf7 from day 1 to day 7, although the expression of Pdcd1 and Havcr2 was also increased (online supplemental figure S2F). Under high expression of Tcf7, flow cytometry results indicated that CD8+ T cells remained in a SLAMF6+PD1+ pre-exhaustion state (online supplemental figure S2G). RNA-seq results further showed that LDL stimulated CD8+ T cells to continuously release a large quantity of cytokines to exert a killing effect (online supplemental figure S2H). To further elucidate the effect of LDL on Tpex cells, we sorted tumor-infiltrating SLAMF6+PD1+CD8+ T cells for stimulation and observed that LDL also enhanced the proliferation of this subset of cells (figure 1K,L).

To test whether LDL-induced TCF1 production and promoted Tex cell proliferation, we used a tumor-bearing mouse model in which CD8+ T cells showed an exhausted phenotype. First, MC38 cells were implanted subcutaneously into C57BL/6j male mice. One week later, PD1 blocking antibodies were injected i.p. at a dose of 12 mg/kg with or without LDL (40 mg/kg) when the tumor area reached 10 mm2 (figure 2A). Our data showed that LDL markedly enhanced the PD1 blocking antibody-mediated antitumor capacity (figure 2B–D). The frequency and total number of CD4+ T and CD8+ T cells were higher in tumor tissues with PD1 blocking antibodies and LDL injection (figure 2E,F).

