Inhibition of Mitochondrial Fission Reverses the Immunoescape of Solid Tumors via IRE1α-XBP-1s-TPP2 axis

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Abstract

Hypoexpression of human major histocompatibility complex (MHC) class I is widely known to be an important strategy of immune evasion in most cases of malignancies that lead to poor prognosis. We demonstrated that mitochondrial dynamics can be exploited as an efficient target in regulating MHC-I expression and cancer immunogenicity. Clinically, MHC-I expression and fragmentation of mitochondria are both closely associated to patient survival but are negatively correlated to one another. Mechanistically, it was observed that endoplasmic reticulum (ER) stress, an integrated signal transduction pathway activated in most rapidly proliferating tumor cells, played a crucial role in connecting mitochondrial fragmentation and cancer cell immunogenicity particularly via the IRE1α-XBP-1 s axis. XBP-1 s, which is activated by imbalanced mitochondrial fission and prolonged oxidative stress, served as a potent transcription factor, promoted the expression of aminopeptidase TPP2 and destructed the applicable antigenic peptide to impede MHC-I complex maturation and the activation of adaptive immune system upon cancer antigen. Our findings highlight the importance of mitochondrial dynamics in determining solid tumor immunogenicity and suggest that mediating mitochondrial fragmentation might provide a novel approach in anti-tumor immunotherapy.

Introduction

Downregulation of MHC-I is often associated with malignant transformation of cells. This abnormality results in a defective synthesis and expression of MHC-Tumor antigen-derived peptide complexes which provide malignant cells with a mechanism to evade the host’s immune system[1]. These peptide complexes are important in the interactions between malignant cells and cognate cytotoxic T cells. Cytotoxic T lymphocytes (CTLs) alongside other lymphocyte subsets are crucial for cancer eradication. CTLs recognize antigenic peptides of 8–10 amino acid (aa) that are derived from tumor-associated antigens (TAAs) and then presented by MHC-I[2]. Loss or downregulation of MHC-I, a condition in which CTLs cannot recognize cancer cells, however, is a deficiency which has not gained sufficient attention on how to figure out its reversal in clinical scenario so far. Immunotherapy is the fourth option in line for cancer therapy, trailing behind surgery, chemotherapy, and radiotherapy. T cell activation to eliminate tumor cells is the most promising anti-cancer strategy since the development of chemotherapy, as demonstrated by the shrinkage of melanoma and non-small cell lung cancer (NSCLC) in response to checkpoint blockers[3]. Unfortunately, existing immunotherapies are either only effective in limited types of tumors, or ultimately develop drug resistance after initial response, both of which prevent them from achieving clinical success. Although little is known about the mechanisms mediating primary and acquired resistance to immunotherapy, downregulation of MHC-I has been reported in resistance to immune checkpoint inhibitors (ICIs) for the cases of lung cancer and melanoma[4]. The above clinical findings have stimulated interests in identifying the molecular mechanisms underlying MHC-I downregulation in malignant cells with the expectation that this information may set the prerequisite for development of strategies to restore MHC-I expression on malignant cells.

Mitochondrion is one of the vital organelles in eukaryotic cells. As a dynamic organelle, it constantly undergoes fission and fusion, and it has the unique ability to regulate its morphology in response to various cellular stimuli[5]. In fact, mitochondrial hyperfragmentation has been observed in several types of cancer cells. It was reported that the inhibition of mitochondrial fission leads to the decrease in cell proliferation and migration among models of lung, colon, gastric, breast and thyroid cancer[6]. This further confirmed the hypothesis that a fragmented mitochondrial phenotype is essential to many cancers, though limited reports had attempted to connect mitochondrial fragmentation with immune escape of cancer cells.

Endoplasmic reticulum (ER) is another central organelle that is closely associated with mitochondria. It functions primarily to process newly synthesized secretory and transmembrane proteins. Rapidly proliferating cancer cells require increased ER activity to facilitate the folding, assembly and transport of membrane and secretory proteins, and are thereby subjected to ER stress[7]. Adaptation to protein folding stress is mediated by the activation of an integrated signal transduction pathway dubbed unfolded protein response (UPR)[8], which results in the activation of three ER transmembrane kinases, namely, protein kinase-like endoplasmic reticulum kinase (PERK), inositol requiring 1α (IRE1α) RNase and activating transcription factor 6 (ATF6), which are otherwise maintained in an inactive state by the association of the chaperone BiP/GRP78 with their luminal domains[9]. ER’s exclusive ability in processing transmembrane protein makes it a major concern in immunology research.

Our present work revealed that mitochondrial fission plays a key role in regulating cancer cell MHC-I membrane expression and immunogenicity by targeting UPR, particularly the IRE1α axis in vitro and in vivo. The current study proposed a novel model whereby mitochondrial fission participates in cancer immune escape, providing promising targets for future clinical therapeutics.

Results
1. Low pSer616 DRP-1 correlates with high MHC-I and indicates better prognosis in cancer patients

The fission process in mammals is mediated by DRP-1 (dynamin-related protein), FIS1 (fission 1 homologue protein), and MFF (mitochondrial fission factor), among which the central player is the highly conserved DRP-1, which belongs to a family of large GTPases that self-assemble to regulate mitochondrial membrane structure[10]. DRP-1 activity is modulated by phosphorylation of regulatory kinases at serine 616. Upon activation, DRP-1 moves from the cytosol to the mitochondrial where it assembles in multimers that constrict and divide the mitochondria[11]. Conversely, phosphorylation of DRP-1 at serine 637 inhibits fission[12]. Tumor samples obtained from 127 patients with head and neck squamous cell carcinoma (HNSCC), 62 patients with NSCLC and 59 patients with malignant melanoma at Sun Yat-sen Memorial Hospital, Sun Yat-sen University (Guangzhou, China) between Jan. 2006 and Dec. 2010 were used for MHC-I and phospho-Ser616 DRP-1 staining, Spearman correlation and Kaplan-Meier survival analysis. Both immunofluorescence (IF) and immunohistochemistry (IHC) staining revealed that the expression of MHC-I was much higher in tumor tissues from patients with longer survival (>5 years, post-surgery). The expression of MHC-I and pSer616 DRP-1 were significantly higher in patients who didn't survive 5 years following surgery (Fig. 1A-C). Correlation among clinicopathological status and the expression of MHC-I or pSer616 DRP-1, univariate and multivariate analysis of factors associated with overall survival of these cancer patients are shown in Supplementary Table 1–2. It was indicated that the expression level of MHC-I and pSer616 DRP-1 have close association with node metastasis but no significant correlation with sex, age and clinical stage (Supplementary Table 1). The levels were also indicators of prognosis in HNSCC, NSCLC and malignant melanoma patients (Supplementary Table 2). Additionally, a Spearman order correlation analysis showed that MHC-I expression was negatively correlated to pSer616 DRP-1 level (Fig. 1D); high expression of MHC-I and low levels of pSer616 DRP-1 are indicators of better overall survival (OS) in these cancer patients respectively (Fig. 1E). Other than collectively demonstrate the clinical significance of MHC-I and pSer616 DRP-1 which were reported previously, the above data also suggest the correlation between mitochondrial fission and MHC-I expression, making mitochondrial fission a plausible leverage in rescuing tumor immunogenicity in solid tumors.

2. Mdivi-1 mediates MHC-I expression in syngeneic tumor models

To investigate the role of DRP-1 in MHC-I antigen expression and its functional properties, we used Mitochondrial division inhibitor 1, Mdivi-1, a selective cell-permeable inhibitor of DRP-1. It inhibits the self-assembly of DRP-1 by blocking DRP-1’s GTPase activity[13]. By using B16F10 melanoma cells expressing the model tumor antigen, chicken-derived ovalbumin (OVA) and C57BL/6 mice, we found that injection of Mdivi-1 via tail vein three days after tumor subcutaneous implantation significantly slowed down the proliferation (or promoted the apoptosis) of tumor compared to vehicle control (DMSO), as indicated by tumor volume measurement (Fig. 2A) and harvested tumor weight (Fig. 2B-C). Flow cytometric analysis of cancer cells isolated from the harvested tumors and stained with mAbs showed that H-2Kb antigen and immunodominant (ID) OVA epitope SIINFEKL[14] expression was upregulated (Fig. 2D) on cancer cells isolated from tumors with reduced size. These phenotypic changes were associated with increased IFN-γ-producing T cells (Fig. 2E) and CTLs infiltration in tumors (Fig. 2F). The above described data were paralleled with those obtained from mice inoculated with tumor cells via the tail vein. Monitoring the latter model with an IVIS Lumina imaging system showed that Mdivi-1 treatment efficiently prevented metastasis spread (Fig. 2G); furthermore, the survival of tumor-bearing mice was prolonged (Fig. 2H). In recent years, Mdivi-1 has emerged as a promising therapeutic agent for stroke, myocardial infarction, neurodegenerative diseases and cancers[15]. For the first time, our research revealed that Mdivi-1’s activity against established tumor is at least partially due to the enhanced membrane expression of MHC-I on cancer cells, which subsequently contributed to recognition by the immune system and CTLs activation.

3. Mdivi-1 improves the adoptive T cell therapy (ACT) in PDX tumor models by upregulating MHC-I

To further investigate the clinical relevance of our findings, we explored whether Mdivi-1 could arouse the immune dormancy and improve ACT effects in cancer patient-derived xenograft (PDX) model implanted in immunocompromised NOD/SCID mice (Fig. 3A). Successful PDX engraftments were established in 11 out of 63 (17.4%) primary HNSCC samples and 6 out of 32 (18.9%) primary NSCLC samples. Similarly, Mdivi-1 treatment effectively inhibited tumor growth, as evaluated through tumor-volume measurements (Fig. 3B) and the harvested PDX tumor weights (Fig. 3C). Double immunostaining in the PDX tissues also showed consistent results as the above, indicating that adoptive transfer with CTLs and Mdivi-1 treatment resulted in massive apoptosis of cancer cells (EpCAM+ TUNEL+) (Fig. 3D and Supplementary Fig. 1A). To further investigate the causes of differences in tumor growth and apoptosis rate, we dissociated the PDX and found that Mdivi-1 treatment significantly upregulated the membrane expression of MHC-I with or without CTL transfer (Fig. 3E) as in syngeneic models, which also corresponded to and could at least partially explain the results of double immunofluorescence staining in PDX tissues depicted above. ACT was significantly upregulated after Mdivi-1 treatment as compared to vehicle control (DMSO) group.
This was proven by more infiltration of CD8+ T cells within cancer nests (Fig. 3F) and more infiltration of IFN-γ-producing T cells[16] (Fig. 3G). Moreover, we isolated the PDX-infiltrating CTLs and observed that perforin and granzyme B, markers associated with cytotoxic activity[17], were upregulated after Mdivi-1 treatment (Fig. 3H-I and Supplementary Fig. 1B). In a word, these findings suggest that the inhibition of mitochondrial fission in PDX that are transferred with tumor-specific CTLs is able to at least partially overcome tumor immune evasion by upregulating MHC-I expression of cancer cells, thus improving the therapeutic effects of ACT on tumors.

