ONCOIMMUNOLOGY
2022, VOL. 11, NO. 1, e2021619 (13 pages)
https://doi.org/10.1080/2162402X.2021.2021619

Taylor & Francis
Taylor & Francis Group

ORIGINAL RESEARCH

OPEN ACCESS Check for updates

Immunomodulation via FGFR inhibition augments FGFR1 targeting T-cell based antitumor immunotherapy for head and neck squamous cell carcinoma

Michihisa Kono[a], Hiroki Komatsuda[a,*], Hidekiyo Yamaki[a,*], Takumi Kumai[a,b], Ryusuke Hayashi[a], Risa Wakisaka[a], Toshihiro Nagato[c], Takayuki Ohkuri[c], Akemi Kosaka[c], Kenzo Ohara[a], Kan Kishibe[a], Miki Takahara[a], Akihiro Katada[a], Tatsuya Hayashi[a,b], Hiroya Kobayashi[c], and Yasuaki Harabuchi[a]

[a]Department of Otolaryngology-Head & Neck Surgery, Asahikawa Medical University, Asahikawa, Japan; [b]Department of Innovative Head & Neck Cancer Research and Treatment, Asahikawa Medical University, Asahikawa, Japan; [c]Department of Pathology, Asahikawa Medical University, Asahikawa, Japan

ABSTRACT

Fibroblast growth factor receptor 1 (FGFR1) is overexpressed in multiple types of solid tumors, including head and neck squamous cell carcinoma (HNSCC). Being associated with poor prognosis, FGFR1 is a potential therapeutic target for aggressive tumors. T cell-based cancer immunotherapy has played a central role in novel cancer treatments. However, the potential of antitumor immunotherapy targeting FGFR1 has not been investigated. Here, we showed that FGFR-tyrosine kinase inhibitors (TKIs) augmented antitumor effects of immune checkpoint inhibitors in an HNSCC mouse model and upregulated tumoral MHC class I and MHC class II expression *in vivo* and *in vitro*. This upregulation was associated with the mitogen-activated protein kinase signaling pathway, which is a crucial pathway for cancer development through FGFR signaling. Moreover, we identified an FGFR1-derived peptide epitope ($FGFR1_{305-319}$) that could elicit antigen-reactive and multiple HLA-restricted $CD4^+$ T cell responses. These T cells showed direct cytotoxicity against tumor cells that expressed FGFR1. Notably, FGFR-TKIs augmented antitumor effects of FGFR1-reactive T cells against human HNSCC cells. These results indicate that the combination of FGFR-TKIs with immunotherapy, such as an FGFR1-targeting peptide vaccine or immune checkpoint inhibitor, could be a novel and robust immunologic approach for treating patients with FGFR1-expressing cancer cells.

ARTICLE HISTORY
Received 10 October 2021
Revised 16 December 2021
Accepted 17 December 2021

KEYWORDS
FGFR1; FGFR tyrosine kinase inhibitor; immunotherapy; peptide vaccine; tumor-associated antigen; head and neck squamous cell carcinoma

Introduction

Head and neck squamous cell carcinoma (HNSCC), which affects the 1) oral cavity, 2) nasal cavity and paranasal sinuses, 3) nasopharynx, 4) oropharynx, 5) hypopharynx, and 6) larynx, annually causes an estimated 300,000 deaths worldwide.[1] Despite advances in surgery and chemoradiotherapy, many patients with HNSCC (especially human papillomavirus (HPV)-negative HNSCC) experience recurrence and metastases. The survival rate of HNSCC patients is less than 50%, which has not changed for decades.[2] Although cetuximab – a drug that targets the epidermal growth factor receptor (EGFR) – is clinically approved, its clinical efficacy is limited in advanced HNSCC patients.[3] Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of cancer and demonstrated that cancer immunotherapy to be effective in clinical practice. However, only a small number of patients (about 20%) benefit from ICIs in various cancers, including HNSCC.[4] Therefore, the development of novel cancer immunotherapy for HNSCC patients is warranted.

The fibroblast growth factor receptor (FGFR) family consists of four main receptor-type tyrosine kinases (FGFR1-4) that are associated with tissue restoration, angiogenesis, and oncogenesis.[5] FGFR1 overexpression causes tumor formation through mitogen-activated protein kinase (MAPK), PI3K/AKT, and JAK/STAT signaling[6,7] and poor outcome in HNSCC.[8] Many clinical trials using FGFR inhibitors have been conducted on various cancers and have shown some efficiency and tolerability.[9–11] However, the combined effect of FGFR inhibitors with other therapies is unknown, and studies investigating the immune effects of FGFR1 inhibition are sparse. As the inhibition of other tyrosine kinase receptors, such as EGFR, augments T cell responses,[12] immunemodulation via FGFR1 blockade is a potential approach for immunotherapy.

Increasing the number of tumor-reactive T cells is the key to successful cancer immunotherapy, as there are only a few T cells and they are weak against tumors. While adoptive cell transfer of tumor-reactive T cells can generate many tumor-reactive T cells, it is difficult to translate this therapy into the clinic because of its complexity and associated high cost. Cancer vaccines, created using tumor antigen-targeted synthetic peptides, are among potential therapies for eliciting tumor-reactive T cell responses against solid tumors. Recently, the development of appropriate adjuvants and

CONTACT Takumi Kumai t-kumai@asahikawa-med.ac.jp Department of Otolaryngology-Head and Neck Surgery, Asahikawa Medical University, Midorigaoka-Higashi 2-1-1-1, Asahikawa 078-8510, Japan

*These authors contributed equally to the work.

Supplemental data for this article can be accessed on the publisher's website.

identification of highly immunogenic antigens has enhanced the antitumor activity of peptide-based cancer vaccines.[13] Although tailor-made peptide vaccines targeting mutation-derived neoantigens have shown potent effects,[14] high costs, and complicated techniques have impeded their acceptance in clinical practice. Thus, peptide vaccines targeting tumor-associated antigens (TAAs), which are expressed in many cancers, are being considered. Identification of effective TAAs and potent adjuvants are required to develop novel and robust cancer immunotherapies.[15]

In this study, we demonstrated that FGFR-tyrosine kinase inhibitors (TKIs) exhibited synergistic activity with ICI in the mouse HNSCC model. FGFR-TKIs amplified the expression of MHC class I and MHC class II in HNSCC cells. The upregulation of MHC class II expression was induced by CIITA, subsequent to inhibition of the FGFR/MAPK pathway. Moreover, we identified a novel FGFR1-derived peptide epitope that could generate antigen-reactive and multiple HLA-DR-restricted $CD4^+$ T cell antitumor responses in healthy donors and HNSCC patients. Notably, we found that FGFR-TKIs augmented the antitumor effects of FGFR-reactive T cells *in vitro*. Overall, these results suggest that FGFR blockade is a novel and suitable combination approach with T cell-based cancer immunotherapy.

Materials and methods

Cell lines and mice

HSC2 (human oral SCC; HLA-DR13), HSC3 (human tongue SCC; HLA-DR15), HSC4 (human tongue SCC; HLA-DR1, 4, and 53), and Sa-3 (human gingival SCC; HLA-DR9, 10, and 53) were supplied by the RIKEN BioResource Center (Tsukuba, Ibaraki, Japan). HPC-92Y (human hypopharyngeal SCC; HLA-DR4, 9, and 53) and CA9-22 (human gingival SCC, HLA-DR 8 and 15) were kindly provided by Dr. Syunsuke Yanoma (Yokohama Tsurugamine Hospital, Yokohama, Japan). SAS (human tongue SCC), SCC152 (HPV-positive human tongue SCC), SCC090 (HPV-positive human hypopharynx SCC), and the T-cell leukemia cell line Jurkat was purchased from the American Type Culture Collection (Manassas, VA, USA). UM-SCC-47 (HPV-positive human tongue SCC) was supplied by Merck Millipore (Burlington, MA, USA). MOC1 (tongue SCC derived from C57BL/6 mice) was supplied by Kerafast Inc. (Boston, MA, USA). L cells (mouse fibroblast cell lines) expressing individual human HLA-DR molecules (HLA-DR4 and 53) were kindly provided by Dr. R. Karr (Karr Pharma, St. Louis, MO) and Dr. Sasazuki (Kyushu University, Fukuoka, Japan). C57BL/6 mice (female, 8 to 10 weeks old) were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan). All mice were maintained in a reactive pathogen-free facility at the Asahikawa Medical University. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Asahikawa Medical University (#20001).

Flow cytometry

Immune cells from mice were stained with PerCP-conjugated anti-CD4 (GK1.5, BioLegend) mAbs, APC/Cy7-conjugated anti-CD8a (53–6.7) mAbs, and the isotype monoclonal mAb. FITC-conjugated anti-I-A/I-E mAbs (M5/114.15.2, BioLegend) was used for negative gating. After pretreatment with 3 μM FGFR1-TKIs (PD173074; AZD4547; Erdafitinib, Selleck Chemicals), 3 μM mitogen-activated protein kinase (MAPK) inhibitor (MEK inhibitor U0126, Promega), MAPK siRNA (SignalSilence® p44/42 MAPK Erk1/2 siRNA, Cell Signaling Technology), 3 μM STAT3 inhibitor (S3I-201, Selleck Chemicals), or 3 μM PI3K inhibitor (BYL719, Selleck Chemicals) for 48 hr, HLA class I and HLA-DR expression on tumor cell lines was assessed via flow cytometry using anti-HLA class I antibodies (Abs) conjugated with fluorescein isothiocyanate (G46-2, BD Pharmingen) and anti-HLA-DR Abs conjugated with phycoerythrin (TU36, BD Pharmingen). HNSCC cell lines were treated with or without 50 U/ml IFN-γ for 48 hr before the assay. IgG1 (MOPC-21, BioLegend) and IgG2a (MOPC-173; BioLegend) were used as isotype controls. Intracellular IFN-γ staining were performed using Perm/Wash™ (BD Pharmingen), Cytofix/Cytoperm™ (BD Pharmingen), APC-conjugated anti-IFN-γ mAbs (4S.B3, BioLegend), and FITC-conjugated anti-granzyme B mAbs (GB11, BioLegend). Samples were analyzed using the CytoFLEX LX flow cytometer and CytExpert (Beckman Coulter).