We also examined the changes of naïve (CD44+CD62L+), TCM (CD44+CD62L−), and TEM (CD44+CD62L−) CD8+ T cells and found that LDL combined with PD1 blocking antibodies had no effect on the proportions of these three types of cells (figure 2G,H). The main effect was on the TCF1+CD8+ T-cell subset, and even PD1+TCF1+CD8+ T cells in a pre-exhaustion state are significantly increased in proportion and number by PD1 blocking antibodies and LDL injection (figure 2I–K). LDL and PD1 blocking antibodies also induced OX40 and CTLA4 expression on CD8+ T cells (figure 2L,M). The production of TNFα and perforin also increased (figure 2N). CD4+ and CD8+ T cells proportions were also increased in both DLNs and spleen (online supplemental figure S2A–G), and an increase in PD1+TCF1+CD8+ T cells was observed in the DLNs (online supplemental figure S3C). However, in splenic lymphocytes, the frequency of PD1+ TCF1+ CD8+ T cells was very low (online supplemental figure S3I). In addition, LDL combined PD1 blocking antibodies did not significantly change the OX40 or CTLA4 expression and the secretion of cytokines on CD8+ T cells in tumor-draining LN and splenic lymphocytes (online...
Figure 1  LDL promoted CD8+ T-cell proliferation and activation in vitro. Splenic lymphocytes were separated and stimulated with anti-CD3 antibodies (5 µg/mL) and anti-CD28 antibodies (2 µg/mL) plus either vehicle control (Ctrl) or LDL (80 µg/mL) for 3 days. CD8+ T cells were sorted from spleen (G–H) or tumor (I–J) by magnetic bead sorting and then activated by anti-CD3 antibodies (1 µg/mL) and anti-CD28 antibodies (0.5 µg/mL) plus either vehicle control (Ctrl) or LDL (80 µg/mL) for 3 days. SLAMF6+PD1+CD8+ T cells were sorted from tumor (K–L) by flow sorting technology and then activated by anti-CD3 antibodies (1 µg/mL) and anti-CD28 antibodies (0.5 µg/mL) plus either vehicle control (Ctrl) or LDL (80 µg/mL) for 3 days. (A, B) Proliferation was assessed via CTV labeling by flow cytometry (A). Statistical analysis of the proliferation rate and total cell number (B) (n=6). (C) TNFα and IFNγ secretion from splenic lymphocytes was assessed by ELISAs (n=4). (D) The indicated genes were detected by qPCR (n=3). (E) Statistical analysis of the frequencies of perforin+ and granzyme B+ CD8+ T cells (n=3) following 3 days of stimulation in vitro with anti-CD3 antibodies (5 µg/mL) and anti-CD28 antibodies (2 µg/mL) plus either vehicle control (Ctrl) or LDL (80 µg/mL). (F) Representative flow plots of CD4+ T cells and CD8+ T cells and the proportions of CD8+ T cells from each group are summarized (n=5). (G, H) Proliferation of CD8+ T-cell from spleen was assayed via CTV labeling by flow cytometry (G). The proliferation rate and total cell number (H) were summarized, and each symbol represents one individual (n=5). (I, J) Proliferation of CD8+ T-cell from tumor was assayed via CTV labeling by flow cytometry (I). The proliferation rate and total cell number (J) were summarized, and each symbol represents one individual (n=5). (K, L) Proliferation of SLAMF6+PD1+CD8+ T cells from tumor was assayed via CTV labeling by flow cytometry (K). The proliferation rate and total cell number (L) were summarized, and each symbol represents one individual (n=4). Data are from one representative of three independent experiments. Error bars represent the SE, and p values were calculated by two-way ANOVA (B, C, E, F right, H, J, L) or two-tailed, unpaired Student’s t-tests (D). NS stands for not significant. See also online supplemental figure S2. ANOVA, analysis of variance; LDL, low-density lipoprotein. CTV, cell trace violet.
Figure 2  LDL-induced tumor-infiltrating TCF1+ T cells and enhanced antitumor immunity. (A–D) MC38 cells were implanted subcutaneously into C57BL/6J male mice. Time course of anti-PD1/LDL therapy initiated at 7, 9, and 11 days post-tumor initiation, composed of three doses (arrows) of IgG or anti-PD1 (12 mg/kg) in the presence of LDL (40 mg/kg) or IgG in C57BL/6J male mice (A). Representative pictures of tumors (B). Tumor growth curve of MC38 cells subcutaneously injected into C57BL/6J male mice (C). Tumor weights (D) were measured and are shown on day 14 post-tumor initiation (n=6). Tumor-infiltrating lymphocytes were separated from tumors on day 14 post-tumor initiation. Frequencies of CD4+ T cells and CD8+ T cells (n=5) (E). Total number of indicated T cell sub-populations among TILs per gram of tumor (F). Frequencies of naïve CD8+ T cells (CD44+CD62L+) and TEM (CD44+CD62L−) were measured (G,H). The proportions of TCF1+ CD8+ T cells (left) and total number of TCF1+ CD8+ T cells among TILs per gram of tumor (right) were summarized (I). The proportions of PD1+ TCF1+ CD8+ T cells (J) and total number of PD1+ TCF1+ CD8+ T cells among TILs per gram of tumor (K) were summarized. Statistical analysis of the frequencies of CTLA4+ CD8+ T cells (L) and OX40+ CD8+ T cells (M) in tumor-infiltrating lymphocytes (n=5). The proportions of Perforin+ CD8+ T cells (left) and TNFα+ CD8+ T cells among TILs (right) were summarized (N). Data are from one representative of three independent experiments. Error bars represent the SE, and p values were calculated by two-way ANOVA. NS stands for not significant. See also online supplemental figure S3. ANOVA, analysis of variance; LDL, low-density lipoprotein.
supplemental figure S3D–FJ,K). There was no impact on the ratio of naïve cells to memory cells (online supplemental figure S3B–H).