4. Mdivi-1 improves the cytotoxic function of CTLs against autologous cancer cells in vitro

The above-described shrinkage of tumor and upregulation of immune response prompted us to determine Mdivi-1’s effect on the cytotoxic function of CTLs in vitro (Fig. 4A). CTLs were primed by tumor-lysate-pulsed DCs (dendritic cells) for 5 days[18] and co-cultured with autologous cancer cells at different E/T (effector/target) ratios. After 12 hours, death of cancer cell (indicated as EpCAM+) was examined by PI and Annexin V uptake using flow cytometry (Supplementary Fig. 1C). The production of Th1 cytokines[19] and residual E/T ratio were quantified using Enzyme-Linked ImmunoSorbent Assay (ELISA) after 24 hours (Fig. 4B) and flow cytometry after 3 days (Fig. 4C) based on CD8 and EpCAM expression, which indicated that the generation of DCs (Supplementary Fig. 1D) and CTLs was feasible and the cytotoxic function of tumor-specific CTLs was enhanced with increasing E/T ratio. Next, in order to determine the appropriate concentration and time of administration for Mdivi-1 in vitro, we performed sequential treatment[20] by using an MTS Assay Kit (Abcam, ab197010) and flow cytometry on HNSCC and NSCLC primary cancer cells and tumor-specific CTLs to evaluate cell viability. It was decided that the treatment regimen of 25–50 µM for 72 hours can efficiently upregulate MHC-I membrane expression (Fig. 4E) with comparatively small anti-proliferative effect (Fig. 4D). By pretreating Mdivi-1 according to the regimen described above, the cytotoxic function of HNSCC and NSCLC primary cancer cells that were co-cultured with tumor-specific CTLs was dramatically enhanced. This was quantified by PI and Annexin V uptake using flow cytometry (Fig. 4F), Th1 cytokines production by specific ELISAs (Fig. 4G) and residual E/T ratio by flow cytometry (Fig. 4H) compared with control group, respectively. In addition, preincubating cancer cells with neutralizing anti-MHC-I antibody[21] abrogated most of the cytotoxicity on target cancer cells. Similar results can be replicated by using melanoma cell line FO-1, a cultured cell line lost MHC-I expression completely due to a defect in B2M gene expression[22], further confirming the restriction and indispensability of MHC-I in cytotoxic function. Together, the data above suggested that inhibition of mitochondrial fission greatly enhances antitumor immunity with comparatively smaller number of CTLs in vitro.

5. Inhibition of mitochondrial fission restores the downregulation of cancer cells membrane expression of MHC-I

Since the restoration of cancer cells’ MHC-I membrane expression and immunogenicity by inhibiting mitochondrial fission had been tested through in vitro and in vivo experiments, we need to further explore the mechanism that contributes to this phenotype. Given that chemical inhibitor may have off-target effects, the results obtained by Mdivi-1 were further confirmed using a DRP-1 siRNA (small interfering RNA, GenePharma, Suzhou, China)[23]. Inhibiting mitochondrial fission by Mdivi-1 or DRP-1 siRNA upregulated MHC-I membrane expression on tongue squamous cell carcinoma (TSCC) cell line SCC-9, CAL-27 and primary HNSCC cancer cells as described previously (Supplementary Fig. 2A and Supplementary Fig. 3A) The results of TSCC cell lines were further verified by immunofluorescence staining (Supplementary Fig. 2B). We then extended the analysis to include additional solid tumors including NSCLC, osteosarcoma, melanoma and mouse melanoma (Supplementary Fig. 2A and Supplementary Fig. 3D). From there, similar phenotype was observed, suggesting that the method of mitochondrial dynamics regulation is universally applicable. However, even though the membrane expression was significantly upregulated, protein level of MHC-I molecules (Supplementary Fig. 2C and Supplementary Fig. 3B) and mRNA level of HLA-A/B/C and B2M (Supplementary Fig. 2D and Supplementary Fig. 3C) stayed almost the same with or without mitochondrial fission interference. Moreover, overexpression of DRP-1 (Generay Biotech, Shanghai, China) exhibited opposite results as that of membrane MHC-I (Supplementary Fig. 3E), whereas similar results were shown for transcription and translation (Supplementary Fig. 3F-G). This paradox implies that the regulation process probably happens after translation.

6. Inhibition of mitochondrial fission alleviates oxidative stress and UPR in cancer cells

Mitochondria are the original source of oxygen free radicals, in particular the generation of reactive oxygen species (ROS). Excessive mitochondrial fission may lead to change of structural organization and arrangement of electron transport chain (ETC) components within the mitochondrial membrane. This organizational arrangement of the ETC may result in perturbation of ETC activity, causing ROS overproduction[24]. ROS are small molecules that are highly active due to the presence of unpaired electrons. They are important mediators of inflammation, and recent findings have linked ER stress to the generation and accumulation of intracellular ROS, a state commonly referred to as oxidative stress. Increased protein-folding in the ER promotes ROS generation which impairs mitochondrial membrane through calcium accumulation in the mitochondria, leading to excessive ROS generation[25]. It is also reported that ER stress may further
induce mitochondrial fission[26]. Through this forward cycle, ROS exacerbates ER stress and further impairs mitochondrial function[10]. It has been widely accepted that inhibition of mitochondrial fission effectively reduces mitochondrial ROS production and thus terminates the abovementioned cycle, followed by attenuated ER stress[27].

It was observed that SCC-9 and CAL-27’s fragmented mitochondria have been distinctly transformed into filamentous phenotype by using Mdivi-1 and siDRP-1 (Fig. 5A). 10-N-nonyl acridine orange, a fluorescent dye that binds to non-oxidized cardiolipin but not to oxidized cardiolipin (Fig. 5B)[28], was evaluated as well as JC-1 (Supplementary Fig. 4A)[29], another key event reflecting oxidative stress, were significantly upregulated with the transformation of mitochondrial morphology. Flow cytometry analysis of intracellular ROS further validates the theory of terminating the ‘stress cycle’ through mitochondrial fission inhibition (Fig. 5C and Supplementary Fig. 4B) as described above, which could further inhibit ER Stress, as indicated by several important markers in immunoblotting (Fig. 5D). In addition, overexpression of DRP-1 efficiently promoted intracellular ROS as expected (Supplementary Fig. 4C). With that, we summarize that inhibiting mitochondrial fission is an effective method to attenuate oxidative stress and ER Stress within cancer cells.

7. IRE1α-XBP-1 s is the most significant regulator axis of MHC-I among UPR

Recently, accumulating evidence revealed the connection between ER stress and MHC-I: The overexpression of an ER stress-inducing misfolded protein or the constitutive expression of nATF6 or XBP-1 s were found to be associated with decreased levels of MHC-I in 293T cells[30]. Similarly, ER stress induced by palmitate or glucose deprivation reduces class I antigen presentation in mouse thymoma cells. Moreover, inhibition of ER stress response in Hereditary Haemochromatosis (HH) cells leads to the restoration of MHC-I[31]. We used dominant negative (DN) plasmids (Addgene 20745, 36954) and siRNA (GenePharma) to interfere with the three signaling pathways and found that IRE1α, the most conserved arm of the UPR[3], is the most significant regulator of MHC-I (Supplementary Fig. 5A). Within minutes of unfolded proteins accumulation, BIP dissociates from PERK, IRE1α and ATF6 and preferentially binds to the unfolded proteins, resulting in the activation of PERK and IRE1α via luminal domain homodimerization and autophosphorylation of ATF6 via proteolytic cleavage[9]. Active IRE1α then excises a 26-base intron in XBP-1 (X-box binding protein 1) mRNA. Re-ligation of spliced XBP-1 shifts the open reading frame, and its translation produces the homeostatic transcription factor XBP-1 s. Spliced XBP-1 transcription factors then translocate into the nucleus where it binds to UPR elements (UPRE) and activates many of the UPR target genes. In this situation, the unspliced XBP-1 which was indicated as XBP-1u, degraded rapidly[32]. Knockdown of XBP-1 s by siRNA (GenePharma) restored MHC-I to the level similar to that of transfected DN IRE1α (Supplementary Fig. 5D). Co-transfection of XBP-1 s and DN IRE1α rescue MHC-I level to normal status, which further confirm this regulatory mechanism (Fig. 5E). Despite observing significant change of membrane MHC-I and attenuation of ER Stress as indicated by GRP78, CHOP[33] and XBP-1 s (Fig. 5D, Supplementary Fig. 5B and 5E), the protein level of MHC-I and mRNA level of HLA-A/B/C and B2M remained unchanged (Supplementary Fig. 5B-C and E-F). In addition, overexpression of XBP-1 s obtained the opposite results of MHC-I membrane expression and ER Stress (Supplementary Fig. 5G-H), yet similar ones of transcription and translation (Supplementary Fig. 5H). Co-transfection of XBP-1 s and siDRP-1 (Fig. 5F) further oriented the research as: Inhibition of mitochondrial fission regulates cancer cell immunogenicity by targeting XBP-1 s other than regulating transcription and translation.

8. Bioinformatic analysis based on microarray indicated TPP2 as the potential target of XBP-1 s

There are several assumptions about how ER stress affects the expression of MHC-I on cell surface, such as (i) to relieve the folding pressure, premature protein is removed from ER including MHC-I[34], (ii) ER stress induced blockage of protein synthesis via PERK-mediated eIF2α phosphorylation contributed to diminished peptide loading[34], (iii) other antigen processing machinery ((APM, such as TAP (transporter associated with antigen presentation) translocates peptides from the cytosol to the ER lumen where loading onto MHC-I molecules takes place and tapasin, plays a decisive role in the formation of low off-rate MHC-I/peptide complexes)) members have been perturbed[30, 35]. However, the molecular mechanism of this phenomenon has never been identified. The expression microarrays indicated that 624 mRNAs were upregulated and 652 mRNAs were found to be significantly downregulated (Fig. 6A) after the knockdown of XBP-1 s. Based on previous observations, downstream genes regulated by XBP-1 s are supposed to be involved in the post-translation procedure, namely translocation, metabolism, protein modification, et al. We next performed the GeneSet Enrichment Analysis (GSEA) with the FDR significance cutoff of 0.05, we found several associated genes which participated in immune, metabolism, post-translation and cancer pathways (Fig. 6C). The identified genes were specifically exhibited on the basis of fold change and p value in Fig. 6B and the associated gene sets were displayed in Fig. 6D-E, with NES indicating normalized enrichment score. We divided these genes into four groups according to their recognized functions in cancer biology (Fig. 6G). Among these genes, tripeptidyl peptidase 2 (TPP2), attracted our attention, as it is a well-recognized mammalian aminopeptidase that removes tripeptides from the N-terminus of longer peptides at neutral pH. It is now well accepted that TPP2 play an essential role in some MHC-I antigen presentation and CD8+ T cells maturation[36]. Other genes such as CUL3, CTSF, MDM2, MRC2, CTSO, AKT2, ATG5, ATG7, UBE2R2 and ERAP1 that possess similar function of degrading or metabolizing specific types of proteins are shown in Fig. 6F. To further identify the functionality of XBP-1, we referred to the human
induces supercomplexes of the ETC thus maximizes OXPHOS (oxidative phosphorylation) activity, enhances ER interactions important for mitochondrial ssion, fusion of mitochondria into linear or tubular networks limits deleterious mutations in mtDNA (mitochondrial DNA), of tumors. This is an important discover compared to the existing research regarding mitochondrial dynamics. Interestingly, constrast to a promising target to regulate immunogenicity of cancer cells which can eventually influence the biological behavior and clinical outcome poor prognosis in HNSCC, NSCLC and melanoma. Our research also revealed for the rst time that mitochondrial ssion can be utilized as mitochondrial ssion through knockdown of DRP-1.