Western blotting

The tumor cell line proteins extracted using the Miute™ Total Protein Extraction Kit (Invent Biotechnologies, Inc.) were subjected to electrophoresis on NuPAGE Bis–Tris gels (Invitrogen, Thermo Fisher Scientific Inc.) and transferred to an Immobilon-P membrane (Merck Millipore). The membrane was incubated with mouse anti-human FGFR1 Abs (M19B2, Novus Biologicals) and mouse anti-human β-actin Abs (C4, Santa Cruz Biotechnology, Santa Cruz, CA) and detected via chemiluminescence using the Amersham ECL Prime Western blotting Detection System (GE Healthcare Life Sciences) and Invitrogen iBright Imaging Systems 1500 (Invitrogen, Thermo Fisher Scientific). Class II transactivator (CIITA) expression was evaluated using mouse anti-CIITA Abs (sc-13556, 7–1 H, Santa Cruz Biotechnology, Santa Cruz, CA). Phosopho-MAPK (pERK1/2) and MAPK(Erk1/2) expression was assessed using rabbit anti-phasopho-p44/42 MAPK (pERK1/2) Ab (Thr202/Tyr204, Cell Signaling Technology) and rabbit anti-p44/42 MAPK(Erk1/2) Ab (137F5, Cell Signaling Technology), respectively. MAPK siRNA was assessed using Lipofectamine ® RNAiMAX Transfection Reagent (Invitrogen, Thermo Fisher Scientific) and OptiMEM I Reduced Serum Medium (Invitrogen, Thermo Fisher Scientific) according to the manufacturer's instructions (Supplemental Figure S1). Protein expression was analyzed using ImageJ.

Synthetic peptides

HLA-DR-binding epitope sequences of FGFR1 were selected using the computer-based algorithms from SYFPEITHI (http://www.syfpeithi.de/)[16] and Immune Epitope Database Analysis Resource (IEDB, RRID:SCR_013182, https://www.iedb.org/).[17] Amino acids were scored for their likelihood of binding to common HLA-DR molecules. We selected $FGFR1_{305-319}$ (LPYVQILKTAGVNTT), as it had a high binding score with multiple HLA-DR molecules (DRB1*0101, DRB1*0401, DRB1*0701, DRB1*1101, and DRB1*1501). The $FGFR1_{305-319}$ peptide was purified by Hokkaido System Science (Sapporo, Japan). Using the same methods, homologous peptides to $FGFR1_{305-319}$ peptide from FGFR3 and FGFR4 protein were identified and synthesized. The PADRE peptide (aK-Cha- VAAWTLKAAa, where "a" denotes D-alanine and "Cha" denotes L-cyclohexylalanine), which can bind with multiple HLA-DR molecules, was used as a positive control.

In vivo assessment of combination therapy with FGFR-TKIs and T-cell based immunotherapy

C57BL/6 mice were intradermally injected with 1×10^6 MOC1 cells. The mice were intraperitoneally administered with PD173074 (20 mg/kg) and anti-PD-1 Ab (200 μg/mouse), three times per week from 18 days after inoculating MOC1 (tumor diameter: 7–8 mm). Tumor growth was monitored every three days by measuring two opposing diameters with a pair of calipers. Tumor volume was calculated as length × $width^2$)/2. Results are presented as mean tumor volume (mm^3) with standard deviation (SD). Tumors were harvested on day 43 for assessment of tumor-infiltrating lymphocytes (TILs) and immunohistochemistry. The TILs were disaggregated from tumor tissues using collagenase (1 mg/ml) and gentlMACS (Miltenyi Biotec, Berguch, Germany) according to the manufacturer's instructions. Surface markers of TILs were assessed via flow cytometry. To assess MHC class I, MHC class II, and PD-L1 expression, formalin-fixed, paraffin-embedded (FFPE) tumor samples were evaluated via immunohistochemistry. Anti-MHC class I (anti-mouse $H\text{-}2K^b$ Ab, AF6-88.5, BioLegend), anti-MHC class II (anti-mouse I-A/I-E Ab, M5/114.15.2, BioLegend), and anti-PD-L1 (10 F.9G2, BioLegend) Abs were used as the primary Abs. FFPE specimens were stained using the VENTANA Benchmark GX (Roche Diagnostics).

In vitro generation of FGFR1-reactive $CD4^+$ T cells

The process used to generate peptide-reactive $CD4^+$ T cell (HTL) lines from healthy donor peripheral blood mononuclear cells (PBMCs), has been previously described in detail.[18] Briefly, dendritic cells (DCs) were induced by stimulating $CD14^+$ cells, isolated using the EasySep™ Human $CD14^+$ Positive Selection Kit (STEMCELL), with GM-CSF (50 ng/ml, PeproTech, Rocky Hill) and IL-4 (1000 IU/ml, PeproTech, Rocky Hill, NJ). HTLs isolated using the EasySep™ Human $CD4^+$ T Cell Isolation Kit (STEMCELL technology) were stimulated by peptide-pulsed autologous DCs for one cycle and γ-irradiated autologous PBMCs for two cycles. HTLs were assessed for production of IFN-γ with $FGFR1_{305-319}$ peptide stimulation using enzyme-linked immunosorbent assay (ELISA) kits (BD Pharmingen), according to the manufacturer's instructions, and then compared to the unstimulated control. Microcultures with a significant increase in IFN-γ production after $FGFR1_{305-319}$ peptide stimulation were subsequently expanded. Finally, $FGFR1_{305-319}$-reactive HTL lines were isolated by limiting dilution.

Measurement of antigen-reactive responses by FGFR1-reactive $CD4^+$ T cell lines

The measurement methods of targeting antigen-reactive responses by HTLs have been previously described in detail.[18] IFN-γ production in the supernatants co-cultured with FGFR1-reactive HTL lines and autologous PBMCs (1×10^5), L-cells (3×10^4), or FGFR1-expressing HNSCC cell lines (3×10^4) as antigen-presenting cells (APCs) was measured using ELISA kits (BD Pharmingen, San Diego, CA). Production of IL-2, TNF-α, and GM-CSF in these supernatants was evaluated using ELISA kits (BD Pharmingen), according to the manufacturer's instructions. Tumor cells were treated with 3 μM FGFR1-TKIs for the indicated experiments. To investigate the reactivity with FGFR3- or FGFR4-derived homologous peptides to FGFR1, FGFR1-induced HTL lines were co-cultured with autologous PBMCs (1×10^5) and $FGFR3_{303-317}$ (TPYVTVLKTAGANTT) or $FGFR4_{313-327}$ (FPYVQVLKTADINSS) peptides. $EGFR_{875-889}$ (KVPIKWMALESILHR) was used as a negative control peptide. To enhance HLA-DR expression, HNSCC cell lines were treated with 500 U/ml IFN-γ (PeproTech) for 48 hr before the assay. HLA restriction was assessed using anti-HLA-DR Ab L243 (HB-55, ATCC) and anti-HLA class I Ab W6/32 (HB-95, ATCC). In the indicated experiments, $MDM2_{32-46}$-reactive HTL lines (H40)[19] were used instead of FGFR1-induced HTL lines.

Cytotoxicity assay

Supernatants of cocultured FGFR1-reactive HTL lines with target tumor cell lines were assessed using granzyme B ELISA kits (MABTECH) according to the manufacturer's instructions. To evaluate the killing activity, target tumor cell lines were labeled using the CellTrace™ CFSE Cell Proliferation Kit (Invitrogen, Thermo Fisher Scientific Inc.). After 6 h of coculturing with various effector/target cell (E:T) ratios of FGFR1-reactive HTL lines, the number of dead tumor cells, labeled using 7-AAD viability staining solution (BioLegend), was quantified via flow cytometry.

FGFR1 peptide-reactive responses by T cells from HNSCC patients

PBMCs from HNSCC patients were co-cultured with $FGFR1_{305-319}$ peptides in 96-well plates, as described previously.[20] The PADRE peptide (capable of binding to all HLA-DR molecules) was co-cultured with PBMCs as a positive control. Briefly, PBMCs (1×10^5) were stimulated with peptides (10 μg/mL) for 2 cycles every 7 days, and IFN-γ

production in the supernatants was measured using ELISA. Anti-HLA-DR Ab L243 (HB-55, ATCC) was applied to confirm that the IFN-γ production is mediated through HLA-DR /peptide/T cell receptor complex. All investigations were approved by the Institutional Ethics Committee of Asahikawa Medical University (#16217), and written informed consent was obtained from all participants.

Statistical analysis

All data were assessed using Student's *t*-test or Fisher's exact test. Statistical significance was set at $p < .05$.

Results

Antitumor effects of combination therapy with FGFR-TKIs and ICI in mouse model

To investigate whether the FGFR inhibition can be applied to cancer immunotherapies as an immunomodulator, we evaluated the immunomodulatory effects of FGFR-TKIs in a mouse model of MOC1, a mouse tongue cancer cell line expressing FGFR1 (Figure 1a). MOC1-inoculated mice were treated with combination therapy using an FGFR-TKI (PD173074) and anti-PD-1 Abs as shown in Figure 1b. The combination therapy showed a synergistic antitumor effect and significantly reduced tumor growth (Figure 1c). FGFR inhibitor recruited CD4+ and CD8 + T cells in tumor microenvironment, which was further increased with the combined blockade of FGFR and PD-1 (Figure 1d). Remarkably, immunohistochemistry of tumors treated with PD173074 revealed a significant upregulation of MHC class I and MHC class II (Figure 1e). *In vitro* analysis also demonstrated that three FGFR-TKIs (PD173074, AZD4547, and erdafitinib) upregulated MHC class I and MHC class II expression on MOC1 cells with or without IFN-γ (figure 1f and g). Moreover, PD-L1 expression in TILs was also increased suggesting that FGFR inhibitor can be a promising adjuvant with PD-1 blockade (Supplemental Figure S2). Because tumors reduce the expression of MHC molecules to escape from immune cells,[21] an immune adjuvant that upregulates expression of MHC molecules on tumor cells is crucial for effective T cell-based cancer immunotherapy. Therefore, FGFR-TKIs might be practical immune adjuvants to combine with T-cell based immunotherapy including ICI via upregulation of MHC expression on tumor cells.