**LRP11 was needed for LDL-mediated stem-like T-cell induction**

To elucidate the mechanism underlying LDL-mediated stem-like T-cell proliferation, we first determined the expression of genes associated with LDL. The data show that many LDL receptor-related proteins (LRPs) were markedly increased in activated CD8^+ T cells (figure 3A). Using a cell-based chimeric receptor screening system,\(^3\) in which chimeric receptors composed of the extracellular domain of LRPs fused to the CD3e intracellular domain could induce β-galactosidase expression on binding to LDL, we found that the LRP11/CD3e chimeric receptor was stimulated by LDL. (figure 3B), suggesting that LRP11 could recognize LDL. To confirm this finding, we used an LRP11 blocking antibody to block LRP11. The in vitro stimulation assay showed that LRP11 blockade offset LDL-induced proliferation and the frequency of CD8^+ T cells (figure 3C,D). LRP11 blockade impaired IFNγ and TNFα secretion (figure 3E) and induced the expression of inhibitory molecule PD1 (online supplemental figure S4A). To explore the role of LRP11 in CD8^+ T cells, we used continuous stimulation.\(^3\) Surprisingly, the frequency of TCF1^+ CD8^+ T cells was decreased by the LRP11 blocking antibody, indicating that LRP11 could regulate TCF1 (figure 3F) during continuous stimulation. Moreover, the frequency of CD8^+ T cells treated with LDL and LRP11 blocking antibodies was different from that of CD8^+ T cells without LDL stimulation, whereas it was increased 4-fold in CD8^+ T cells with LDL stimulation (figure 3G). Next, we designed LRP11-specific shRNA and used the knockdown approach to examine the specific role of LRP11 (online supplemental figure S4B). Indeed, knockdown of LRP11 impaired CD8^+ T-cell proliferation and Tf7^+ expression (figure 3H and online supplemental figure S4B).

We next explored the physiological role of LRP11 in T cells in tumor tissues using LRP11 agonistic antibodies. First, MC38 cells were implanted subcutaneously into C57BL/6J male mice. One week later, PD1 blocking antibodies were injected i.p. at a dose of 12 mg/kg with or without LRP11 agonistic antibodies (20 mg/kg) when the tumor area reached 10 mm^2 (figure 4A). Tumor-bearing model experiments showed that LRP11 agonistic antibodies enhanced antitumor immunity and limited tumor growth, and the best outcomes were observed when the two drugs were used in combination (figure 4B–D). Consistent with LDL stimulation, LRP11 agonistic antibodies increased the frequency and total number of CD8^+ T cells in tumor tissues (figure 4E,F). There was no impact on the ratio of naïve cells to memory cells (figure 4G). LRP11 agonistic antibodies combined with PD1 blocking antibodies increased the frequency and total number of TCF1^+ CD8^+ T cells and PD1^+ TCF1^+ CD8^+ T cells in tumor tissues (figure 4H–J), the expression of OX40, Perforin and TNFα on CD8^+ T cells also increased (figure 4K,L).

All these results suggest that LRP11 enhanced exhausted CD8^+ T-cell proliferation and cytotoxic function. At the same time, we examined the changes in these parameters in the DLNs and spleen. In the DLNs, both the LRP11 agonist alone and the combination with PD1 antibody increased the proportion of tumor-infiltrating CD8^+ T cells and CD4^+ T cells (online supplemental figure S5A), as well as the release of perforin (online supplemental figure S5F). However, the impact on the PD1^+ TCF1^+ CD8^+ T cell subset was only observed with the combination therapy (online supplemental figure S5D). Other parameters did not show any significant changes in the DLNs (online supplemental figure S5B,C,E). Similarly, in the spleen, the same trend was observed as in the LDL. In the case of combination therapy, both the TCF1^+ CD8^+ T cell subset and the PD1^+ TCF1^+ CD8^+ T cell subset showed a decrease (online supplemental figure S5I,J), while other parameters did not show any changes in the spleen (online supplemental figure S5G,H,K,L).

**LRP11-induced stem-like T cells through MAPK13**

As reported, LRP11 is a membrane molecule, but TCF1 is located in the nucleus. We hypothesized that there is a shuttle factor linking LRP11 in the cytoplasm and TCF1 in the nucleus. To test this hypothesis, we applied IP and mass spectrometry (MS) using an LRP11 antibody. The IP-MS data show that LRP11 bound to mitogen-activated protein kinase (MAPK) 13 (online supplemental extended data table 1), which is located in both the cytoplasm and nucleus and acts as a kinase to phosphorylate transcription factors. To confirm this, we used a MAPK13 antibody to perform an IP assay. The IP data showed that MAPK13 interacted with LRP11 and TCF1 (figure 5A). To determine the role of MAPK13 in T-cell activation, we used a MAPK13 inhibitor, MPAK13-IN-1, to inhibit MAPK13 activation. The in vitro stimulation assay showed that MAPK13 inhibition decreased LDL-induced proliferation and the frequency of T cells (figure 5B,C). Furthermore, MAPK13 inhibition impaired IFNγ and TNFα secretion (figure 5D). During continuous stimulation, MAPK13 inhibition reduced the proportion of CD8^+ T cells and PD1^+ TCF1^+ CD8^+ T cells (figure 5E,F). MAPK13 knockdown with MAPK13-specific shRNA decreased LDL-induced proliferation, frequency of CD8^+ T cells and Tf7^+ expression (figure 5G,H and online supplemental figure S4C).