Mitochondrial ssion is also implicated in the release of cytochrome C into the cytosol to trigger apoptosis, or for the destruction of damaged cellular organelles. For example, mitochondrial ssion produces smaller, fragmented mitochondria, which are important for mitochondrial metabolism and apoptosis. The mitochondrial ssion and fusion are both closely related to important physiological processes. For instance, mitochondrial ssion and fusion are both closely related to important physiological processes. A complex contains multiple copies of the same enzyme in a single membrane, allowing for the coordinated regulation of substrate flow and product formation. The detailed mechanism of present study is shown in Fig. 7A.

To further investigate its speci c role in antigen processing and presentation, we used B16F10 cell line that was stably transfected with either shTpp2 or shCtrl (small hairpin RNA, Genechem, Shanghai, China). When transiently transfected with OVA (Generay) and SIINFEKL plasmids (Cat. No. 102944, Addgene), it was observed that knockdown of Tpp2 e ciently promoted the SIINFEKL-H-2Kb complex expression (Fig. 7B). The abovementioned stably transfected cells were then co-cultured with the isolated OVA-speci c CTLs derived from OT-1 transgenic mice (Fig. 7B)[37]. We tested the production of mouse IFN-γ (Fig. 7D) and the speci c lysis of cancer cells (Fig. 7E) after co-culturing for a predetermined time and E/T ratio. It was revealed that Tpp2 knockdown signi cantly promoted recognition of the epitope by CTLs. The results above were all evidences that point to the predominant role of Tpp2 in destroying peptides, i.e. cleave them to sizes smaller than required to bind to MHC-I (i.e.: < 8–10 amino acids)[14]. Given the fact that TPP2 regulates the immunogenicity of cancer cells by degrading antigenic peptides, it can be inferred and experimentally veri ed that the inhibition of mitochondrial ssion would not affect the expression of other immunological-related or unrelated transmembrane proteins (Fig. 7F). The precision in this method avoids any off-target occurrences and side effects. Suppressing the degradation of antigenic peptides is a possible mechanism to which mitochondrial dynamics regulate cancer immunogenicity. The detailed mechanism of present study is exhibited in Fig. 7G.

9. TPP2 prevents MHC-1 maturation by the destruction of antigenic peptides

Antigen presentation is a sophisticated process that involves a number of crucial events: (i) MHC-I folding and maturation, (ii) peptide generation and shuttle into the ER, (iii) assembly of the APM, (iv) trafficking of peptide loaded MHC-I through the Golgi apparatus to cell surface and (v) ultimately its recognition by CD8+ T lymphocytes. During this delicate process, any corrupted intracellular events could lead to the generation of high off-rate MHC-I/peptide complexes that dissociate prematurely during their journey towards cell surface[31]. Co-transfection of siTPP2 and XBP-1 s rescued MHC-I to its unaffected level in TSCCs and B16F10, further veri ed that TPP2 is the downstream gene regulated by XBP-1 s and is involved in MHC-I maturation (Fig. 7A). To further investigate its speci c role in antigen processing and presentation, we used B16F10 cell line that was stably transfected with either shTpp2 or shCtrl (small hairpin RNA, Genechem, Shanghai, China). When transiently transfected with OVA (Generay) and SIINFEKL plasmids (Cat. No. 102944, Addgene), it was observed that knockdown of Tpp2 e ciently promoted the SIINFEKL-H-2Kb complex expression (Fig. 7B). The abovementioned stably transfected cells were then co-cultured with the isolated OVA-speci c CTLs derived from OT-1 transgenic mice (Fig. 7B)[37]. We tested the production of mouse IFN-γ (Fig. 7D) and the speci c lysis of cancer cells (Fig. 7E) after co-culturing for a predetermined time and E/T ratio. It was revealed that Tpp2 knockdown signi cantly promoted recognition of the epitope by CTLs. The results above were all evidences that point to the predominant role of Tpp2 in destroying peptides, i.e. cleave them to sizes smaller than required to bind to MHC-I (i.e.: < 8–10 amino acids)[14]. Given the fact that TPP2 regulates the immunogenicity of cancer cells by degrading antigenic peptides, it can be inferred and experimentally veri ed that the inhibition of mitochondrial ssion would not affect the expression of other immunological-related or unrelated transmembrane proteins (Fig. 7F). The precision in this method avoids any off-target occurrences and side effects. Suppressing the degradation of antigenic peptides is a possible mechanism to which mitochondrial dynamics regulate cancer immunogenicity. The detailed mechanism of present study is exhibited in Fig. 7G.

Discussion

Immune escape of tumor is the central mechanism that leads to difficulty in early diagnosis, in nite proliferation and metastasis without restriction and ultimately death of patients. Loss or downregulation of membrane MHC-I has always been considered to be an important cause of tumor escaping from immunological surveillance[2] and is also frequently found in solid tumors, including malignant melanoma, breast cancer, stomach cancer, colon cancer, and bladder cancer[1]. In this study, we characterized MHC-I membrane expression as a prognosis indicator in HNSCC, NSCLC and melanoma patients, which has con rmed the ndings of several previous research[1–2, 4]. However, there has been little research on the restoration of MHC-I expression to reverse the immunescape phenotype and promote CTLs recognition and eradication of cancer cells.

Mitochondria are the indispensable regulatory centers shared by all eukaryotic cells and have always been the focus of basic scienti c research. In recent years, the unique mitochondrial dynamics has gradually received more and more attention in addition to studies on mitochondrial metabolism and apoptosis. The mitochondrial fission and fusion are both closely related to important physiological processes. For example, mitochondrial fission produces smaller, fragmented mitochondria, which are important for mitochondrial movement to regions of high energy demand or to allow for equal mitochondrial distribution to daughter cells following mitosis[38]. Mitochondrial fission is also implicated in the release of cytochrome C into the cytosol to trigger apoptosis, or for the destruction of damaged cellular organelles[39]. More importantly, impaired fusion and enhanced fission have been frequently observed in solid tumors[40]. Several studies have demonstrated that mitochondrial fission is required to maintain the metastasis potential and proliferating rate of breast, thyroid, and glioblastoma cancer cells[41]. It was also observed that tumor growth may be blocked by inhibition of mitochondrial fission through knockdown of DRP-1[6]. In the present study, we have also determined that pSer616 DRP-1 is a indicator of poor prognosis in HNSCC, NSCLC and melanoma. Our research also revealed for the rst time that mitochondrial fission can be utilized as a promising target to regulate immunogenicity of cancer cells which can eventually in uence the biological behavior and clinical outcome of tumors. This is an important discover compared to the existing research regarding mitochondrial dynamics. Interestingly, constrast to mitochondrial fission, fusion of mitochondria into linear or tubular networks limits deleterious mutations in mtDNA (mitochondrial DNA), induces supercomplexes of the ETC thus maximizes OXPHOS (oxidative phosphorylation) activity, enhances ER interactions important for...
Ca^{2+} flux and protect mitochondria from autophagic degradation\cite{42}. In addition, mitochondria elongate as a survival mechanism in response to nutrient starvation and stress, linking fusion to cell longevity and persistence\cite{43}. This indicates that inhibiting mitochondrial fission namely promoting fusion, can dramatically mitigate the damage that traditional methods such as chemotherapy and radiotherapy poses on non-transformed cells, which was a major concern of side effect and an obstacle hampering many therapies from clinical application.

ER stress caused by abnormal accumulation of unfolded proteins in ER is a hallmark feature of secretory cells and many diseases, including diabetes, neuro-degeneration, and cancers\cite{8}. The UPR can promote survival under conditions of transient and mild ER stress. For example, ER stress promotes angiogenesis through stimulating VEGF expression and secretion. It also induces cancer cell dormancy through G1 arrest in response to decreased cyclin D1 downstream of PERK activation. The robust upregulation of GRP78 and other ER chaperones by the UPR can enhance the ER protein folding capacity and reestablish ER homeostasis, which protects the cancer cells from apoptosis and allows for recurrence once favorable growth conditions return\cite{44}. However, if ER stress is prolonged and severe, coupled with failure of compensatory mechanisms, apoptosis will be initiated through activation of transcription factor CHOP by downstream signaling from PERK via ATF4. Although the molecular mechanisms underlying this switch remain poorly understood, each apical UPR sensor holds a dualistic role in propagating adaptive as well as toxic signals\cite{32}. Thus, from a ‘simplistic’ point of view one would like to promote severe ER stress in cancer cells by therapeutics that either block the pro-survival pathways and/or promote the pro-apoptotic signals emanating from the UPR, such as using proteasome inhibitors like HIV protease inhibitors\cite{48} to further increase the protein burden in the already challenged ER, or HSP90 inhibitors which can activate all three UPR branches\cite{46}. The above methods are all plotting to further aggravate ER stress, although they do cause damage to cancer cells, it is inevitable that non-transformed cells may severely suffer as well. In recent years, there have been several reports regarding small molecule inhibitors targeting UPR, such as the IRE1α-specific inhibitor 4μ8c\cite{31}, ER Stress inhibitor TUDCA\cite{31}, inhibitor of PERK’s kinase domain named GlaxoSmithKline (GSK)\cite{47}, et al. However, their application is limited due to off-target effect and pancreatic toxicity\cite{52}. Moreover, the molecular insights on apoptosis/survival decisions during ER stress are still too limited and the risk exists that the anticancer drug in question might actually end up blocking ER stress-mediated apoptosis, thereby promoting rather than preventing tumor progression. Not surprising that there are conflicting data in literature considering the impact of inhibiting PERK or IRE1α in cancer therapy\cite{48}. The treatment strategy proposed by us of targeting mitochondrial fission rather than ER stress subtly bypass the above drawbacks we may encounter. Regulating mitochondrial fission is an easier, safer and more controllable way as compared to ER stress.