FGFR1 blockade upregulates tumor HLA expression through MAPK signaling pathway

Next, we evaluated the effects of FGFR-TKIs as immune mediators in human HNSCC cells *in vitro*. FGFR1 was expressed in most of the human HNSCC cells tested in this study (Figure 2a), and FGFR-TKIs inhibited the tumor proliferation (Supplemental Figure S3). As well as in the mouse model, three FGFR-TKIs enhanced HLA class I and HLA-DR

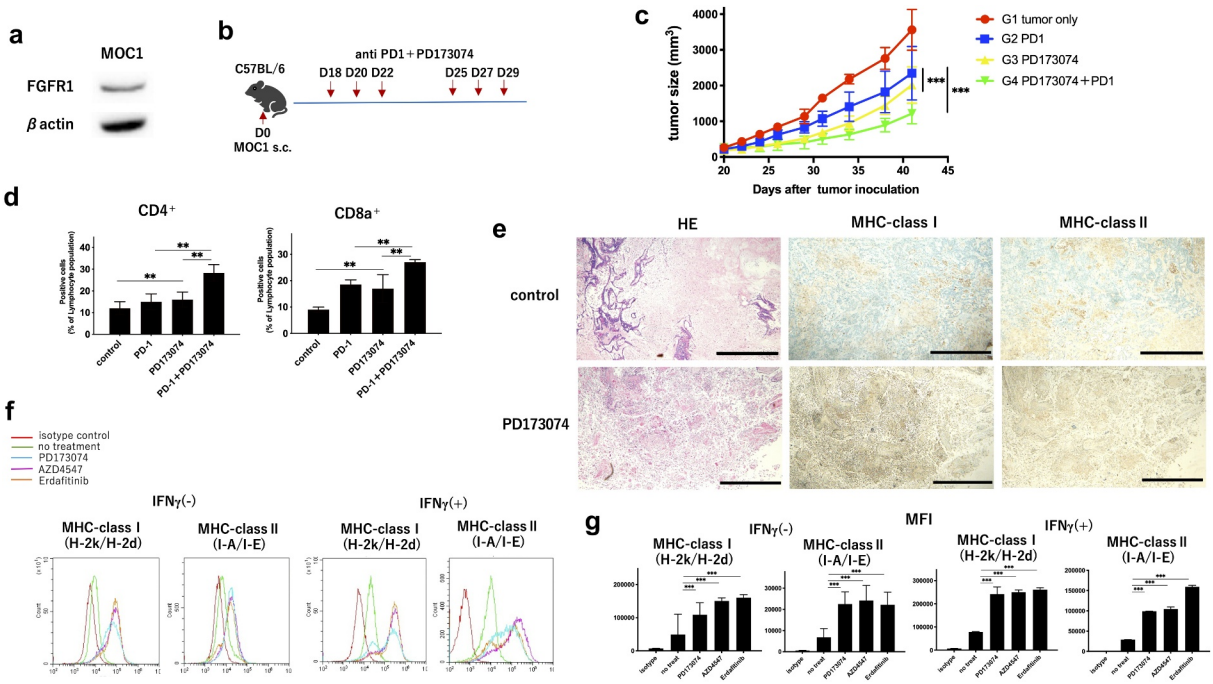


Figure 1. FGFR1 as an immune adjuvant to combine with ICI in HNSCC mouse models. (a) FGFR1 expression in mouse HNSCC cell lines (MOC1) was examined by Western blotting. (b) Experimental schema. C57BL/6 mice were intradermally injected with MOC1 ($1x10^6$). PD173074 (20 mg/kg) and anti-PD-1 Ab (200 μg/mice) was administered 3 times per week from day 18 (tumor size: 7–8 mm). (c) Tumor growth curves. Control (Red), anti-PD-1 Ab monotherapy (Blue), PD173074 monotherapy (Yellow), and combination therapy with PD173074 and anti-PD-1 Ab (Green) (n = 4 or 5 /group). Bars and error bars indicate the mean and SD, respectively (*p < .05, **p < .01, ***<0.001, one-way ANOVA). (d) The mice were sacrificed on day 42, and the percentages of $CD4^+$ T cells and $CD8^+$ T cells in TILs were evaluated with flow cytometry. (e) A representative image of MHC-class I or MHC-class II expression in immunohistochemistry on tumor (Day 42). MHC-class I (central) and MHC-class II (right) was enhanced by PD173074. H&E staining was shown in the left. Scale bars represent 100 μm. (f, g) MHC-class I and MHC-class II expression on MOC1 incubated with 3 μM FGFR-TKIs for 48 hr were evaluated by flow cytometry. MOC1 was treated with or without 50 U/ml IFN-γ for 48 hr before the assay. Red: isotype control, Green: untreated tumor cell lines, Blue: treated with PD173074. Pink: treated with AZD4547. Orange: treated with Erdafitinib. (f) Representative data of flow cytometry. (g) Averages values of mean fluorescence intensity (MFI) by FGFR-TKIs. (*p < .05, **p < .01, ***<0.001, Student's *t* test).

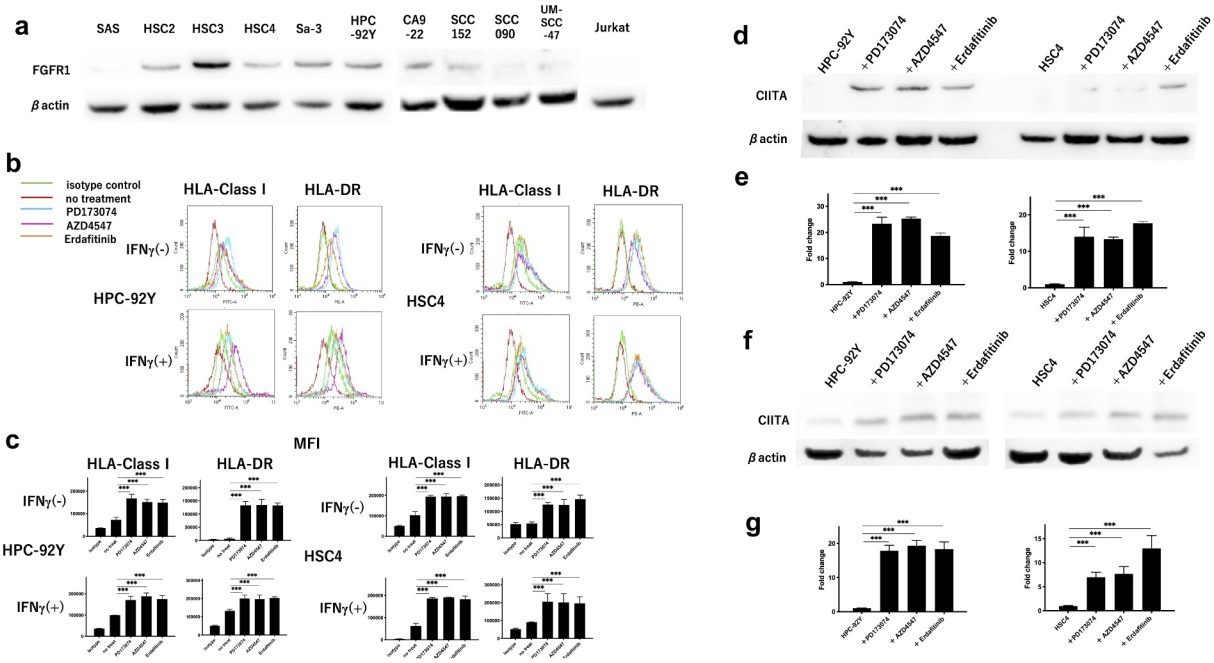


Figure 2. The changes of HLA and CIITA expression on HNSCC cell lines by FGFR1-TKIs. (a) FGFR1 expression in human HNSCC cell lines was examined by Western blotting. Jurkat (leukemia cells) was used as a negative control. (b, c) HLA-class I and HLA-DR and expression on HNSCC cell lines incubated with 3 μM FGFR-TKIs for 48 hr were evaluated by flow cytometry. HNSCC cell lines were treated with or without 50 U/ml IFN-γ for 48 hr before the assay. Green: isotype control, Red: untreated tumor cell lines, Blue: treated with PD173074. Pink: treated with AZD4547. Orange: treated with Erdafitinib. (b) Representative data of flow cytometry. (c) Averages values of mean fluorescence intensity (MFI). (d-g) FGFR-TKIs (3 μM) upregulated CIITA expression in HNSCC cell lines. HNSCC cell lines were treated with or without 50 U/ml IFN-γ for 48 hr before the assay. (d) Representative data of Western blotting without IFN-γ. (e) Quantitative analysis of protein expression. (f) Representative data of Western blotting with IFN-γ. (g) Quantitative analysis of protein expression. Each data was representative in the triplicate experiments. Bars and error bars show the mean and SD, respectively. (*p < .05, **p < .01, ***<0.001, Student's *t* test).

expression on human FGFR1-expressed tumor cell lines (Figure 2b and c). Since IFN-γ is a potent activator of HLA expression, the increase of HLA expression by FGFR inhibitor was maintained even in the presence of IFN-γ. Because Class II transactivator (CIITA) is a master regulator of MHC Class II gene, we next evaluated the expression of CIITA in tumor cells treated with FGFR inhibitors. CIITA expression was induced by FGFR-TKIs (Figure 2d and e). Since IFN-γ alone could induce CIITA expression (Supplemental Figure 4), FGFR-TKIs further increased the effect of IFN-γ (figure 2f and g).

Pathways downstream of FGFR include the MAPK, STAT3, and PI3K signaling pathways, which are crucial for oncogenesis via FGFR signaling. To elucidate the mechanism by which pathway is responsible for FGFR-TKIs-induced HLA expression, tumor cells were treated with MAPK, STAT3, or PI3K inhibitors. Notably, the MAPK inhibitor enhanced HLA class I and HLA-DR expression on tumor cell lines (Figure 3a and b). As a proof of concept, the phosphorylated MAPK was inhibited by FGFR inhibitor (Supplemental Figure S5). The upregulation of HLAs via MAPK inhibition was further confirmed by silencing MAPK gene (Figure 3c and d), whereas STAT3 or PI3K inhibition had no effect on HLA expression in tumors (Figure 3e and f). The expression of HLAs was upregulated with FGFR inhibition even in the presence of IFN- γ, a potent inducer of HLAs (Supplemental Figure S6). MAPK inhibiton also upregulated CIITA expression suggesting that the FGFR1/MAPK pathway might suppresses HLA Class II expression through inhibiting CIITA (Figure 3g and h). These results suggest that FGFR inhibits HLA class I and CIITA/HLA class II expression in tumors through MAPK signaling, and FGFR-TKIs could be immune adjuvants for T-cell-based immunotherapy by upregulating antigen presentation on tumor.

Generation of FGFR1-reactive CD4$^+$ T cell lines

As FGFR-TKIs should be used in FGFR-expressing tumors, it is rational to target FGFR as an immune antigen for combined immunotherapy with FGFR-TKIs. Using computer-based algorithm, we selected peptide FGFR1$_{305\text{-}319}$ (LPYVQILKTAGVNTT) as a potential candidate to elicit CD4$^+$ T cell responses (Supplemental Figure S7). FGFR1$_{305\text{-}319}$ peptide-reactive HTL lines were induced by repeatedly stimulating CD4$^+$ T cells from healthy donors with the FGFR1$_{305\text{-}319}$ peptide. FGFR1$_{305\text{-}319}$ peptide-reactive HTL lines (K1, K2, and K3) released IFN-γ in a peptide dose-dependent manner (Figure 4a). This response was inhibited by anti-HLA-DR Abs but not by anti-HLA class I Abs suggesting that the peptide recognition of HTLs was restricted by HLA class II (Figure 4b). To identify the HLA-DR alleles responsible for the interactions of these HTL lines with the peptide, L-cells transfected with a single HLA-DR allele gene were used as APCs. As shown in Figure 4c, the HTL lines K1 and K3 were restricted to L-cells expressing HLA-DR4, and K2 was restricted to L-cells expressing HLA-DR53. This result suggests