MAPK13 is a serine/threonine protein kinase. We next tested TCF1 phosphorylation in CD8^+ T cells with LDL stimulation. We found that LDL promoted TCF1 phosphorylation, which was eliminated by LRP11 blocking or MAPK13 inhibition (figure 5I). Notably, the TCF1 level correlated with its phosphorylation and was decreased by LRP11 blockade or MAPK13 inhibition (figure 5J and online supplemental figure S4D). These data demonstrated that the LRP11-MAPK13 axis induced TCF1 phosphorylation and activation. To explore the interaction between TCF1 and MAPK13, we constructed
Figure 3  LRP11 was required during LDL-induced TCF1⁺ T cells. (A) Splenic lymphocytes were separated and stimulated with anti-CD3 antibodies (5 µg/mL) and anti-CD28 antibodies (2 µg/mL) plus either vehicle control (Ctrl) or LDL (80 µg/mL) for 3 days. The indicated genes associated with LDL were detected by qPCR (n=3). (B) Indicated genes fused with CD3 chimeric receptor reporters were stimulated with LDL or vehicle control for 24 hours, and then, β-galactosidase activity was measured. Each symbol represents one individual (n=6). (C, D) Splenic lymphocytes were separated and stimulated with anti-CD3 antibodies (5 µg/mL) and anti-CD28 antibodies (2 µg/mL) plus LDL (80 µg/mL) in the presence of LRP11 (3 µg/mL) blocking antibodies for 3 days. Proliferation was assessed via CTV labeling by flow cytometry (C). The proliferation rate and total cell number (D) are summarized, and each symbol represents one individual (n=5). (E) CD8⁺ T cells were sorted and cultured in the presence of anti-CD3 antibodies (0.5 µg/mL), anti-CD28 antibodies (0.5 µg/mL), IL-2 (20 U/mL) and IL-12 (10 ng/mL) plus LDL (80 µg/mL) for 72 hours and subsequently assessed by flow cytometry, and the proliferation rate and total cell number were summarized. All data are the mean±SEM and were analyzed by two-tailed, unpaired Student’s t-tests (A, B) or two-way ANOVA (D–E, F–H right). The data are from one of three independent experiments. NS stands for not significant. ANOVA, analysis of variance; LDL, low-density lipoprotein. CTV, cell trace violet.
Figure 4  LRP11 activation induced tumor-infiltrating TCF1+ T cells and enhanced antitumor immunity. (A–D) Experimental design. MC38-bearing C57BL/6J male mice were injected i.p. with LRP11 agonistic antibodies (20 mg/kg) with or without αPD1 antibodies (12 mg/kg) or vehicle control (IgG). Time course of LRP11 agonistic antibody with or without αPD1 antibodies or IgG therapy initiated at 7, 9, and 11 days post-tumor initiation, comprised three doses (arrows) in C57BL/6J male mice (A). Representative pictures of tumors are shown (B). Tumor weights (C) were measured and are shown on day 13 post-tumor (n=5). Tumor growth curves (D) of MC38 subcutaneously injected into C57BL/6J male mice were measured and are shown at individual points (n=5). (E) The proportions of tumor-infiltrating CD4+ and CD8+ T cells were measured. Total number of indicated T cell subpopulations among TILs per gram of tumor (F). Frequencies of naïve CD8+ T cells (CD44−CD62L+), Tcm (CD44+CD62L−) and Tem (CD44+CD62L+) were measured (G). The proportions of TCF1+ CD8+ T cells (H) and total number of TCF1+ CD8+ T cells among TILs per gram of tumor (I) were summarized. The proportions of PD1+ TCF1+ CD8+ T cells (left) and total number of PD1+ TCF1+ CD8+ T cells among TILs per gram of tumor (right) were summarized (J). Statistical analyses of the frequencies of OX40+ PD1+ TILs (n=5) (K). The proportions of Perforin+ CD8+ T cells (left) and TNFα+ CD8+ T cells among TILs (right) were summarized (L). The results are expressed as the mean±SEM. Statistics were calculated by two-way ANOVA. NS, not significant. See also online supplemental figure S5. ANOVA, analysis of variance; i.p., intraperitoneally.
MAPK13 was needed in LDL-induced TCF1⁺ T cells. (A) Splenic CD8⁺ T cells were sorted and stimulated with anti-CD3 antibodies (1 μg/mL) and anti-CD28 antibodies (0.5 μg/mL) for 3 days. IP was performed using anti-MAPK13 antibodies, and LRP11, TCF1, and MAPK13 were detected (left) and quantitative assessment of protein expression were summarized (right) (n=3). The data are from one of three independent experiments. (B, C) Splenic lymphocytes were separated and stimulated with anti-CD3 antibodies (5 μg/mL) and anti-CD28 antibodies (2 μg/mL) plus LDL (80 μg/mL) in the presence of MAPK13 inhibitor (25 μM) for 3 days. Proliferation was assessed via CTV labeling by flow cytometry (B). The proliferation rate and total cell number are summarized (n=5) (C). (D) TNFα and IFNγ secretion was assessed by ELISAs. Each symbol represents one individual (n=4). (E) The proportions of CD8⁺ T cells in splenic lymphocytes from each group are summarized (n=5). (F) Gating scheme for PD1⁺ TCF1⁺ CD8⁺ T-cell sorting, and all cells were previously gated on CD8⁺ T cells (left). The proportions of PD1⁺ TCF1⁺ CD8⁺ T cells from each group are summarized (n=5). (G, H) Naive CD8⁺ T cells were isolated, activated and retrovirally transduced with pCDH-H1-EF1-Puro (scramble) or carrying murine MAPK13 shRNA. Cells were cultured with LDL (80 μg/mL) for 72 hours and subsequently assessed by flow cytometry (G). The proliferation rate and total cell number are summarized (n=5) (H). (I) Western blot analyses of TCF1 (C63D9) phosphorylation from nuclear extracts of splenic CD8⁺ T cells and quantitative assessment of protein expression were summarized (right) (n=3). Splenic CD8⁺ T cells were separated and stimulated with anti-CD3 antibodies (1 μg/mL) and anti-CD28 antibodies (0.5 μg/mL) plus LDL (80 μg/mL) in the presence of LRP11 blocking antibodies (3 μg/mL) or MAPK13 inhibitor (25 μM) for 3 days. (J) IP was performed to detect the interaction between MAPK13 and the indicated TCF1 mutants and quantitative assessment of protein expression were summarized (right) (n=3). Error bars represent the SE, and p values were calculated by two-way ANOVA. ANOVA, analysis of variance; IP, intraperitoneally; LDL, low-density lipoprotein. CTV, cell trace violet.
TCF1 truncating mutants (online supplemental figure S4E). IP data show that the C-terminus was needed for their binding (figure 5J). Moreover, TCF1 knockdown decreased LDL-induced proliferation and the frequency of CD8 T cells (online supplemental figure S4F,G).