Rescuing loss or downregulation of MHC-I is a promising method to initiate a cytotoxic immune response against the transformed cells. A strong link between UPR and the MHC-I antigen presentation pathway defects was established by previous research\cite{30} and our study. However, the broad impact of UPR on distinct cellular mechanisms, combined with complexity of the antigen presentation pathway, does not make the establishment of a mechanistic link between ER stress response activation and MHC-I impairment an easy task\cite{31}. By using expression microarray, bioinformatic analysis, ChIP-qPCR and luciferase reporter assay, TPP2 has emerged as a plausible link between MHC-I expression and UPR, especially XBP-1 s. Peptides that bind to MHC-I are produced from intracellular proteins as a byproduct of protein catabolism. The major protease responsible for the initial cleavage of cellular proteins into oligopeptides is the proteasome. The majority of peptides produced by proteasomes are very rapidly hydrolyzed into amino acids by the concerted action of aminopeptidases and endopeptidases in the cytosol. However, a small fraction of peptides escape destruction and are transported by TAP into ER where ones of the right size and sequence bind to newly assembled MHC-I. However, proteasomes more frequently generate peptides (~10–20%) that are too long to bind to MHC-I molecules but can serve as potential antigenic precursors. These long precursors can be converted to MHC-I binding peptides by aminopeptidases, or may be completely degraded to amino acids by aminopeptidases and endopeptidases\cite{49}. When closely examined, most aminopeptidases preferentially degrade relatively short peptides. They have little or no activity on peptides that are longer than about 16 amino acids when tested in vitro, while TPP2 works as an exception for its ability to degrade longer peptides\cite{50}. TPP2, the aminopeptidase is reported to be involved in the generation of many epitopes recognized by CD8⁺ T lymphocytes, however, it also destroys epitope-containing peptides. Due to the fact that very few peptides suffice for immune surveillance by CD8⁺ T cells, the productive/destructive balance is not always positive for the generation of MHC-I ligands\cite{51}. Although the exact effect of TPP2 on class I antigen processing and presentation is still controversial, our data suggested a moderate yet a predominantly destructive role of TPP2. Meanwhile, we have also put forward several post-translation related genes and pathways that require further exploration.

**Methods**

**Patients and tissue samples**
127 HNSCC samples, 62 NSCLC samples and 59 melanoma samples collected at Sun Yat-sen Memorial Hospital, Sun Yat-sen University (Guangzhou, China) between January 2006 and December 2010 were used for MHC-I, pSer616 DRP-1 staining, Spearman correlation and Kaplan-Meier survival plotting. The date of death was obtained from patient records or through follow-up telephone calls. Survival time was calculated from the date of surgery to the date of death or to the last follow-up. Each patient has been followed up for at least 60 months. Additionally, tumor samples and peripheral blood samples obtained from 109 patients with HNSCC and 60 patients with NSCLC at Sun Yat-sen Memorial Hospital, Sun Yat-sen University between April 2015 and October 2018 were used for primary cancer cell and T cell isolation and analysis. 15–20 mL peripheral blood was obtained from each patient. The clinical features of these patients are provided in Supplementary Table 3-4. All samples were collected from patients who had provided informed consent, and all related procedures were performed with the approval of the internal review and ethics board of Sun Yat-sen Memorial Hospital.

**Cell lines**

Human cancer cell lines SCC-9 (TSCC), CAL-27 (TSCC), A549 (NSCLC), Saos-2 (osteosarcoma) and mouse cancer cell line B16F10 (melanoma) were purchased from American Type Culture Collection (ATCC). M21 (melanoma), Colo38 (melanoma) and FO-1 (melanoma) were generous gifts from Dr. Soldano Ferrone (Massachusetts General Hospital, Harvard Medical School). Cells were cultured in DMEM, DMEM/F12 or RPMI 1640 (Thermo Scientific) supplemented with 10% fetal bovine serum (FBS) (Gibco).

**Syngeneic models**

All mouse experiments were reviewed and approved by the ethics boards and the Clinical Research Committee of Sun Yat-sen Memorial Hospital. Female C57BL/6 mice of 5–6 weeks old that purchased from Laboratory Animal Center, Sun Yat-sen University were used as syngeneic models, with 6 mice per experimental group. B16F10 cells stably expressing OVA were constructed by transfecting with OVA lentiviral (Cat. No. 113030, Addgene). 2 × 10^5 B16F10-OVA cells were resuspended in 150 µL PBS and then injected subcutaneously into the flanks of the C57BL/6 mice. 3 days after tumor implantation, Mdivi-1 (Cat. No. HY-15886, MedChemExpress, 2.5 mg/kg) or the vehicle control (DMSO) was given by tail vein injection for 5 days in a 7-day cycle daily. Tumor growth was monitored and recorded with calipers every two days after tumor implantation. The survival endpoint was when tumor reached a diameter of 15 mm. Tumor volumes were calculated with the formula: 0.5 × length × (width)^2. To further investigate the effect of Mdivi-1 on tumor metastasis, 2 × 10^5 cells were resuspended in 200 µL PBS and inoculated via tail vein, Mdivi-1 was then given the same way as described above.

**IVIS Lumina imaging**

B16F10-OVA cells transfused to C57BL/6 mice via tail vein were pre-transfected with lentivirus carrying luciferase reporter gene to examine the metastasis of tumor through IVIS Lumina imaging. Mice were given D-Luciferin (150 mg/kg i.p., 15 minutes before imaging), anesthetized (3% pentobarbital) and imaged with Xenogen IVIS Lumina system (Caliper Life Sciences). Bioluminescent flux (photons/s/cm^2/steradian) was determined to observe tumor migration.

**Primary cells isolation from tumors and peripheral blood of patients with cancer**

Tumors were cut into small fragments (approximately 1 mm^3) and incubated for 30 minutes with collagenase type I and III (Worthington Biochemical), in RPMI 1640 medium containing 2% FBS (5 ml/g tumor tissue) at 37°C. The tumor pieces were transferred to a tissue digestion C-tube (Miltenyi Biotec) and further dissociated enzymatically and mechanically on a gentleMACS Dissociator (Miltenyi Biotec) to obtain a single-cell suspension. Primary cancer cells were purified with EpCAM^+ microbeads (Cat. No. 130-061-101, Miltenyi Biotec). CD3^+ T cells were isolated from peripheral blood with EasySep™ Human T Cell Isolation Kit (Cat. No. 17951, Stemcell) according to the manufacturer's instructions. The isolated cancer cells were discarded after ten passages.

**Preparation of cancer cells lysate**

The isolated autologous cancer cells were incubated with 0.01% EDTA-solution for 5 minutes, carefully detached with a cell scraper, washed twice in PBS and resuspended at a density of 5 × 10^6/ml in serum-free medium. The cell suspensions were frozen at -80°C and disrupted by four freeze-thaw cycles. For the removal of crude debris, the lysate was centrifuged for 10 minutes at 300 g. The supernatant was collected and passed through a 0.2 µm filter. The protein concentration of the lysate was determined by commercial assay (Cat. No. 5000002, BioRad).

**Generation of DCs and tumor-specific CTLs**

DCs were generated as previously described with slight modifications[18, 21]. In brief, peripheral blood mononuclear cells (PBMCs) were isolated from the peripheral blood of patients with HNSCC or NSCLC by SepMate™ tube for density gradient centrifugation (Cat. No. 86450,
Stemcell) and were subsequently allowed to adhere in culture flasks for 1 hour. The initial adherent cell fraction was harvested and cultured in DMEM containing 50 ng/ml GM-CSF, 20 ng/ml IL-4 (PeproTech) and 10% heat-inactivated FBS for 6 days. The cultures were replaced with fresh medium and cytokines every three days, and cell differentiation was monitored through light microscopy. DCs were matured through incubation with 100 ng/ml LPS (Sigma) and 500 U/ml IFN-γ (PeproTech) for 48 hours and then pulsed for 24 hours with cancer cell lysates (200 µg protein/ 1 × 10^6 cells/ ml) as prepared above. DCs maturation were confirmed to be ≥ 90% pure by flow cytometric analysis for specific functional markers CD80, CD83 and CD86 (Supplementary Fig. 1D). To generate tumor-specific CTLs, we incubated the isolated CD3+ T cells with antigen-specific DCs (5:1) in RPMI 1640 medium supplemented with 25 U/ml IL-2 (Peprotech) and 10% heat-inactivated FBS for 5 days. The generated CTLs’ ability to kill autologous cancer cells was determined by PI and Annexin V uptake of cancer cells co-cultured (Supplementary Fig. 1C), Th1 cytokine production (Fig. 4B) and residual E/T ratio (Fig. 4C).

**Patient-derived xenograft (PDX) implantation**

Primary specimens were collected from patients with HNSCC and NSCLC who underwent tumor resection at Sun Yat-sen Memorial Hospital, Sun Yat-Sen University. Four-week-old female NOD/SCID mice purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and maintained under pathogen-free conditions were used for PDX transplantation, with three mice per experimental group. The PDX procedure was performed as previously described[20]. Briefly, a small incision was made on the flank of anaesthetized NOD/SCID mice. The primary HNSCC and NSCLC samples were then minced into fragments of 2–3 mm^3 and implanted into the subcutaneous tissue. The incision was then closed with sutures. The time from patient collection to mouse implantation ranged from 1–3 hours. Tumor formation was monitored with calipers per week following implantation.

**Adoptive cell therapy (ACT)**

CD3+ T cells were isolated from peripheral blood of the same patients with HNSCC or NSCLC. Mature DCs were generated and verified as described above, then incubated with autologous CD3+ T cells (DC/T cell ratio 1:5) for 5 days. Then, 2.5 × 10^6 T cells and 0.5 × 10^6 DCs were intravenously transfused into each PDX-bearing mouse via tail vein after palpable tumor formation. Mdivi-1 (2.5 mg/kg) or the vehicle control (DMSO) was given on the same day as CTL transfer by tail vein injection for 5 days daily in a 7-day cycle. Tumor growth was monitored and recorded with calipers weekly after ACT. The survival endpoint was when tumors reached a diameter of 15 mm. The dissociation of harvested PDXs was the same as primary cell isolation as described above. The tumor infiltrated T cells (TILs) were purified with CD8 microbeads (Cat. No. 130-045-201, Miltenyi Biotech). Tumor volumes were calculated with the formula: 0.5 × length×(width)^2.