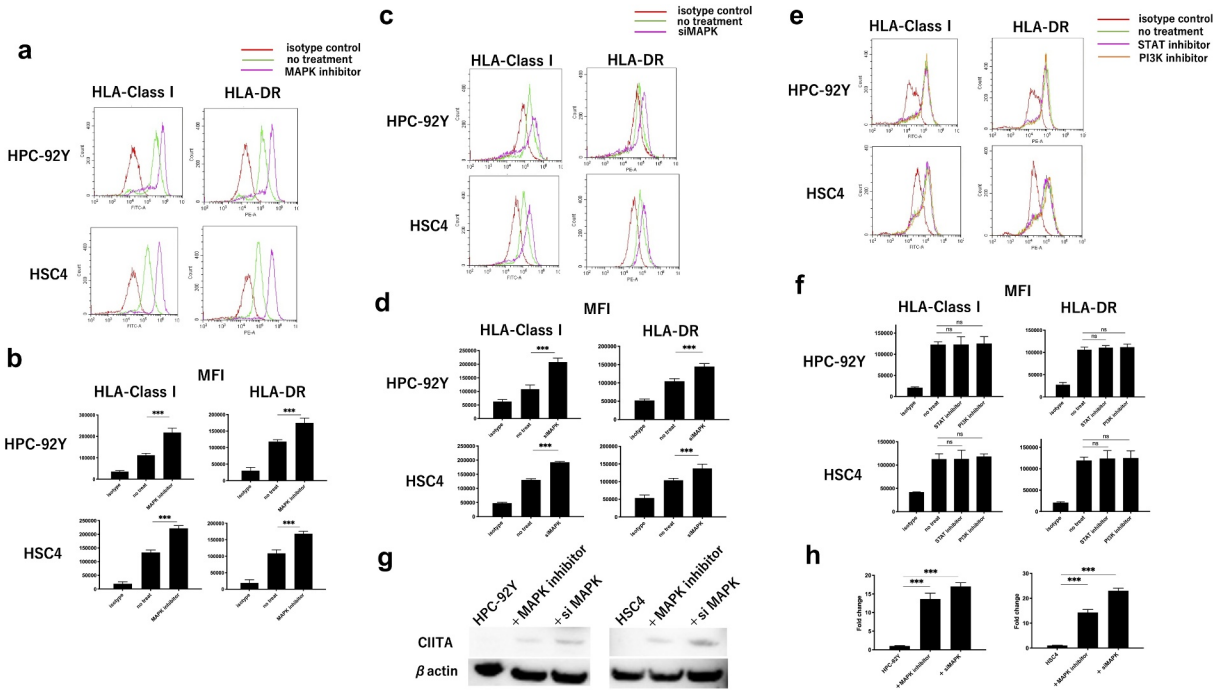


Figure 3. Upregulation of HLAs and CIITA expression on HNSCC cell lines by MAPK inhibition. (a, b) HLA-DR and HLA-class I expression on HNSCC cell lines incubated with 3 μM MAPK inhibitor and 50 U/ml IFN-γ for 48 hr were evaluated by flow cytometry. Red: isotype control, Green: untreated tumor cell lines, Pink: treated with MAPK inhibitor. (a) Representative data of flow cytometry. (b) Averages values of mean fluorescence intensity (MFI). (c, d) HLA-DR and HLA-class I expression on HNSCC cell lines incubated with siMAPK and 50 U/ml IFN-γ for 48 hr were evaluated by flow cytometry. Red: isotype control, Green: untreated tumor cell lines, Pink: treated with MAPK inhibitor. (c) Representative data of flow cytometry. (d) Averages values of mean fluorescence intensity (MFI). (e, f) HLA-DR and HLA-class I expression on HNSCC cell lines incubated with 3 μM STAT inhibitor or PI3K inhibitor and 50 U/ml IFN-γ for 48 hr were evaluated by flow cytometry. Red: isotype control, green: untreated tumor cell lines, pink: treated with STAT inhibitor. Orange: treated with PI3K inhibitor. (e) Representative data of flow cytometry. (f) Averages values of mean fluorescence intensity (MFI). (g, h) HNSCC cell lines were treated with 3 μM MAPK inhibitor or siMAPK, and class II transactivator (CIITA) expression was examined. (g) Representative data of Western blotting. (h) Quantitative analysis of protein expression. Each data was representative in the triplicate experiments. Bars and error bars show the mean and SD, respectively. (*p < .05, **p < .01, ***<0.001, Student's *t* test).

that the $FGFR1_{305\text{-}319}$ peptide was capable of inducing T cells that are restricted to multiple HLA-DR molecules. The amino acid sequence of the identified FGFR1 peptide epitope has homologous regions in the FGFR3 and FGFR4 proteins (Figure 4d). Interestingly, FGFR1-reactive T cell lines (K1) recognized homologous FGFR3- and FGFR4-derived peptides (Figure 4e). The response to FGFR3- or FGFR4-derived peptide was inhibited by anti-HLA-DR Abs, and irrelevant EGFR-derived peptide could not activate HTLs suggesting that FGFR1-reactive T cell lines could specifically recognize FGFR family protein (Figure 4f). Collectively, the $FGFR1_{305\text{-}319}$ epitope peptide could be applied as a cancer vaccine against tumors expressing FGFR3 or FGFR4 in addition to FGFR1.

Direct tumor recognition and cytotoxic activity by $FGFR1_{305\text{-}319}$-reactive $CD4^+$ T cell lines

To assess whether $FGFR1_{305\text{-}319}$-reactive HTLs could directly recognize tumor cells, HTL lines were co-cultured with FGFR1-expressing HNSCC cell lines. As shown in Figure 5a, $FGFR1_{305\text{-}319}$-reactive HTLs responded to HLA-DR-matched tumor cells but not to HLA-DR-unmatched tumor cells. T cell responses were inhibited with anti-HLA-DR but not with anti-HLA Class I Abs suggesting that T cells react to tumor in the context of HLA-DR (Supplemental Figure S8). Moreover, a $FGFR1_{305\text{-}319}$-reactive HTL line (K1) co-cultured with HLA-DR-matched tumor cells produced granzyme B (Figure 5b). In addition to IFN-γ granzyme B, various Th1 cytokines including IL-2 and TNF-α were produced from $FGFR1_{305\text{-}319}$-reactive HTLs in response to tumor (Supplemental Figure S9 and S10), indicating that FGFR1-induced HTL lines are cytotoxic HTLs.[22] As shown in Figure 5c and d, direct tumor cytotoxicity was observed in these HTLs. These results suggest that the $FGFR1_{305\text{-}319}$ peptide could be a potent antitumor vaccine for generating cytotoxic HTLs that directly kill tumors.

$FGFR1_{305\text{-}319}$-reactive T cells in periphery blood from HNSCC patients

In a clinical setting, the existence of FGFR1-reactive precursor T cells in cancer patients is essential for the translation of cancer peptide vaccines targeting FGFR1. Accordingly, we assessed the presence of FGFR1-reactive T cells via short-term stimulation of PBMCs, isolated from six HNSCC patients, with $FGFR1_{305\text{-}319}$ peptide. PBMCs from untreated HNSCC patients were stimulated with peptides for two cycles every 7 days, and the production of IFN-γ was measured. As shown in Figure 6a and B, T cells from HNSCC patients responded to the $FGFR1_{305\text{-}319}$ peptide, indicating that precursor T cells that can react to the FGFR1 peptide vaccine exist in patients with HNSCC.

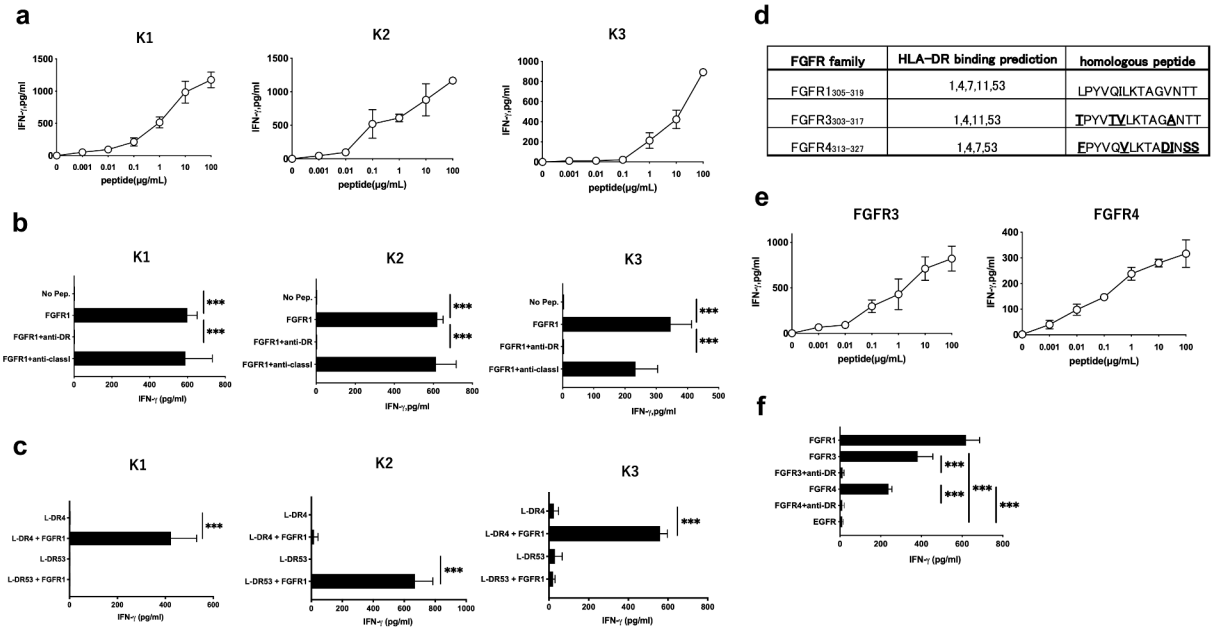


Figure 4. Generation of FGFR1$_{305\text{-}319}$-reactiveCD4$^+$ T cell lines. (a) FGFR1$_{305\text{-}319}$-reactive CD4$^+$ T cell lines (K1, K2, and K3) were assessed for IFN-γ production in response to irradiated autologous PBMCs as APCs with several concentrations of FGFR1$_{305\text{-}319}$ peptide. (b) HLA restriction analysis of the FGFR1$_{305\text{-}319}$-reactive CD4$^+$ T cell lines. Peptide-reactive responses in the FGFR1$_{305\text{-}319}$-reactive CD4$^+$ T cell lines were evaluated by co-cultured with irradiated autologous PBMCs as APCs in the context of anti-HLA-DR mAb or anti-HLA class I mAb. (c) Assessment of restrictive HLA-DR allele in the FGFR1$_{305\text{-}319}$-reactive CD4$^+$ T cell lines. Each T cells was co-culturing with L-cells expressing individual HLA-DR as APCs. IFN-γ production in the supernatants was assessed by ELISA after co-culturing with APCs for 48 hr. (d) Peptide sequences of FGFR1$_{305\text{-}319}$ and its homologous FGFR family-derived peptide. Underlined letters indicate amino acids that are different from FGFR1$_{305\text{-}319}$ peptide. (e) Evaluation of FGFR1$_{305\text{-}319}$-reactive CD4$^+$ T cell response to the homologous FGFR family-derived peptides. FGFR1$_{305\text{-}319}$-reactive CD4$^+$ T cell lines (K1) were evaluated for IFN-γ production in response to irradiated autologous PBMCs as APCs with FGFR3$_{303\text{-}317}$ peptide or FGFR4 $_{313\text{–}327}$ peptide. (f) HLA restriction analysis of the FGFR1$_{305\text{-}319}$-reactive CD4$^+$ T cell lines to FGFR3 or FGFR4 peptide. FGFR3$_{303\text{-}317}$ or FGFR4$_{313\text{-}327}$ peptide-reactive responses in the FGFR1$_{305\text{-}319}$-reactive CD4$^+$ T cell lines were assessed by co-cultured with irradiated autologous PBMCs as APCs in the context of anti-HLA-DR mAb or anti-HLA class I mAb. EGFR$_{875\text{-}889}$ peptide was used as negative control peptide. Each data was representative in the triplicate experiments. Bars and error bars show the mean and SD, respectively. (*p < .05, **p < .01, ***<0.001, Student's *t* test).