To explore the role of MAPK13 in stem-like T cells in tumor tissues, we inhibited MAPK13 during PD1 blockade immunotherapy in an MC38 tumor-bearing mouse model. The tumor grew faster in mice treated with the MAPK13 inhibitor (figure 6A–D), showing that MAPK13 was needed for LDL-enhanced antitumor immunity. We next determined the frequency of CD4 T or CD8 T cells and found that tumor-infiltrating CD8 T cells were slightly decreased when MAPK13 was inhibited (figure 6E). In addition, OX40 and CTLA4 on CD8 T cells were induced by PD1 blockade and LDL (figure 6F). Interestingly, the MAPK13 inhibitor reduced PD1 TCF1 CD8 T cells in tumor tissues (figure 6G), tumor-draining LNs (online supplemental figure S6H) and splenic lymphocytes (online supplemental figure S6I). OX40 and CTLA4 showed no changes in the DLNs and spleen (online supplemental figure S6F,G), the frequency of CD8 T cells in DLNs was increased by PD1 blockade and LDL and decreased when MAPK13 was inhibited (online supplemental figure S6D). However, there were no alterations in the proportions of CD8 T cells and CD4 T cells in the spleen (online supplemental figure S6E). These data were consistent with the B16-F10 melanoma-bearing mouse model (online supplemental figure S6A-C).