**EpCAM and TUNEL co-staining**

Frozen biopsy samples of harvested PDXs were routinely cut into 4-µm-thick sections. The sections were then mounted on glass slides and fixed with 4% paraformaldehyde for 3 minutes at room temperature. TUNEL staining was performed with an In Situ Cell Death Detection Kit (Cat. No. 11684817910, Roche) according to the manufacturer’s instructions. The sections were then washed twice with 0.01 mM PBS at pH 7.4, then stained with rabbit anti-human EpCAM (Cat. No. ab213500, Abcam, 1:100) overnight at 4°C and subsequently by Alexa Fluor 594-conjugated secondary antibody (Cat. No. A-11012, Thermo Fisher Scientific) for 1 hour at room temperature. DAPI was then used to counterstain the nuclei, and the images were acquired with upright fluorescence microscope (Axio Imager A2, ZEISS).

**Enzyme-linked ImmunoSpot (ELISpot)**

To analyze the tumor infiltrated T cells, single cell suspensions were generated as previously described from harvested tumors or PDXs and were rested overnight to get rid of living tumor cells via plastic adherence. Viable cells were separated via density gradient centrifugation and added to the ELISpot plate[16]. The number of IFN-γ-producing T cells was determined with IFN-γ ELISpot kit (Cat. No. 2210001 and 211001, Dakewe) according to the manufacturer’s protocol.

**Cytotoxic and co-culture assays**

Primary specimens were collected as described above. 46 HNSCC and 28 NSCLC samples with autologous peripheral blood were used for co-culture analysis. Tumor-specific CTLs, generated by incubation with tumor antigen pulsed DCs as described above, were co-cultured with the autologous cancer cells at indicated E/T ratio. Cancer cells were pretreated with Mdivi-1 at 50 µM for 72 hours or left untreated before co-culture. After 12 hours, EpCAM+ cells were harvested and death was assessed by flow cytometry with apoptosis detection kit (Cat. No. 88-8007-74, eBioscience) that stains for Annexin V and PI, according to the manufacturer’s instructions. The percentages of apoptotic cells include the percentages of early (Annexin V^+ PI^-) and late apoptotic cells (Annexin V^+ PI^+). Specific apoptosis was calculated as: percentage of induced apoptosis-percentage of spontaneous apoptosis)/(100%-percentage of spontaneous apoptosis) ×
100% as previously described[52]. We mainly focused on late apoptosis. Supernatant was collected at 24 hours after co-culture to measure IFN-γ, IL-2 and TNF-α release using specific ELISAs (Cat. No. ELH-IFNg-1, ELH-IL2-1 and ELH-TNFa-1, Raybiotech). After 72 hours of culture at 37 °C, adherent cancer cells and T cells were collected altogether and residual cancer cells and T cells were assessed by flow cytometric analysis based on EpCAM and CD8 expression respectively. To determine MHC-I restriction of cancer cells lysis, the target cells were preincubated with MHC-I blocking antibody w6/32 (Cat. No. sc-32235, Santa Cruz, 10 µg/mL) for 2 hours at 37 °C before co-culture[21]. The result was further verified by using immunodeficient FO-1 melanoma cell line[53].

**Mitochondrial staining**

To visualize the changes in mitochondrial morphology, we planted cells onto coverslips and treated as described. Then, the cells were stained for 30 minutes with 0.02 µM MitoTracker Red CMXRos (Cat. No. M7512, ThermoFisher) at 37 °C protected from light. The images were acquired with laser scanning confocal microscopy (LSM 800 with Airyscan, Zeiss).

**Measurements of oxidative stress**

To evaluate the oxidative stress within cancer cells, we tested several indicators as follow: oxidation of cardiolipin, intracellular ROS and mitochondrial membrane potential (ΔΨm)[28]. We assessed cardiolipin oxidation by using fluorescent dye NAO (Cat. No. A1372, ThermoFisher), which binds to non-oxidized cardiolipin, but not oxidized cardiolipin. We planted cells onto coverslips and treated as described, then incubated cells with 100 nM NAO for 15 minutes at 37 °C protected from light. The images were acquired with laser scanning confocal microscopy (LSM 800 with Airyscan, Zeiss). Intracellular ROS production was measured by flow cytometry using the oxidation sensitive dye DCFH-DA (Cat. No. S0033, Beyotime Biotechnology). The cells were seeded in 6-well plates and treated as described. After washing with preheated PBS for three times, the cells were incubated with DCFH-DA at 37 °C for 25 minutes and detached, suspended to be tested. The green fluorescence was measured using FITC channel. Similarly, mitochondrial membrane potential (ΔΨm) was measured by flow cytometry using MitoScreen (JC-1) Kit (Cat. No. 551302, BD Pharmingen) according to the manufacturer's protocol.

**In vitro antigen presentation assays**

5–6 weeks old female OT-1 transgenic mice were purchased from Model Animal Research Center, Nanjing University (Nanjing, China). The OVA-specific CD8+ T cells were isolated from spleens of OT-1 mice by negative selection using CD8a+ T Cell Isolation Kit (Cat. No. 130-104-075, Miltenyi Biotec). The isolated CD8+ T cells were cultured in RPMI 1640 medium containing 10% FBS and 100 U/ml IL-2 with activation by anti-CD3/CD28 beads (Cat. No. 11452D, ThermoFisher)[37]. B16F10 was stably transfected with shTpp2 provided by Genechem (Shanghai, China) and transiently transfected with OVA (Generay) or SIINFEKL (Cat. No. 102944, Addgene) with the same backbone which was GFP labeled.

The anti-CD3/CD28 activated CTLs described above were co-cultured with pretreated B16F10 at indicated E/T ratio. After 12 hours, GFP+ cells were harvested and death was assessed by flow cytometry with apoptosis detection kit (Cat. No. 88-8007-74, eBioscience) that stains for annexin V and PI, according to the manufacturer’s instructions. The calculation of specific death is described above. Supernatant was collected at 24 hours of culture to measure IFN-γ using specific ELISA (Cat. No. ELM-IFNg-1, Raybiotech).

**Bioinformatic analysis**

We obtained XBP-1 ChIP-seqs of T47D, HS578T and MDA-MB-231 cells from ENCODE, then processed them by ENCODE processing pipeline. To predict the potential XBP-1 binding sites at TPP2 promoter region, we used motif-counter (https://bio.tools/motifcounter) to scan TPP2 promoter region from both strands with XBP-1 motif obtained from JASPAR database.

**Chromatin immunoprecipitation assays (ChIP)**

ChIP assays were performed as previously described[54]. Briefly, cells (5 × 10^6) were washed with PBS and incubated for 10 min with 1% formaldehyde at room temperature. Crosslinking was halted with 0.1 M glycine for 5 min. The cells were washed twice with PBS and lysed for 1 h at 4 °C in a lysis buffer, then sonicated into chromatin fragments with an average length of 500–800 bp, as assessed via agarose gel electrophoresis. The samples were precleared with Protein-A agarose (Roche) for 1 h at 4 °C on a rocking platform. Then, 5 µg of specific antibodies was added and the samples rocked overnight at 4 °C. Immunoprecipitated DNA was purified using the QiAquick PCR purification kit (Qiagen) according to the manufacturer’s protocol. The final ChIP DNA was then used as a template in qPCR with the primers in Table S 5. ChIP-grade anti-XBP-1 antibody (Santa cruz, sc-8015) and anti-RNA polymerase II antibody (Abcam, ab5131) were used in this study.

**Luciferase reporter assay**
A luciferase assay was carried out as previously described with modifications[55]. Briefly, pGL4-TPP2-wide type(-wt) or mutant (mut) was obtained by cloning a 2000 bp DNA fragment (from TPP2 transcriptional starting site) into the pGL4.20-Basic vector upstream of the luciferase reporter gene. The pGL4.20 derived reporter vectors were transfected into cells and the stable cell lines were obtained through puromycin selection for two weeks. The pRL-TK plasmid delivering Renilla Luciferase was co-transfected as a control. The luciferase activities were measured using a Dual Luciferase Reporter Assay Kit (Promega), and the target effect was presented as the luciferase activity of the reporter vector with the target sequence relative to that without the target sequence.

Flow cytometry

Cells were stained with H-2Kb-BV421 (Cat. No. 562942, BD Pharmingen), SIINFEKL-H-2Kb-PE (Cat. No. 12-5743-82, eBioscience), anti-Mouse CD8a-APC (Cat. No. 553035, BD Pharmingen), anti-OVA-H-2Kb tetramer-BV421 (Cat. No. TB-5001-4, MBL), HLA-ABC-PE (Cat. No. 560168, BD Pharmingen), Perforin-BV421 (Cat. No. 563393, BD Pharmingen), Granzyme B-FITC (Cat. No. 560211, BD Pharmingen), CD8-PE-CF594 (Cat. No. 562282, BD Pharmingen), EpCAM-BV510 (Cat. No. 563181, BD Pharmingen), ITGB1-PE (Cat. No. 555443, BD Pharmingen), Tfr1-APC (Cat. No. 561940, BD Pharmingen), E-Cadherin-BV421 (Cat. No. 743712, BD Pharmingen), CD80-PE (Cat. No. 560925, BD Pharmingen), CD83-BV421 (Cat. No. 562630, BD Pharmingen) and CD86-FITC (Cat. No. 560958, BD Pharmingen). For intracellular staining, cells were pretreated with the Intracellular Fixation and Permeabilization Kit (Cat. No. 88-8824, eBioscience) according to the manufacturer's instructions. Cells were subsequently analyzed with multicolor flow cytometry (BD, FACSVerse).

Quantitative real-time PCR (qRT-PCR)

qRT-PCR was performed using SYBR Green Real-time PCR Master Mix (ReverTra Ace, Toyobo) and LightCycler 480 (Roche, Basel, Switzerland) according to the manufacturer's instructions. The sequences of all the involved primers are listed in Supplementary Table 5. The relative expression levels of control were set to 1.

Immunoblotting

Protein extracts were resolved through 8% SDS–polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes (BioRad), probed with an antibody directed against MHC-I (Cat. No. sc-32235, Santa Cruz, 1:200), IRE1α (Cat. No. 3294, Cell Signaling Technology, 1:1000), pIRE1α (Cat. No. 3294, Cell Signaling Technology, 1:1000), XBP-1 (Cat. No. 4494, Cell Signaling Technology, 1:3200), EpCAM (Cat. No. ab213500, Abcam, 1:100) and CD8 (Cat. No. ab93278, Abcam, 1:500). The accuracy of automated measurements was confirmed through independent evaluation by two pathologists. Cells stained with the indicated antibodies were counted at 400 × magnification at least ten fields per section. The stained slides were imaged using an upright fluorescence microscope (Axio Imager A2, ZEISS).