Synergistic antitumor effects of FGFR-TKIs with tumor-reactive CD4$^+$ T cells

Finally, we evaluated the antitumor effects of combination therapy with FGFR1-reactive HTLs and FGFR-TKIs. IFN-γ production in FGFR1$_{305\text{-}319}$-reactive HTL lines was augmented by use of FGFR-TKIs (Figure 7a). To determine whether FGFR inhibition functions as an immune adjuvant for FGFR-irrelevant immunotherapy, MDM2-reactive T cells were used as effector cells.[19] As FGFR-TKIs did not alter MDM2 expression in HNSCC cells (Supplemental Figure S11), tumor recognition by MDM2$_{32\text{-}46}$-reactive HTLs (H40) was increased by FGFR-TKIs (Figure 7b) suggesting that FGFR blockade can be applied to any T-cell-based immunotherapy. The production of granzyme B and the killing activity of FGFR1$_{305\text{-}319}$-reactive HTL lines were also enhanced by FGFR-TKIs (Figure 7c-e). Based on these findings, FGFR-TKIs and cancer peptide vaccines (such as the FGFR1$_{305\text{-}319}$ peptide), could be a potent combination therapy against FGFR1-expressing tumors.

Discussion

In this study, we elucidated two aspects of FGFR1 in cancer immunology: as an immune adjuvant and as a target antigen of a peptide vaccine. To the best of our knowledge, there have been no reports elucidating the antitumor T cell response elicited by FGFR1-derived peptides, and only a few studies have investigated the immunomodulatory effects of FGFR1 inhibition. According to the Human Protein Atlas database and previous studies, overexpression of FGFR1 occurs in more than 70% of HNSCC cases.[23,24] The expression of FGFR1 is related to poor prognosis in HNSCC,[25,26] especially in HPV-negative HNSCC, but not in HPV-positive HNSCC.[24,27] Because HPV-negative HNSCC exhibits poor response to standard therapies, developing novel treatments targeting FGFR1 could be a potential approach to treat patients with HNSCC. FGFR1 amplification is associated with poor prognosis in most types of cancer, such as melanoma, lung cancer, pancreatic cancer, and glioblastoma.[28–31] Activation of FGFR1 leads to tumor development through its downstream signaling pathways: the Ras-dependent MAPK, PI3K/AKT, and JAK/STAT pathways.[7] As these networks contribute to aggressive tumor behavior by activating tumor cell proliferation, survival, differentiation, and migration,[32] the development of FGFR1-targeting therapy would be beneficial for patients with aggressive tumors.

Immunomodulatory adjuvants are required for efficient immunotherapy to induce immune cold to hot tumors. Since adjuvants such as Toll-like receptor (TLR) ligands have improved clinical outcomes, chemotherapy and molecular-targeted drugs may also act as immune adjuvants in combined immunotherapy.[13] Few studies have investigated the effects of FGFR-TKIs on tumor immunity. In this study, we showed that FGFR-TKIs upregulated HLA-DR and HLA class I expression

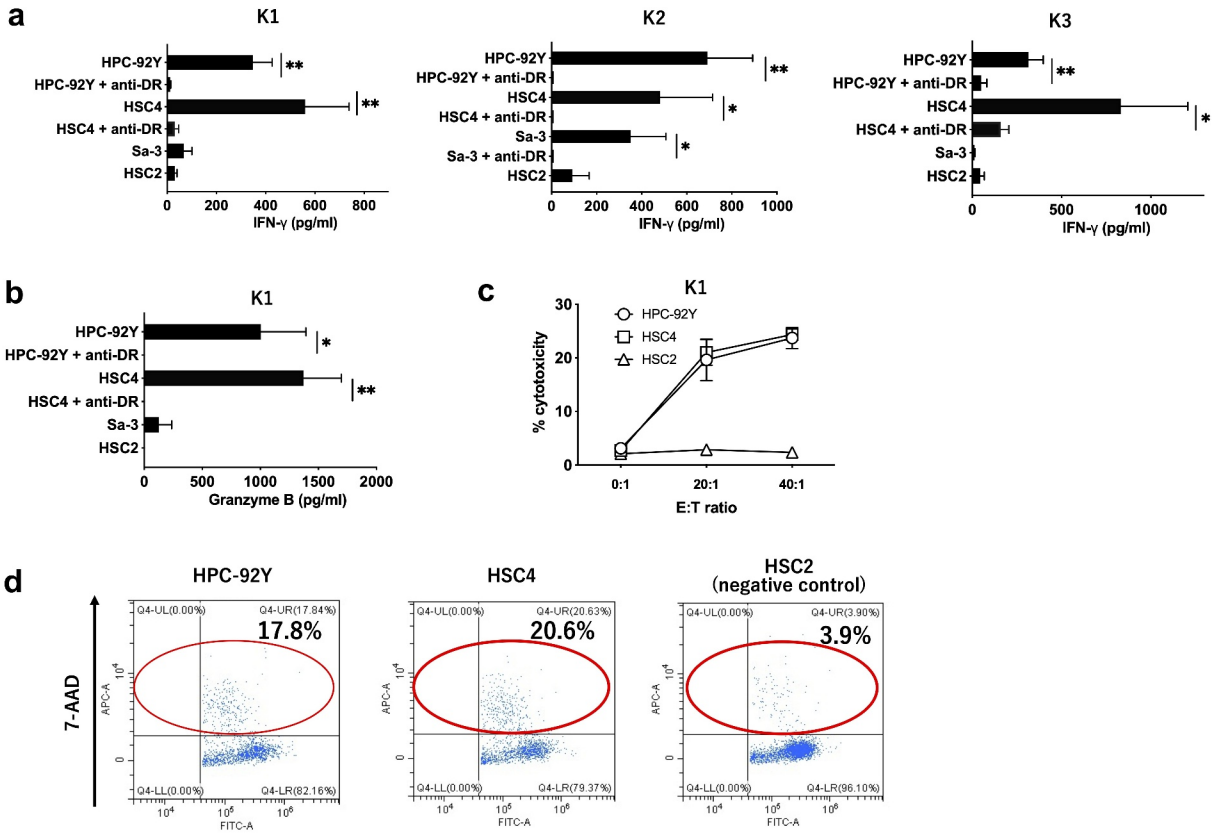
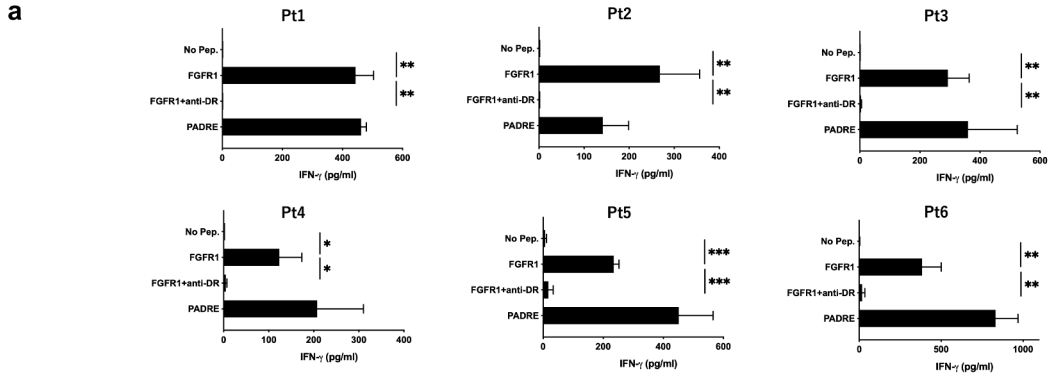


Figure 5. Direct killing of FGFR1 expressing HNSCC cells by FGFR1$_{305\text{-}319}$-reactiveCD4$^+$ T cell lines. (a) FGFR1$_{305\text{-}319}$-reactive CD4$^+$ T cell lines were co-cultured with HLA-DR matched or unmatched HNSCC cell lines expressing FGFR1 for 48 hr. K1 and K3 were restricted to HLA-DR4, and K2 was restricted to HLA-DR53. The cell lines used were HSC2 (HLA-DR13), HSC3 (HLA-DR15), HSC4 (HLA-DR1,4, and 53), Sa-3 (HLA-DR9, 10, and 53), and HPC-92Y (HLA-DR4, 9, and 53). HNSCC cell lines were treated with 500 U/ml IFN-γ for 48 hr before the assay. IFN-γ production in the supernatants was evaluated by ELISA. (b) Granzyme-B production from FGFR1$_{305\text{-}319}$-reactive CD4$^+$ T cell line (K1: HLA-DR4 restricted) was assessed in supernatants co-cultured with HLA-DR matched or unmatched HNSCC cell lines. (c) Killing activity of FGFR1$_{305\text{-}319}$-reactive CD4$^+$ T cell line (K1) was evaluated by co-culturing with CSFE-labeled HNSCC cell lines for 6 hr with various E: T (Effector: Target cells) ratio, and measuring percentages of CFSE$^+$ 7-AAD$^+$ dead cells with flow cytometry. (d) Representative data of flow cytometry in the killing assay (Effector to target ratio was 20:1). Each data was representative in the triplicate experiments. Bars and error bars show the mean and SD, respectively. (*p < .05, **p < .01, ***<0.001, Student's *t* test).

in tumor cell lines, such that enhanced HLA-DR expression augmented tumor recognition and killing by FGFR1-reactive HTLs. This effect was also observed in FGFR1-irrelevant antigen-reactive T cells, indicating that FGFR1 blockade can be applied to any tumor vaccine as an immune adjuvant. As FGFR1 blockade alone could directly suppress tumor proliferation and induce cell death, FGFR1 TKIs may damage tumor cells in several ways: 1) direct killing of tumor cells; 2) increased prevalence of dead tumor cells can be a source of tumor antigens in APCs; 3) upregulation of MHC expression in tumor cells, followed by antitumor T-cell activation. Thus, FGFR inhibition has the potential to be used as an adjuvant immunotherapy.