**DISCUSSION**

Recent advances highlight the critical role of TCF1 in stem-like T cells in tumor tissues and chronic viral infection. In contrast to the detailed characterization of TCF1, which controls transcriptional reprogramming in tumor-infiltrating stem-like T cells, much less is known about the regulation of TCF1, which is located in the nucleus. Here, we found that LRP11, a membrane receptor, functioned as a sensor to receive extracellular signals and activated TCF1. LRP11 activation enhanced TCF1 CD8 T-cell-mediated antitumor immunity and enhanced the antitumor capacity of PD1 blockade immunotherapy. Mechanistically, LRP11 activation induced MAPK13 activation. Then, MAPK13 phosphorylates TCF1. This leads to enhanced proliferation and cytotoxicity of CD8 T cells, and an extended duration of their effector function in tumors. It also increases the proportion of tumor infiltrating stem-like T cells, leading to a stronger antitumor immunity (figure 7).

Stem-like or progenitor T cells in tumors correlate with long-term maintenance of T-cell responses and better patient outcomes after immunotherapy. Although exhausted and dysfunctional signals, such as persistent antigen exposure, high inhibitory ligands and cytokines, which promote CD8 T-cell exhaustion, are enriched in tumors, stem-like T cells maintain the proportion and response to PD1 blockade. Importantly, TCF1 acts as a master regulator to control T-cell fate and development. Emerging data show that TCF1 CD8 T cells reside in specific tissue niches in tumors or viral infections. How TCF1 CD8 T cells receive signals in niches is unclear. Here, we found that tumor-infiltrating TCF1 CD8 T cells were induced by LDL stimulation through LRP11. LRP11 blockade offset LDL-mediated TCF1 CD8 T-cell induction, whereas LRP11 activation by agonistic antibodies promoted the expansion and differentiation of tumor-infiltrating TCF1 CD8 T cells and enhanced antitumor immunity. In our in vivo experiments, we found that the increase of TCF1 CD8 T-cell subset was only significant when LDL was used in combination with anti-PD1 antibodies. When LDL was used alone, this subset of cells did not show as significant a change as observed in vitro. This may be due to the fact that the dose of LDL used in vitro was higher, with 80 µg/mL of LDL equivalent to the LDL content in high-fat diet mice, thus having a stronger stimulating effect on cell proliferation, whereas the dosage administered in vivo was only equivalent to the normal mouse serum content, hence having a less significant effect. Furthermore, several reports have shown that TCF1 CD8 T cells in DLNs migrate to tumors or the pancreas, where they differentiate further and function. In our study, we also observed an increased proportion of this cell subset in the DLNs. However, we did not observe a significant increase in the expression of OX40, perforin, and TNFα secretion. We also considered that the DLNs mainly transport lymphocytes to the tumor, where they eventually develop and mature, expressing more activation markers.
Figure 6  MAPK13 was needed during PD1 blocking immunotherapy. MC38 colon carcinoma-bearing C57BL/6J male mice were injected i.p. α-PD1 antibodies (12 mg/kg) plus LDL (40 mg/kg) in the presence or absence of a MAPK13 inhibitor (20 mg/kg) (A). (B–D) Representative pictures of tumors are shown (B). Tumor growth curve of MC38 cells subcutaneously implanted into C57BL/6J male mice (n=6) (D). (E, F) Tumor-infiltrating CD8+ T cells, CD4+ T cells (E), CTLA4+CD8+ T cells, and OX40+CD8+ T cells (F) were assessed by flow cytometry (n=5). (G) Tumor-infiltrating lymphocytes were separated from tumors on Day 14 post-tumor initiation. Representative plots of the coexpression pattern of TCF1 with the effector molecule PD1 in CD8+ T cells from the indicated groups (left). Frequencies and total number were summarized in addition (right). (H–J) RNA-seq experiment was performed with samples obtained from CD8+ T cells sorted from spleens. Splenic CD8+ T cells were sorted and stimulated with anti-CD3 antibodies (1 µg/mL) and anti-CD28 antibodies (0.5 µg/mL) plus LDL (80 µg/mL) in the presence of MAPK13 inhibitor (25 µM) for 3 days. Then collected cells for RNA-seq. The samples were sequenced using Illumina NovSeq 6000 platform. (H) Volcano plot showing RNA-seq with increased expression (red) and decreased expression (blue) (FDR-adjusted p<0.05) of splenic CD8+ T cells in the presence of LDL (80 µg/mL) vs LDL (80 µg/mL) plus MAPK13 inhibitor (25 µM). (I) Heatmaps representing major differential gene expression of cytokines for splenic CD8+ T cells in the presence of LDL (80 µg/mL) compared with LDL (80 µg/mL) plus MAPK13 inhibitor (25 µM). (J) Pathway enrichment analysis of RNA-seq showing the top 20 downregulated pathways of splenic CD8+ T cells in the presence of LDL (80 µg/mL) vs LDL (80 µg/mL) plus MAPK13 inhibitor (25 µM) ranked by adjusted p value. Data are from one representative of three independent experiments. Error bars represent the SEM, and p values were calculated by two-way ANOVA. NS stands for not significant. See also online supplemental figure S6. ANOVA, analysis of variance; LDL, low-density lipoprotein.
and releasing more tumor-killing cytokines. One curious observation is that when the PD1 antibodies were used in combination with LDL or agLRP11, there was a decrease in the proportion of PD1+TCF1+CD8+ T cells in the spleen. We speculate that this could be due to the migration of this cell subset in the spleen to the tumor site, where they exert their function. We propose that LRP11 functions in a TCF1-dependent manner and induces TCF1 activation, leading to the expansion and differentiation of tumor-infiltrating TCF1+ CD8+ T cells.