Immunofluorescence and immunohistochemistry staining

Antigen retrieval was performed by incubation of the slides in a pressure cooker for 5 minutes in 0.01 M citrate buffer, pH 6.0 and subsequent treatment with 3% hydrogen peroxide for 5 minutes. Slides were incubated overnight at 4°C with antibodies as follow: HC-10 + HC-A2 (targeting MHC-I, a generous gift from Dr. Koichi Sakakura, Massachusetts General Hospital, Harvard Medical School), pSer616 DRP-1 (Cat. No. bs-12702R-HRP, Bioss, 1:300) and EpCAM (Cat. No. ab213500, Abcam, 1:100). Immunohistochemical staining was performed according to the manufacturer's instructions. In order to simplify the analysis, expression of MHC-I and pSer616 DRP-1 were scored based on both intensity (0–3) and extent of staining (1–3). A multiplicative staining score was calculated by multiplying the intensity and extent scores to yield scores on a 9-point scale from 1 to 9. When dealing with multiple scores per patient, the individual scores were averaged to obtain a final score. Cases were divided based on staining scores into 3 groups: weak (0–2), moderate (3–5) and strong (6–9)[19]. For immunofluorescence, specimens were incubated with MHC-I (Cat. No. sc-32235, Santa Cruz, 1:200), pSer616 DRP-1 (Cat. No. 4494, Cell Signaling Technology, 1:3200), EpCAM (Cat. No. ab213500, Abcam, 1:100) and CD8 (Cat. No. ab93278, Abcam, 1:500). The accuracy of automated measurements was confirmed through independent evaluation by two pathologists. Cells stained with the indicated antibodies were counted at 400 × magnification at least ten fields per section. The stained slides were imaged using an upright fluorescence microscope (Axio Imager A2, ZEISS).

Statistics

All data are expressed as the mean ± standard error of mean (s.e.m.). All statistical analyses were performed in SPSS Windows version 13.0. Spearman correlation analysis was used to assess the relationship between MHC-I and pSer616 DRP-1 expression. Kaplan–Meier survival curves were plotted and log-rank tests were performed. All experiments were performed at least in triplicates and the exact numbers of independent experiments with similar results are indicated in the figure legends. All statistical analyses of experiments were performed with two-tailed Student's T tests unless otherwise stated. p < 0.05 was considered statistically significant.
| Characteristics  | MHC-I (multiplicative score) | P     | pSer616 DRP-1 (multiplicative score) | P     |
|------------------|------------------------------|-------|-------------------------------------|-------|
|                  | No. of score < 5 | No. of score > 5 | No. of score < 5 | No. of score > 5 |
| **Table S1.1**   |                 |       |                                     |       |
| **HNSCC (n = 127)** |                 |       |                                     |       |
| **Characteristics** |                 |       |                                     |       |
| Sex              |                 |       |                                     |       |
| Male             | 36 (45.0)       | 44 (55.0) | 46 (57.5)                           | 34 (42.5) |
| Female           | 29 (61.7)       | 18 (38.3) | 22 (46.8)                           | 25 (53.2) |
| Age              |                 |       |                                     |       |
| < 50             | 27 (54.0)       | 23 (46.0) | 26 (52.0)                           | 24 (48.0) |
| ≥ 50             | 38 (49.4)       | 39 (50.6) | 42 (54.5)                           | 35 (45.5) |
| Node metastasis  | 0.000           |       | 0.030                               |       |
| N0               | 13 (23.6)       | 42 (76.4) | 36 (65.5)                           | 19 (34.5) |
| N1               | 52 (72.2)       | 20 (27.8) | 32 (44.4)                           | 40 (55.6) |
| Clinical stage   | 0.778           |       | 0.001                               |       |
| T                | 32 (53.3)       | 28 (46.7) | 22 (36.7)                           | 38 (63.3) |
| ≥ T              | 33 (49.3)       | 34 (50.7) | 46 (68.7)                           | 21 (31.3) |
| **Table S1.2**   |                 |       |                                     |       |
| **NSCLC (n = 62)** |                 |       |                                     |       |
| **Characteristics** |                 |       |                                     |       |
| Sex              |                 |       |                                     |       |
| Male             | 25 (56.8)       | 19 (43.2) | 23 (52.3)                           | 21 (47.7) |
| Female           | 12 (66.7)       | 6 (33.3)  | 10 (55.6)                           | 8 (44.4)  |
| Age              | 0.809           |       | 0.937                               |       |
| < 50             | 12 (60.0)       | 8 (40.0)  | 10 (50.0)                           | 10 (50.0) |
| ≥ 50             | 25 (59.5)       | 17 (40.5) | 23 (54.8)                           | 19 (45.2) |
| Node metastasis  | 0.000           |       | 0.019                               |       |
| N0               | 8 (28.6)        | 20 (71.4) | 20 (71.4)                           | 8 (28.6)  |
| N1               | 29 (85.3)       | 5 (14.7)  | 13 (38.2)                           | 21 (61.8) |
| Clinical stage   | 0.834           |       | 0.021                               |       |
| T                | 20 (62.5)       | 12 (37.5) | 12 (37.5)                           | 20 (62.5) |
| ≥ T              | 17 (56.7)       | 13 (43.3) | 21 (70.0)                           | 9 (30.0)  |
Table S1.3
Melanoma (n = 59)

| Characteristics | MHC-I (multiplicative score) | P | pSer616 DRP-1 (multiplicative score) | P |
|-----------------|-----------------------------|---|-------------------------------------|---|
|                 | No. of score < 5            | No. of score > 5 | No. of score < 5 | No. of score > 5 |
| Sex             |                             |    |                                    |    |
| Male            | 20 (66.7)                   | 10 (33.3) | 12 (40.0) | 18 (60.0) |
| Female          | 14 (48.3)                   | 15 (51.7) | 7 (24.1)   | 22 (75.9) |
| Age             |                             |    |                                    |    |
| ≤ 50            | 25 (49.1)                   | 13 (50.9) | 12 (31.6) | 26 (68.4) |
| ≥ 50            | 9 (40.0)                    | 12 (60.0) | 7 (33.3)   | 14 (66.7) |
| Node metastasis|                             |    |                                    |    |
| N0              | 15 (38.5)                   | 24 (61.5) | 6 (15.4)   | 33 (84.6) |
| N1              | 19 (95.0)                   | 1 (5.0)   | 13 (65.0)  | 7 (35.0)  |
| Clinical stage  |                             |    |                                    |    |
| I               | 21 (65.6)                   | 11 (34.4) | 9 (28.1)   | 23 (71.9) |
| II              | 13 (48.1)                   | 14 (51.9) | 10 (37.0)  | 17 (63.0) |

Univariate and Multivariate Analysis of Factors Associated with Overall Survival of Cancer Patients
| Variables                              | No. of cases | HR (95%CI)     | P     |
|----------------------------------------|--------------|----------------|-------|
| **Univariate analysis**                |              |                |       |
| Sex                                    | 80/47        | 0.885 (0.593–1.320) | 0.549 |
| Male vs. Female                        |              |                |       |
| Age (years)                            | 50/77        | 0.916 (0.618–1.360) | 0.665 |
| ≤50 vs. ≥50                            |              |                |       |
| Node metastasis                        | 55/72        | 17.351 (9.112–33.040) | <0.001|
| N0 vs. N+                              |              |                |       |
| Clinical stage                         | 60/67        | 11.817 (6.866–20.338) | <0.001|
| II vs. III                             |              |                |       |
| MHC-I score                            | 65/62        | 0.398 (0.265–0.598) | <0.001|
| <5 vs. >5                              |              |                |       |
| pSer616 DRP-1 score                    | 68/59        | 1.617 (1.182–2.213) | 0.016 |
| <5 vs. >5                              |              |                |       |
| **Multivariate analysis**              |              |                |       |
| Node metastasis                        | 55/72        | 73.271 (22.298–240.769) | <0.001|
| N0 vs. N+                              |              |                |       |
| Clinical stage                         | 60/67        | 115.805 (36.014–372.379) | <0.001|
| II vs. III                             |              |                |       |
| MHC-I score                            | 65/62        | 0.024 (0.007–0.081) | <0.001|
| <5 vs. >5                              |              |                |       |
| pSer616 DRP-1 score                    | 68/59        | 0.055 (0.017–0.179) | <0.001|
| <5 vs. >5                              |              |                |       |
Table S2.2
NSCLC (n = 62)

| Variables                  | No. of cases | HR (95%CI)        | P     |
|----------------------------|--------------|------------------|-------|
| **Univariate analysis**    |              |                  |       |
| Sex                        | 44/18        | 0.767 (0.425–1.385) | 0.379 |
| Male vs. Female             |              |                  |       |
| Age (years)                | 20/42        | 1.401 (0.775–2.534) | 0.264 |
| ≤50 vs. ≥50                |              |                  |       |
| Node metastasis            | 28/34        | 8.029 (3.833–16.822) | <0.001|
| N0 vs. N+                  |              |                  |       |
| Clinical stage             | 32/30        | 14.254 (6.261–32.453) | <0.001|
| □ vs. □                    |              |                  |       |
| MHC-I score                | 37/25        | 0.554 (0.311–0.986)  | 0.044 |
| < 5 vs. >5                 |              |                  |       |
| pSer616 DRP-1 score        | 33/29        | 1.420 (1.018–1.982)  | 0.021 |
| < 5 vs. >5                 |              |                  |       |
| **Multivariate analysis**  |              |                  |       |
| Node metastasis            | 28/34        | 42.813 (11.631-157.592) | <0.001|
| N0 vs. N+                  |              |                  |       |
| Clinical stage             | 32/30        | 51.703 (14.969–178.580) | <0.001|
| □ vs. □                    |              |                  |       |
| MHC-I score                | 37/25        | 0.057 (0.009–0.361)   | 0.001 |
| < 5 vs. >5                 |              |                  |       |
| pSer616 DRP-1 score        | 33/29        | 0.216 (0.075–0.618)   | 0.004 |
| < 5 vs. >5                 |              |                  |       |
| Variables                  | No. of cases | HR (95%CI)            | P     |
|----------------------------|--------------|-----------------------|-------|
| **Univariate analysis**    |              |                       |       |
| Sex                        | 30/29        | 0.875 (0.507–1.511)   | 0.632 |
| Male vs. Female            |              |                       |       |
| Age (years)                | 38/21        | 0.850 (0.479–1.508)   | 0.578 |
| <50 vs. ≥50                |              |                       |       |
| Node metastasis            | 39/20        | 1.919 (1.019–3.614)   | 0.043 |
| N0 vs. N+                  |              |                       |       |
| Clinical stage             | 32/27        | 3.173 (1.807–5.572)   | <0.001|
| < vs. ≥                    |              |                       |       |
| MHC-I score                | 34/25        | 0.472 (0.267–0.833)   | 0.010 |
| < 5 vs. >5                 |              |                       |       |
| pSer616 DRP-1 score        | 19/40        | 2.522 (1.519–4.187)   | 0.026 |
| < 5 vs. >5                 |              |                       |       |
| **Multivariate analysis**  |              |                       |       |
| Node metastasis            | 39/20        | 14.294 (4.790–42.654) | <0.001|
| N0 vs. N+                  |              |                       |       |
| Clinical stage             | 32/27        | 9.997 (4.354–22.957)  | <0.001|
| < vs. ≥                    |              |                       |       |
| MHC-I score                | 34/25        | 0.025 (0.008–0.078)   | <0.001|
| < 5 vs. >5                 |              |                       |       |
| pSer616 DRP-1 score        | 19/40        | 4.157 (2.519–6.860)   | 0.008 |
| < 5 vs. >5                 |              |                       |       |

| No. | Gender | Age (y) | Clinical Diagnosis | Pathological Diagnosis | TNM Stage | PDX succeeded or not |
|-----|--------|---------|--------------------|-------------------------|-----------|---------------------|
| HNSCC-01 | Male | 47      | Left tongue carcinoma | Squamous cell carcinoma (Moderately differentiated) | T1N0M0 | No |