The expression of MHC class II is controlled by CIITA, the expression of which is induced by IFN-γ.[33,34] As FGFR-TKIs upregulated HLA-DR and CIITA expression in the absence of IFN-γ, IFN-γ would be dispensable for CIITA expression with respect to FGFR inhibition. Pannini et *al* showed that CIITA expression in macrophages is inhibited by TLR2-induced MAPK signaling.[35] We demonstrated that CIITA expression in tumor cells was enhanced by inhibition of MAPK/ERK via small molecular inhibitors or siRNA. As MAPK signaling is a common pathway downstream of various proteins such as FGFR and EGFR, which inhibition upregulates HLA class II,[12,36] MAPK signaling would be a key pathway in tumor-mediated suppressed expression of MHC. Since Dennison et *al*. have demonstrated that MEK1 knockout tumors upregulate MHC class I expression, CD8 T cell infiltration, and T cell activation,[37] our results further verified this issue not only in mouse models but also in human cells. Given the aforementioned finding, in future studies, it would be appropriate to examine whether other reagents that inhibit the MAPK pathway can change tumor MHC expression and T cell infiltration.

Akhand et *al* have shown that FGFR inhibitors augment the antitumor effect of anti-PD-1 Abs in breast cancer models by enhancing the intratumoral infiltration of lymphocytes and reducing myeloid suppressor cells.[38] In addition, Palakurthi et *al* reported that combination therapy with anti-PD-1 Abs and FGFR inhibition led to increased T cell infiltration to support enhanced survival in lung cancer models.[39] Concordant with these findings, our results elucidated that the basis of these synergistic effects of FGFR inhibitors is the upregulation of MHC expression in tumors. As T cells are depleted in microenvironment with FGFR3-expressing tumors,[40] and FGFR inhibition activates T cells in the FGFR2 model,[39] the FGFR family may suppress MHC expression on tumors to evade immune



b

	Sex	Age (years)	Stage	Tumor site	No antigen	FGFR1	FGFR1 + anti-DR	PADRE
					IFN- γ (pg/ml)			
1	M	67	T4N1M0	Oropharynx p16 +	<	442 ± 82	<	460 ± 30
2	M	55	T1N3M0	Nasopharynx	<	269 ± 98	<	121 ± 82
3	M	68	T3N2bM0	Oral cavity	<	390 ± 72	<	360 ± 188
4	F	64	T4aN2cM0	Larynx	<	124 ± 59	15 ± 10	178 ± 89
5	M	76	T1N0M0	Hypopharynx	<	235 ± 21	25 ± 18	451 ± 132
6	M	59	T3N2cM0	Larynx	<	384 ± 131	28 ± 22	823 ± 149

Figure 6. The existence of FGFR1-reactive precursor T cells in HNSCC patients. (a) PBMCs from HNSCC patients were co-cultured with $FGFR1_{305-319}$ peptides for 2 cycles every one week. T cell response to $FGFR_{305-319}$ peptide was assessed by measuring IFN-γ production in the supernatants using ELISA. Anti-HLA-DR mAb was used to assess HLA restriction of the T cells. PADRE peptide was used as a positive control. Each data was representative in the triplicate experiments. Bars and error bars show the mean and SD, respectively. (*p < .05, **p < .01, ***<0.001, Student's *t* test). (b) The clinical characteristics and peptide-reactivity of the 6 HNSCC patients. <: less than the lower limit of detection.

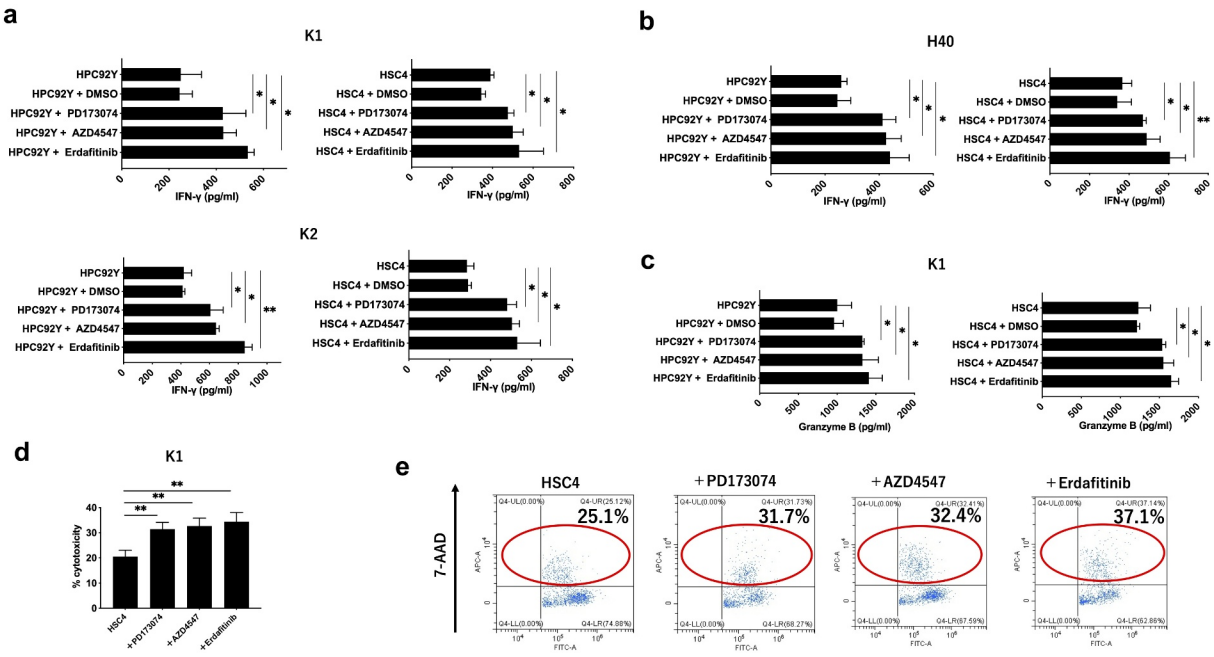


Figure 7. Synergistic antitumor effects of FGFR inhibitor with tumor-reactive T cells. (a, b) (a) Responses of $FGFR1_{305-319}$-reactive $CD4^+$ T cells (K1 and K2) or (b) $MDM2_{32-46}$-reactive $CD4^+$ T cells (H40) to tumor cell lines pretreated by FGFR-TKIs was evaluated by measuring IFN-γ production. HNSCC cell lines were treated with 50 U/ml IFN-γ for 48 hr before the assay. DMSO was used as a negative control. (c) Granzyme B production from $FGFR1_{305-319}$-reactive $CD4^+$ T cells (K1) against tumor cells pretreated by FGFR-TKIs was evaluated. DMSO was used as a negative control. (d) Killing ability of $FGFR1_{305-319}$-reactive $CD4^+$ T cells (K1) to tumor cells pretreated by FGFR-TKIs. T cell lines K1 was co-cultured with CSFE-labeled FGFR-TKIs pretreated tumor cells for 6 hr. The percentages of dead cells were measured using 7-AAD staining by flow cytometry. Effector to target ratio was 20:1. Symbols and error bars indicate the mean and SD, respectively. (e) Representative data of flow cytometry. Each data was representative in the triplicate experiments. Bars and error bars show the mean and SD, respectively. (*p < .05, **p < .01, ***<0.001, student's *t* test).

surveillance. Although the antitumor activity of CTLs is outside the scope of this study, upregulation of HLA class I by FGFR1 inhibition may have a positive effect on CTLs. In addition to T cells, inhibition of FGFR in tumor-associated macrophages increases the M1/M2 macrophage ratio.[41] Thus, FGFR inhibition can be applied for immunomodulation, and further studies

are required to elucidate the effect of combined immunotherapy with FGFR inhibition in clinical settings (FGFR inhibitor with PD-1 blockade: ClinicalTrials.gov Identifier: NCT05004974).

We identified a novel T cell epitope peptide derived from FGFR1, which elicited antitumor HTL responses. Thus far, $CD8^+$ cytotoxic T lymphocytes (CTLs) have been considered to play central roles in T cell-based immunotherapy. However, tumor cells escape from CTLs through multiple mechanisms, such as downregulation of MHC class I expression.[42] Recent evidence has shown that HTLs are essential for successful tumor clearance.[43] Antigen-reactive HTLs are required to activate CTL and NK cells by producing Th1 cytokines, maturation and activation of macrophages through the CD40L/CD40 pathway, establishes long-lived antitumor memory responses and augments immunosurveillance.[44–46] In addition to their helper function, HTLs exhibit a direct cytotoxic function.[22] Antitumor responses by immunotherapy requires both antigen-reactive CTLs and HTLs, even in tumors that do not express MHC class II molecules.[47] Our *in silico* sequencing suggested that the $FGFR1_{305\text{-}319}$ peptide is capable of binding to multiple common HLA-DR alleles (DRB1*0101, DRB1*0401, DRB1*0701, DRB1*1101, and DRB1*1501). In this study, we have shown that the $FGFR1_{305\text{-}319}$ peptide could bind to HLA-DR4 and -DR53. This suggests that the $FGFR1_{305\text{-}319}$ peptide could be applied to a large population of patients. Moreover, the $FGFR1_{305\text{-}319}$ peptide possesses a potential HLA-A0201-binding epitope ($FGFR1_{308\text{-}315}$: VQILKTAG). As long peptides containing HTL and CTL epitopes have shown high efficacy in clinical applications,[48] the potential of the FGFR1 peptide to induce both CTLs and HTLs should be considered in the future. In addition, the FGFR1 peptide epitope has amino acid sequences homologous to the FGFR3 and FGFR4 peptide epitopes that may bind to multiple HLA-DRs. Because FGFR1-reactive HTLs can react with FGFR3- and FGFR4-derived peptides, the FGFR1 peptide vaccine can be applied to FGFR family (FGFR1, FGFR3, and FGFR4)-expressing tumors. The disadvantage of targeting TAAs is the risk of damage to normal tissues. However, we have shown that the precursor of FGFR1-reactive T cells were present in healthy donors and cancer patients without autoimmune diseases, suggesting that the risk of autoreactivity is relatively low. Because the migrating TAA-reactive T cells from thymus have moderate- or low-affinity T cell receptors, normal tissues with low FGFR1 expression[24,49] might be ignored from these T-cells. Beside normal tissues, our results showed that the T cells elicited by FGFR1 peptide could recognize and kill tumors with high FGFR1 expression indicating that FGFR1 peptide-based vaccine can induce antitumor T cells without damaging normal tissues. As FGFR inhibitors have been considerably safe in clinical trials,[50] targeting FGFR1 as an immunogen might be a safe and effective approach.

In summary, we demonstrated that FGFR-TKIs augmented antitumor effects of ICI in HNSCC mouse models by upregulating the expression of MHC class I and MHC class II *in vitro* and *in vivo*. This upregulation was mediated through the inhibition of the MAPK signaling pathway, but not that of the STAT and PI3K signaling pathways. Furthermore, we identified a novel helper epitope from FGFR1 that could elicit antigen-reactive T cell responses. FGFR1-reactive T cells were restricted to common HLA-DRs, and exhibited direct tumor cell recognition and cytotoxic activity against FGFR1-expressing HNSCC cells. The precursor T cells that react to the FGFR1 epitope peptide were detected in HNSCC patients, suggesting this epitope to be a potential candidate for peptide vaccines. Notably, FGFR-TKIs augmented the antitumor effect of FGFR1-reactive T cells against human HNSCC. These results suggest that FGFR-TKIs are potential immune adjuvants for T-cell-based immunotherapy. Combination therapy with TKIs and cancer vaccines or ICI could be a novel and potent immunotherapeutic approach to treat aggressive cancers with FGFR expression.