Due to the different cellular locations of LRP11 and TCF1, a shuttle molecule is needed for this effect. MAPK13, which is distributed in the cytoplasm and nucleus, was identified to interact with LRP11 and TCF1. MAPK13 is a p38 MAP kinase that responds to extracellular stimuli and then directly phosphorylates and activates transcription factors, leading to cell proliferation. Our data suggested that MAPK13 bound to TCF1 and was needed for LDL-induced TCF1 phosphorylation, suggesting that MAPK13 directly phosphorylated TCF1. TCF1 resides in the nucleus, and its phosphorylation is less discussed. In the embryonic ectoderm, the TCF family members LEF1, TCF4, and TCF3 are phosphorylated after Wnt stimulation. Of note, at early stages of T-cell development, TCF1 is rapidly induced by NOTCH signaling and binds to β-catenin, which is activated by the Wnt cascade induced by Frizzled and LRP5/6 receptors interacting with extracellular Wnt ligands, to promote chromatin accessibility. Here, our data showed that TCF1 displayed increased phosphorylation after LDL stimulation. All these data show that TCF1 phosphorylation enhances TCF1 epigenetic and transcriptional reprogramming in stem-like CD8+ T cells.

Thus, we identified LRP11 as a cell surface regulator of stem-like CD8+ T cells. LRP11 activation promoted TCF1-mediated transcriptional reprogramming in stem-like CD8+ T cells. These data extend our understanding of the regulation of TCF1+ stem-like CD8+ T cells. Moreover, our data indicate that MAPK13 can be activated by LRP11 activation and phosphorylated TCF1. Thus, our findings highlight the role of the LRP11-MAPK13 axis in TCF1+ stem-like CD8+ T cells, which are optimal targets for tumor immunotherapy and other chronic diseases.
REFERENCES