**Clinical Samples for Co-culture Used in Fig. 4**
| Variables  | No. of cases | HR (95%CI)                  | P                      |
|------------|--------------|-----------------------------|------------------------|
| HNSCC-02   | Male         | 66                          | Right buccal mucosa carcinoma (Moderately differentiated) | T2N0M0 | No |
| HNSCC-03   | Female       | 74                          | Left maxillary gingival carcinoma (Moderately differentiated)    | T1N0M0 | No |
| HNSCC-04   | Female       | 56                          | Right tongue carcinoma (Highly differentiated)                   | T1N0M0 | Yes |
| HNSCC-05   | Female       | 54                          | Left tongue carcinoma (Moderately differentiated)                 | T4N2M0 | No |
| HNSCC-06   | Male         | 73                          | Left tongue carcinoma (Moderately differentiated)                 | T2N2M0 | No |
| HNSCC-07   | Male         | 52                          | Post-surgery ipsilateral lymph node metastasis of right mouth floor carcinoma (Moderately differentiated) | T2N2M0 | No |
| HNSCC-08   | Male         | 45                          | Right tongue carcinoma (Moderately differentiated)                | T1N0M0 | No |
| HNSCC-09   | Female       | 63                          | Left tongue carcinoma (Highly differentiated)                     | T1N0M0 | No |
| HNSCC-10   | Male         | 65                          | Right oropharyngeal carcinoma (Moderately differentiated)         | T2N1M0 | No |
| HNSCC-11   | Male         | 49                          | Left mouth floor carcinoma (Highly differentiated)                | T3N1M0 | No |
| HNSCC-12   | Male         | 71                          | Left tongue carcinoma (Poorly differentiated)                    | T3N2M0 | Yes |
| HNSCC-13   | Female       | 60                          | Total tongue carcinoma (Moderately differentiated)                | T4N1M0 | Yes |
| HNSCC-14   | Male         | 56                          | Right tongue carcinoma (Moderately differentiated)                | T2N2M0 | No |
| Variables  | No. of cases | HR (95%CI) | P |
|------------|--------------|------------|---|
| HNSCC-15   | Female 51    | Left buccal mucosa carcinoma | Squamous cell carcinoma (Moderately differentiated) | T3N2M0 No |
| HNSCC-16   | Female 58    | Right tongue carcinoma | Squamous cell carcinoma (Highly differentiated) | T1N0M0 Yes |
| HNSCC-17   | Male 39      | Left mandibular gingival carcinoma | Squamous cell carcinoma (Poorly differentiated) | T4N1M0 No |
| HNSCC-18   | Female 50    | Left tongue carcinoma | Squamous cell carcinoma (Moderately differentiated) | T2N2M0 No |
| HNSCC-19   | Male 51      | Right tongue carcinoma | Squamous cell carcinoma (Poorly differentiated) | T3N1M0 No |
| HNSCC-20   | Male 73      | Left mouth floor carcinoma | Squamous cell carcinoma (Poorly differentiated) | T3N2M0 No |
| HNSCC-21   | Male 65      | Right mandibular gingival carcinoma | Squamous cell carcinoma (Highly differentiated) | T2N2M0 No |
| HNSCC-22   | Male 84      | Left tongue carcinoma | Squamous cell carcinoma (Highly differentiated) | T1N0M0 No |
| HNSCC-23   | Female 73    | Right mouth floor carcinoma | Squamous cell carcinoma (Moderately differentiated) | T3N2M0 No |
| HNSCC-24   | Female 39    | Total tongue carcinoma | Squamous cell carcinoma (Moderately differentiated) | T4N1M0 No |
| HNSCC-25   | Male 62      | Right tongue carcinoma | Squamous cell carcinoma (Highly differentiated) | T2N0M0 No |
| HNSCC-26   | Male 67      | Left maxillary gingival carcinoma | Squamous cell carcinoma (Moderately differentiated) | T3N1M0 No |
| HNSCC-27   | Female 65    | Right buccal mucosa carcinoma | Squamous cell carcinoma (Moderately differentiated) | T2N1M0 No |
| HNSCC-28   | Male 79      | Left maxillary gingival carcinoma | Squamous cell carcinoma (Poorly differentiated) | T3N1M0 No |

Clinical Samples for Co-culture Used in Fig. 4
| Variables | No. of cases | HR (95%CI) | P |
|-----------|-------------|------------|---|
| HNSCC-29  | Male 75     | Left oropharyngeal carcinoma | Squamous cell carcinoma (Poorly differentiated) | T3N3M0 Yes |
| HNSCC-30  | Male 48     | Left tongue carcinoma | Squamous cell carcinoma (Highly differentiated) | T2N0M0 No |
| HNSCC-31  | Female 53   | Left tongue carcinoma | Squamous cell carcinoma (Highly differentiated) | T1N0M0 No |
| HNSCC-32  | Female 66   | Right tongue carcinoma | Squamous cell carcinoma (Moderately differentiated) | T2N2M0 No |
| HNSCC-33  | Male 67     | Total tongue carcinoma | Squamous cell carcinoma (Moderately differentiated) | T4N1M0 No |
| HNSCC-34  | Male 60     | Right mouth floor carcinoma | Squamous cell carcinoma (Moderately differentiated) | T1N1M0 No |
| HNSCC-35  | Female 49   | Left maxillary sinus carcinoma with brain metastasis | Squamous cell carcinoma (Poorly differentiated) | T4N1M1 Yes |
| HNSCC-36  | Female 51   | Right tongue carcinoma | Squamous cell carcinoma (Moderately differentiated) | T2N0M0 No |
| HNSCC-37  | Male 60     | Right tongue carcinoma | Squamous cell carcinoma (Moderately differentiated) | T2N1M0 No |
| HNSCC-38  | Male 72     | Left buccal mucosa carcinoma | Squamous cell carcinoma (Poorly differentiated) | T3N1M0 No |
| HNSCC-39  | Female 59   | Right maxillary gingival carcinoma | Squamous cell carcinoma (Moderately differentiated) | T3N0M0 No |
| HNSCC-40  | Male 64     | Left mouth floor carcinoma | Squamous cell carcinoma (Highly differentiated) | T2N0M0 No |
| HNSCC-41  | Male 76     | Left tongue carcinoma | Squamous cell carcinoma (Moderately differentiated) | T3N0M0 Yes |
| HNSCC-42  | Female 57   | Right tongue carcinoma | Squamous cell carcinoma (Poorly differentiated) | T3N1M0 No |

Clinical Samples for Co-culture Used in Fig. 4
| Variables   | No. of cases | HR (95%CI) |
|-------------|--------------|------------|
| HNSCC-43    | Male 52      | Right tongue carcinoma (Highly differentiated) |
|             |              | T2N0M0     | No |
| HNSCC-44    | Female 68    | Left maxillary sinus carcinoma (Moderately differentiated) |
|             |              | T3N0M0     | No |
| HNSCC-45    | Female 64    | Right buccal mucosa carcinoma (Moderately differentiated) |
|             |              | T2N2M0     | No |
| HNSCC-46    | Male 70      | Left tongue carcinoma (Highly differentiated) |
|             |              | T2N0M0     | No |
| HNSCC-47    | Male 45      | Left tongue carcinoma (Highly differentiated) |
|             |              | T1N0M0     | No |
| HNSCC-48    | Female 62    | Total tongue carcinoma (Moderately differentiated) |
|             |              | T4N2M0     | No |
| HNSCC-49    | Female 60    | Right tongue carcinoma (Moderately differentiated) |
|             |              | T2N1M0     | Yes |
| HNSCC-50    | Male 81      | Left mouth floor carcinoma (Poorly differentiated) |
|             |              | T3N0M0     | No |
| HNSCC-51    | Male 75      | Left maxillary sinus carcinoma (Poorly differentiated) |
|             |              | T4N2M0     | No |
| HNSCC-52    | Female 72    | Right tongue carcinoma (Moderately differentiated) |
|             |              | T3N1M0     | Yes |
| HNSCC-53    | Male 73      | Left tongue carcinoma (Moderately differentiated) |
|             |              | T2N2M0     | No |
| HNSCC-54    | Male 68      | Right mouth floor carcinoma (Highly differentiated) |
|             |              | T2N0M0     | No |
| HNSCC-55    | Female 65    | Right mandibular gingival carcinoma (Poorly differentiated) |
|             |              | T3N0M0     | Yes |
| HNSCC-56    | Female 65    | Left tongue carcinoma (Moderately differentiated) |
|             |              | T2N0M0     | No |

Clinical Samples for Co-culture Used in Fig. 4
| Variables  | No. of cases | HR (95%CI) | P                      |
|------------|--------------|------------|------------------------|
| HNSCC-57   | Male 73      | Right maxillary gingival carcinoma | Squamous cell carcinoma (Poorly differentiated) | T4N1M0 | No |
| HNSCC-58   | Male 70      | Right tongue carcinoma | Squamous cell carcinoma (Moderately differentiated) | T2N0M0 | No |
| HNSCC-59   | Female 64    | Left mouth floor carcinoma | Squamous cell carcinoma (Moderately differentiated) | T2N0M0 | No |
| HNSCC-60   | Male 67      | Right maxillary sinus carcinoma | Squamous cell carcinoma (Highly differentiated) | T1N1M0 | Yes |
| HNSCC-61   | Male 58      | Left tongue carcinoma | Squamous cell carcinoma (Highly differentiated) | T1N0M0 | No |
| HNSCC-62   | Female 49    | Right mouth floor carcinoma | Squamous cell carcinoma (Moderately differentiated) | T2N1M0 | No |
| HNSCC-63   | Female 45    | Right tongue carcinoma | Squamous cell carcinoma (Highly differentiated) | T2N2M0 | No |
| NSCLC-01   | Male 62      | Left lung non small cell lung cancer | Squamous cell carcinoma (Moderately differentiated) | T2N0M0 | No |
| NSCLC-02   | Female 43    | Left lung non small cell lung cancer | Squamous cell carcinoma (Poorly differentiated) | T4N0M0 | No |
| NSCLC-03   | Female 45    | Right lung non small cell lung cancer with multiple bone metastases | Adenocarcinoma (Poorly differentiated) | T4N3M1 | No |
| NSCLC-04   | Male 55      | Right lung non small cell lung cancer with brain metastasis | Adenocarcinoma (Moderately differentiated) | T4N3M1 | No |
| NSCLC-05   | Male 65      | Right lung non small cell lung cancer | Squamous cell carcinoma (Moderately differentiated) | T3N0M0 | No |
| NSCLC-06   | Female 79    | Right lung non small cell lung cancer | Squamous cell carcinoma (Moderately differentiated) | T4N0M0 | Yes |
| NSCLC-07   | Female 74    | Right lung non small cell lung cancer | Squamous cell carcinoma (Moderately differentiated) | T4N3M0 | No |