Acknowledgments

The authors also thank Dr. Hajime Kamada (Hokuto Social Medical Corporation) for his excellent suggestions for the manuscript.

Authors' contributions

Acquisition, analysis, and interpretation of data: MK, TK, RH, HKom, HY, HK, RW, and KO. Statistical analysis of data: KK, MT, and AKa. Material support: TN, TO, AKo, and HKob. Development of methodology: TK, TH and YH. Conception, design and supervision of the study: TK. Writing of the paper: MK and TK. Review of the paper: TK and YH.

Availability of data and material

All data relevant to the study are included in the article or uploaded as supplementary information.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by JSPS KAKENHI Grant Number 20K09724 and 21H03082.

ORCID

Takumi Kumai http://orcid.org/0000-0002-3411-671X
Toshihiro Nagato http://orcid.org/0000-0001-8326-8296

Ethics approval, consent to participate and consent for publication

All experiments were approved by the institutional ethics committee on the Asahikawa Medical University (#16217). The study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The patients have given their written informed consent to participate and publish their case.

Abbreviations

APC	antigen-presenting cell
CIITA	class II transactivator
CTL	$CD8^+$ cytotoxic T lymphocyte
EGFR	epidermal growth factor receptor
E: T	Effector: Target
FGFR	fibroblast growth factor receptor
HNSCC	head and neck squamous cell carcinoma
HTL	$CD4^+$ helper T lymphocyte

(*Continued*)

APC	antigen-presenting cell
CIITA	class II transactivator
CTL	$CD8^+$ cytotoxic T lymphocyte
EGFR	epidermal growth factor receptor
E: T	Effector: Target
FGFR	fibroblast growth factor receptor
HNSCC	head and neck squamous cell carcinoma
HTL	$CD4^+$ helper T lymphocyte
ICI	immune checkpoint inhibitor
mAb	monoclonal antibody
MAPK	mitogen-activated protein kinase
MFI	mean fluorescence intensity
PBMC	peripheral blood mononuclear cell
TAA	tumor-associated antigen
TKI	tyrosine kinase inhibitor

References

1. Chow LQM. Head and neck cancer. reply. N Engl J Med. 2020 May 14;382(20):e57. PubMed PMID: 32402180. doi:10.1056/NEJMc2001370.
2. Conley BA. Treatment of advanced head and neck cancer: what lessons have we learned? J Clin Oncol. 2006 Mar 1;24(7):1023–1025. PubMed PMID: 16505419. doi:10.1200/JCO.2005.05.0682.
3. Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, Jones CU, Sur R, Raben D, Jassem J, et al. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med. 2006 Feb 9;354(6):567–578. PubMed PMID: 16467544. doi:10.1056/NEJMoa053422.
4. Ferris RL, Blumenschein G Jr., Fayette J, Guigay J, Colevas AD, Licitra L, Harrington K, Kasper S, Vokes EE, Even C, et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. N Engl J Med. 2016 Nov 10;375(19):1856–1867. PubMed PMID: 27718784; PubMed Central PMCID: PMCPMC5564292. doi:10.1056/NEJMoa1602252.
5. Dieci MV, Arnedos M, Andre F, Soria JC. Fibroblast growth factor receptor inhibitors as a cancer treatment: from a biologic rationale to medical perspectives. Cancer Discov. 2013 Mar;3(3):264–279. PubMed PMID: 23418312. doi:10.1158/2159-8290.CD-12-0362.
6. Brooks AN, Kilgour E, Smith PD. Molecular pathways: fibroblast growth factor signaling: a new therapeutic opportunity in cancer. Clin Cancer Res. 2012 Apr 1;18(7):1855–1862. PubMed PMID: 22388515. doi:10.1158/1078-0432.CCR-11-0699.
7. Beenken A, Mohammadi M. The FGF family: biology, pathophysiology and therapy. Nat Rev Drug Discov. 2009 Mar;8(3):235–253. PubMed PMID: 19247306; PubMed Central PMCID: PMCPMC3684054. doi:10.1038/nrd2792.
8. Hierro C, Rodon J, Tabernero J. Fibroblast growth factor (FGF) receptor/FGF inhibitors: novel targets and strategies for optimization of response of solid tumors. Semin Oncol. 2015 Dec;42(6):801–819. PubMed PMID: 26615127. doi:10.1053/j.seminoncol.2015.09.027.
9. Pal SK, Rosenberg JE, Hoffman-Censits JH, Berger R, Quinn DI, Galsky MD, Wolf J, Dittrich C, Keam B, Delord J-P, et al. Efficacy of BGJ398, a fibroblast growth factor receptor 1–3 inhibitor, in patients with previously treated advanced urothelial carcinoma with FGFR3 alterations. Cancer Discov. 2018 Jul;8(7):812–821. PubMed PMID: 29848605; PubMed Central PMCID: PMCPMC6716598. doi:10.1158/2159-8290.CD-18-0229.
10. Voss MH, Hierro C, Heist RS, Cleary JM, Meric-Bernstam F, Tabernero J, Janku F, Gandhi L, Iafrate AJ, Borger DR, et al. A phase i, open-label, multicenter, dose-escalation study of the oral selective FGFR inhibitor debio 1347 in patients with advanced solid tumors harboring FGFR gene alterations. Clin Cancer Res. 2019 May 1;25(9):2699–2707. PubMed PMID: 30745300. doi:10.1158/1078-0432.CCR-18-1959.
11. Bahleda R, Meric-Bernstam F, Goyal L, Tran B, He Y, Yamamiya I, Benhadji KA, Matos I, Arkenau H-T. Phase I, first-in-human study of futibatinib, a highly selective, irreversible FGFR1-4 inhibitor in patients with advanced solid tumors. Ann Oncol. 2020 Oct;31(10):1405–1412. PubMed PMID: 32622884. doi:10.1016/j.annonc.2020.06.018.
12. Kumai T, Matsuda Y, Oikawa K, Aoki N, Kimura S, Harabuchi Y, Celis E, Kobayashi H. EGFR inhibitors augment antitumour helper T-cell responses of HER family-specific immunotherapy [research support, N.I.H., extramural research support, non-U.S. gov't]. Br J Cancer. 2013 Oct 15;109(8):2155–2166. PubMed PMID: 24045666; PubMed Central PMCID: PMC3798972. eng. doi:10.1038/bjc.2013.577.
13. Kumai T, Kobayashi H, Harabuchi Y, Celis E. Peptide vaccines in cancer-old concept revisited. Curr Opin Immunol. 2017 Apr;45:1–7. PubMed PMID: 27940327; PubMed Central PMCID: PMCPMC5449210. doi:10.1016/j.coi.2016.11.001.
14. Ott PA, Hu-Lieskovan S, Chmielowski B, Govindan R, Naing A, Bhardwaj N, Margolin K, Awad MM, Hellmann MD, Lin JJ, et al. A phase Ib trial of personalized neoantigen therapy plus Anti-PD-1 in patients with advanced melanoma, non-small cell lung cancer, or bladder cancer. Cell. 2020 Oct 15;183(2):347–362 e24. PubMed PMID: 33064988. doi:10.1016/j.cell.2020.08.053.
15. Kumai T, Fan A, Harabuchi Y, Celis E. Cancer immunotherapy: moving forward with peptide T cell vaccines. Curr Opin Immunol. 2017 Aug;47:57–63. PubMed PMID: 28734176; PubMed Central PMCID: PMCPMC5626598. doi:10.1016/j.coi.2017.07.003.
16. Rammensee H, Bachmann J, Emmerich NP, Bachor OA, Stevanović S. SYFPEITHI: database for MHC ligands and peptide motifs [research support, Non-U.S. gov't review]. Immunogenetics. 1999 Nov;50(3–4):213–219. PubMed PMID: 10602881; eng. doi:10.1007/s002510050595.
17. Vita R, Mahajan S, Overton JA, Dhanda SK, Martini S, Cantrell JR, Wheeler DK, Sette A, Peters B. The immune epitope database (IEDB): 2018 update. nucleic. Acids Res. 2019 Jan 8;47(D1):D339–D343. PubMed PMID: 30357391; PubMed Central PMCID: PMCPMC6324067. doi:10.1093/nar/gky1006.
18. Kobayashi H, Wood M, Song Y, Appella E, Celis E. Defining promiscuous MHC class II helper T-cell epitopes for the HER2/neu tumor antigen. Cancer Res. 2000 Sep 15;60(18):5228–5236. PubMed PMID: 11016652.
19. Kono M, Kumai T, Hayashi R, Yamaki H, Komatsuda H, Wakisaka R, Nagato T, Ohkuri T, Kosaka A, Ohara K, et al. Interruption of MDM2 signaling augments MDM2-targeted T cell-based antitumor immunotherapy through antigen-presenting machinery. Cancer Immunol Immunother. 2021 Apr 18;70(12):3421–3434. doi:10.1007/s00262-021-02940-5. PubMed PMID: 33866408.
20. Kumai T, Ohkuri T, Nagato T, Matsuda Y, Oikawa K, Aoki N, Kimura S, Celis E, Harabuchi Y, Kobayashi H, et al. Targeting HER-3 to elicit antitumor helper T cells against head and neck squamous cell carcinoma. Sci Rep. 2015 Nov 5;5(1):16280. PubMed PMID: 26538233; PubMed Central PMCID: PMCPMC4633732. doi:10.1038/srep16280.
21. Vertuani S, Triulzi C, Roos AK, Charo J, Norell H, Lemonnier F, Pisa P, Seliger B, Kiessling R. HER-2/neu mediated down-regulation of MHC class I antigen processing prevents CTL-mediated tumor recognition upon DNA vaccination in HLA-A2 transgenic mice. Cancer Immunol Immunother. 2009 May;58(5):653–664. PubMed PMID: 18820911. doi:10.1007/s00262-008-0587-1.
22. Quezada SA, Simpson TR, Peggs KS, Merghoub T, Vider J, Fan X, Blasberg R, Yagita H, Muranski P, Antony PA, et al. Tumor-reactive CD4+ T cells develop cytotoxic activity and eradicate large established melanoma after transfer into lymphopenic hosts. J Exp Med. 2010 Mar 15;207(3):637–650. PubMed PMID: 20156971; PubMed Central PMCID: PMCPMC2839156. doi:10.1084/jem.20091918.

23. Digre A, Lindskog C. The human protein Atlas-spatial localization of the human proteome in health and disease. Protein Sci. 2021 Jan;30(1):218–233. PubMed PMID: 33146890; PubMed Central PMCID: PMCPMC7737765. doi:10.1002/pro.3987.
24. Koole K, Brunen D, van Kempen PM, Noorlag R, de Bree R, Lieftink C, van Es RJJ, Bernards R, Willems SM. FGFR1 is a potential prognostic biomarker and therapeutic target in head and neck. Squamous Cell Carcinoma Clin Cancer Res. 2016 Aug 1;22(15):3884–3893. PubMed PMID: 26936917. doi:10.1158/1078-0432.CCR-15-1874.
25. Mariz B, Soares CD, de Carvalho MGF, Jorge-Júnior J. FGF-2 and FGFR-1 might be independent prognostic factors in oral tongue squamous cell carcinoma. Histopathology. 2019 Jan;74 (2):311–320. PubMed PMID: 30129658. doi:10.1111/his.13739.
26. Dubot C, Bernard V, Sablin MP, Vacher S, Chemlali W, Schnitzler A, Pierron G, Ait Rais K, Bessoltane N, Jeannot E, et al. Comprehensive genomic profiling of head and neck squamous cell carcinoma reveals FGFR1 amplifications and tumour genomic alterations burden as prognostic biomarkers of survival. Eur J Cancer. 2018 Mar;91. 47–55. PubMed PMID: 29331751. doi:10.1016/j.ejca.2017.12.016.
27. Seiwert TY, Zuo Z, Keck MK, Khattri A, Pedamallu CS, Stricker T, Brown C, Pugh TJ, Stojanov P, Cho J, et al. Integrative and comparative genomic analysis of HPV-positive and HPV-negative head and neck squamous cell carcinomas. Clin Cancer Res. 2015 Feb 1;21 (3):632–641. PubMed PMID: 25056374; PubMed Central PMCID: PMCPMC4305034. doi:10.1158/1078-0432.CCR-13-3310.
28. Lin WM, Baker AC, Beroukhim R, Winckler W, Feng W, Marmion JM, Laine E, Greulich H, Tseng H, Gates C, et al. Modeling genomic diversity and tumor dependency in malignant melanoma. Cancer Res. 2008 Feb 1;68(3):664–673. PubMed PMID: 18245465. doi:10.1158/0008-5472.CAN-07-2615.
29. Weiss J, Sos ML, Seidel D, Peifer M, Zander T, Heuckmann JM, Ullrich RT, Menon R, Maier S, Soltermann A, et al. Frequent and focal FGFR1 amplification associates with therapeutically tractable FGFR1 dependency in squamous cell lung cancer. Sci Transl Med. 2010 Dec 15;2(62):62ra93. PubMed PMID: 21160078; PubMed Central PMCID: PMCPMC3990281. doi:10.1126/scitranslmed.3001451.
30. Lehnen NC, Von Massenhausen A, Kalthoff H, Zhou H, Glowka T, Schütte U, Höller T, Riesner K, Boehm D, Merkelbach-Bruse S, et al. Fibroblast growth factor receptor 1 gene amplification in pancreatic ductal adenocarcinoma. Histopathology. 2013 Aug;63 (2):157–166. PubMed PMID: 23808822. doi:10.1111/his.12115.
31. Rand V, Huang J, Stockwell T, Ferriera S, Buzko O, Levy S, Busam D, Li K, Edwards JB, Eberhart C, et al. Sequence survey of receptor tyrosine kinases reveals mutations in glioblastomas. Proc Natl Acad Sci U S A. 2005 Oct 4;102(40):14344–14349. PubMed PMID: 16186508; PubMed Central PMCID: PMCPMC1242336. doi:10.1073/pnas.0507200102.
32. Haugsten EM, Wiedlocha A, Olsnes S, Wesche J. Roles of fibroblast growth factor receptors in carcinogenesis. Mol Cancer Res. 2010 Nov;8(11):1439–1452. PubMed PMID: 21047773. doi:10.1158/1541-7786.MCR-10-0168.
33. Masternak K, Muhlethaler-Mottet A, Villard J, Zufferey M, Steimle V, Reith W. CIITA is a transcriptional coactivator that is recruited to MHC class II promoters by multiple synergistic interactions with an enhanceosome complex. Genes Dev. 2000 May 1;14 (9):1156–1166. PubMed PMID: 10809673; PubMed Central PMCID: PMCPMC316580. doi:10.1101/gad.14.9.1156.
34. Steimle V, Siegrist CA, Mottet A, Lisowska-Grospierre B, Mach B. Regulation of MHC class II expression by interferon-gamma mediated by the transactivator gene CIITA. Science. 1994 Jul 1;265(5168):106–109. PubMed PMID: 8016643. doi:10.1126/science.8016643.
35. Pennini ME, Pai RK, Schultz DC, Boom WH, Harding CV. Mycobacterium tuberculosis 19-kDa lipoprotein inhibits IFN-γ-Induced chromatin remodeling of MHC2TA by TLR2 and MAPK signaling. J Immunol. 2006 Apr 1;176(7):4323–4330. PubMed PMID: 16547269. doi:10.4049/jimmunol.176.7.4323.
36. Pollack BP, Sapkota B, Cartee TV. Epidermal growth factor receptor inhibition augments the expression of MHC class I and II genes. Clin Cancer Res. 2011 Jul 1;17(13):4400–4413. PubMed PMID: 21586626. doi:10.1158/1078-0432.CCR-10-3283.
37. Dennison L, Ruggieri A, Mohan A, Leatherman J, Cruz K, Woolman S, Azad N, Lesinski GB, Jaffee EM, Yarchoan M, et al. Context-Dependent immunomodulatory effects of MEK inhibition are enhanced with T-cell agonist therapy. Cancer Immunol Res. 2021 Aug 13;9(10):1187–1201. doi:10.1158/2326-6066.CIR-21-0147. PubMed PMID: 34389557.
38. Akhand SS, Liu Z, Purdy SC, Abdullah A, Lin H, Cresswell GM, Ratliff TL, Wendt M. Pharmacologic inhibition of FGFR modulates the metastatic immune microenvironment and promotes response to immune checkpoint blockade. Cancer Immunol Res. 2020 Dec;8 (12):1542–1553. PubMed PMID: 33093218; PubMed Central PMCID: PMCPMC7710538. doi:10.1158/2326-6066.CIR-20-0235.
39. Palakurthi S, Kuraguchi M, Zacharek SJ, Zudaire E, Huang W, Bonal DM, Liu J, Dhaneshwar A, DePeaux K, Gowaski MR, et al. The combined effect of FGFR inhibition and PD-1 blockade promotes tumor-intrinsic induction of antitumor immunity. Cancer Immunol Res. 2019 Sep;7(9):1457–1471. PubMed PMID: 31331945. doi:10.1158/2326-6066.CIR-18-0595.
40. Robinson BD, Vlachostergios PJ, Bhinder B, Liu W, Li K, Moss TJ, Bareja R, Park K, Tavassoli P, Cyrta J, et al. Upper tract urothelial carcinoma has a luminal-papillary T-cell depleted contexture and activated FGFR3 signaling. Nat Commun. 2019 Jul 5;10(1):2977. PubMed PMID: 31278255; PubMed Central PMCID: PMCPMC6611775. doi:10.1038/s41467-019-10873-y.
41. Im JH, Buzzelli JN, Jones K, Franchini F, Gordon-Weeks A, Markelc B, Chen J, Kim J, Cao Y, Muschel RJ, et al. FGF2 alters macrophage polarization, tumour immunity and growth and can be targeted during radiotherapy. Nat Commun. 2020 Aug 13;11 (1):4064. PubMed PMID: 32792542; PubMed Central PMCID: PMCPMC7426415. doi:10.1038/s41467-020-17914-x.
42. O'Donnell JS, Teng MWL, Smyth MJ. Cancer immunoediting and resistance to T cell-based immunotherapy. Nat Rev Clin Oncol. 2019 Mar;16(3):151–167. PubMed PMID: 30523282. doi:10.1038/s41571-018-0142-8.
43. Melssen M, Slingluff CL Jr. Vaccines targeting helper T cells for cancer immunotherapy. Curr Opin Immunol. 2017 Aug;47:85–92. PubMed PMID: 28755541; PubMed Central PMCID: PMCPMC5757837. doi:10.1016/j.coi.2017.07.004.
44. Lhuillier C, Rudqvist NP, Yamazaki T, Zhang T, Charpentier M, Galluzzi L, Dephoure N, Clement CC, Santambrogio L, Zhou XK, et al. Radiotherapy-exposed CD8+ and CD4+ neoantigens enhance tumor control. J Clin Invest. 2021 Mar 1;131(5): PubMed PMID: 33476307; PubMed Central PMCID: PMCPMC7919731. doi:10.1172/JCI138740.
45. Marty Pyke R, Thompson WK, Salem RM, Font-Burgada J, Zanetti M, Carter H. Evolutionary pressure against MHC class II binding cancer mutations. Cell. 2018 Oct 4;175(2):416–428 e13. PubMed PMID: 30245014; PubMed Central PMCID: PMCPMC6482006. doi:10.1016/j.cell.2018.08.048.
46. Lorvik KB, Hammarstrom C, Fauskanger M, Haabeth OAW, Zangani M, Haraldsen G, Bogen B, Corthay A. Adoptive transfer of tumor-specific Th2 cells eradicates tumors by triggering an in situ inflammatory immune response. Cancer Res. 2016 Dec 1;76(23):6864–6876. PubMed PMID: 27634753. doi:10.1158/0008-5472.CAN-16-1219.
47. Alspach E, Lussier DM, Miceli AP, Kizhvatov I, DuPage M, Luoma AM, Meng W, Lichti CF, Esaulova E, Vomund AN, et al. MHC-II neoantigens shape tumour immunity and response to immunotherapy. Nature. 2019 Oct;574(7780):696–701. PubMed PMID: 31645760; PubMed Central PMCID: PMCPMC6858572. doi:10.1038/s41586-019-1671-8.
48. Rabu C, Rangan L, Florenceau L, Fortun A, Charpentier M, Dupré E, Paolini L, Beauvillain C, Dupel E, Latouche J-B, et al. Cancer vaccines: designing artificial synthetic long peptides to improve presentation of class I and class II T cell epitopes by

dendritic cells. Oncoimmunology. 2019;8(4):e1560919. PubMed PMID: 30906653; PubMed Central PMCID: PMCPMC6422379. doi:10.1080/2162402X.2018.1560919.

49. Peng R, Chen Y, Wei L, Li G, Feng D, Liu S, Jiang R, Zheng S, Chen Y. Resistance to FGFR1-targeted therapy leads to autophagy via TAK1/AMPK activation in. Gastric Cancer. 2020 Nov;23(6):988–1002. PubMed PMID: 32617693. doi:10.1007/s10120-020-01088-y.

50. Meric-Bernstam F, Bahleda R, Hierro C, Sanson M, Bridgewater J, Arkenau H-T, Tran B, Kelley RK, Park JO, Javle M, et al. Futibatinib, an irreversible FGFR1-4 inhibitor, in patients with advanced solid tumors harboring FGF/FGFR aberrations: a phase I dose-expansion study. Cancer Discov. 2021 Sep 22; PubMed PMID: 34551969. doi:10.1158/2159-8290.CD-21-0697.