Clinical Samples for Co-culture Used in Fig. 4
| Variables | No. of cases | HR (95% CI) | P       |
|-----------|--------------|-------------|---------|
| NSCLC-08  | Female 48    | Left lung non small cell lung cancer | Adenocarcinoma (Moderately differentiated) | T2N0M0 | No |
| NSCLC-09  | Male 64      | Right lung non small cell lung cancer | Pleomorphic carcinoma | T4N2M0 | No |
| NSCLC-10  | Male 63      | Left lung non small cell lung cancer | Adenocarcinoma (Moderately differentiated) | T3N1M0 | No |
| NSCLC-11  | Male 54      | Left lung non small cell lung cancer | Adenocarcinoma (Poorly differentiated) | T4N1M0 | No |
| NSCLC-12  | Male 72      | Right lung non small cell lung cancer | Adenocarcinoma (Moderately differentiated) | T4N0M0 | Yes |
| NSCLC-13  | Female 63    | Right lung non small cell lung cancer with brain metastasis | Adenocarcinoma (Poorly differentiated) | T4N3M1 | No |
| NSCLC-14  | Female 39    | Left lung non small cell lung cancer with multiple bone metastases | Squamous cell carcinoma (Poorly differentiated) | T4N2M1 | No |
| NSCLC-15  | Female 37    | Left lung non small cell lung cancer | Adenocarcinoma (Poorly differentiated) | T4N3M0 | No |
| NSCLC-16  | Male 55      | Right lung non small cell lung cancer | Squamous cell carcinoma (Poorly differentiated) | T4N2M0 | No |
| NSCLC-17  | Female 38    | Left lung non small cell lung cancer with multiple bone metastases | Magnocellular carcinoma of lung | T4N3M1 | No |
| NSCLC-18  | Female 80    | Right lung non small cell lung cancer | Adenocarcinoma (Poorly differentiated) | T3N0M0 | No |
| NSCLC-19  | Male 47      | Right lung non small cell lung cancer | Squamous cell carcinoma (Poorly differentiated) | T4N0M0 | No |
| NSCLC-20  | Male 52      | Right lung non small cell lung cancer | Adenocarcinoma (Highly differentiated) | T2N0M0 | Yes |
| NSCLC-21  | Male 63      | Right lung non small cell lung cancer with brain metastasis | Magnocellular carcinoma of lung | T4N2M1 | No |
| NSCLC-22  | Female 42    | Left lung non small cell lung cancer | Squamous cell carcinoma (Moderately differentiated) | T3N1M0 | No |

Clinical Samples for Co-culture Used in Fig. 4
| Variables | No. of cases | HR (95%CI) | P |
|-----------|-------------|-----------|---|
| NSCLC-23  | Female 50   | Left lung non small cell lung cancer | Adenocarcinoma (Moderately differentiated) | T3N0M0 No |
| NSCLC-24  | Male 72     | Right lung non small cell lung cancer | Adenocarcinoma (Moderately differentiated) | T3N1M0 Yes |
| NSCLC-25  | Male 66     | Left lung non small cell lung cancer | Squamous cell carcinoma (Moderately differentiated) | T4N0M0 No |
| NSCLC-26  | Female 71   | Left lung non small cell lung cancer | Squamous cell carcinoma (Poorly differentiated) | T4N1M0 No |
| NSCLC-27  | Male 50     | Right lung non small cell lung cancer | Adenocarcinoma (Moderately differentiated) | T4N2M0 No |
| NSCLC-28  | Female 45   | Right lung non small cell lung cancer with brain metastasis | Squamous cell carcinoma (Poorly differentiated) | T4N1M1 Yes |
| NSCLC-29  | Female 59   | Left lung non small cell lung cancer | Magnocellular carcinoma of lung | T4N3M0 No |
| NSCLC-30  | Female 67   | Left lung non small cell lung cancer | Adenocarcinoma (Moderately differentiated) | T3N1M0 Yes |
| NSCLC-31  | Male 53     | Left lung non small cell lung cancer with bilateral lung metastases and multiple bone metastases | Squamous cell carcinoma (Poorly differentiated) | T4N2M1 No |
| NSCLC-32  | Female 72   | Right lung non small cell lung cancer | Adenocarcinoma (Moderately differentiated) | T4N2M0 No |

**Clinical Samples for Co-culture Used in Fig. 4**

| No.     | Gender | Age (y) | Clinical Diagnosis | Pathological Diagnosis | TMN Stage |
|---------|--------|---------|--------------------|-----------------------|-----------|
| HNSCC-64| Male   | 69      | Left mandibular gingival carcinoma | Squamous cell carcinoma (Highly differentiated) | T2N2M0    |
| HNSCC-65| Female | 63      | Left tongue carcinoma | Squamous cell carcinoma (Highly differentiated) | T1N0M0    |
| HNSCC-66| Female | 47      | Left tongue carcinoma | Squamous cell carcinoma (Moderately differentiated) | T1N2M0    |
| Variables | No. of cases | HR (95%CI) | P  |
|-----------|-------------|------------|----|
| HNSCC-67  | Male        | 54         | Bilateral mouth floor carcinoma (Moderately differentiated) | T4N2M0 |
| HNSCC-68  | Female      | 72         | Left tongue carcinoma (Highly differentiated) | T2N0M0 |
| HNSCC-69  | Female      | 51         | Right buccal mucosa carcinoma (Highly differentiated) | T4N2M0 |
| HNSCC-70  | Male        | 47         | Right tongue carcinoma (Moderately differentiated) | T4N0M0 |
| HNSCC-71  | Male        | 44         | Post-surgery recurrent oropharyngeal carcinoma of right tongue carcinoma (Highly differentiated) | T4N0M0 |
| HNSCC-72  | Male        | 69         | Right mandibular gingival carcinoma (Highly differentiated) | T3N0M0 |
| HNSCC-73  | Male        | 55         | Post-surgery ipsilateral lymph node metastasis of left mandibular gingival carcinoma (Moderately differentiated) | T1N1M0 |
| HNSCC-74  | Female      | 60         | Left buccal mucosa carcinoma (Moderately differentiated) | T2N0M0 |
| HNSCC-75  | Female      | 72         | Right tongue carcinoma (Poorly differentiated) | T3N0M0 |
| HNSCC-76  | Male        | 55         | Left maxillary gingival carcinoma (Moderately differentiated) | T2N0M0 |
| HNSCC-77  | Male        | 60         | Left tongue carcinoma (Highly differentiated) | T1N0M0 |
| HNSCC-78  | Male        | 62         | Left mouth floor carcinoma (Moderately differentiated) | T2N1M0 |
| HNSCC-79  | Female      | 50         | Right tongue carcinoma (Moderately differentiated) | T2N0M0 |

Clinical Samples for Co-culture Used in Fig. 4
| Variables   | No. of cases | HR (95%CI)                      | P                                      |
|-------------|--------------|---------------------------------|----------------------------------------|
| HNSCC-80    | Male         | 47                              | Right oropharyngeal carcinoma          |
|             |              |                                 | Squamous cell carcinoma (Moderately   |
|             |              |                                 | differentiated)                        |
| HNSCC-81    | Female       | 78                              | Left tongue carcinoma with lung        |
|             |              |                                 | metastasis                             |
|             |              |                                 | Squamous cell carcinoma (Poorly        |
|             |              |                                 | differentiated)                        |
| HNSCC-82    | Male         | 63                              | Total tongue carcinoma                 |
|             |              |                                 | Squamous cell carcinoma (Moderately    |
|             |              |                                 | differentiated)                        |
| HNSCC-83    | Male         | 55                              | Left tongue carcinoma                  |
|             |              |                                 | Squamous cell carcinoma (Poorly        |
|             |              |                                 | differentiated)                        |
| HNSCC-84    | Male         | 57                              | Right maxillary gingival carcinoma     |
|             |              |                                 | Squamous cell carcinoma (Moderately    |
|             |              |                                 | differentiated)                        |
| HNSCC-85    | Female       | 69                              | Right tongue carcinoma                 |
|             |              |                                 | Squamous cell carcinoma (Highly        |
|             |              |                                 | differentiated)                        |
| HNSCC-86    | Female       | 50                              | Left tongue carcinoma                  |
|             |              |                                 | Squamous cell carcinoma (Moderately    |
|             |              |                                 | differentiated)                        |
| HNSCC-87    | Male         | 57                              | Left mouth floor carcinoma             |
|             |              |                                 | Squamous cell carcinoma (Moderately    |
|             |              |                                 | differentiated)                        |
| HNSCC-88    | Male         | 61                              | Right mandibular gingival carcinoma    |
|             |              |                                 | Squamous cell carcinoma (Moderately    |
|             |              |                                 | differentiated)                        |
| HNSCC-89    | Female       | 80                              | Left tongue carcinoma                  |
|             |              |                                 | Squamous cell carcinoma (Moderately    |
|             |              |                                 | differentiated)                        |
| HNSCC-90    | Male         | 71                              | Left maxillary gingival carcinoma      |
|             |              |                                 | Squamous cell carcinoma (Moderately    |
|             |              |                                 | differentiated)                        |
| HNSCC-91    | Male         | 49                              | Right oropharyngeal carcinoma          |
|             |              |                                 | Squamous cell carcinoma (Poorly        |
|             |              |                                 | differentiated)                        |
| HNSCC-92    | Female       | 75                              | Left buccal mucosa carcinoma           |
|             |              |                                 | Squamous cell carcinoma (Moderately    |
|             |              |                                 | differentiated)                        |
| HNSCC-93    | Female       | 68                              | Left maxillary sinus carcinoma         |
|             |              |                                 | Squamous cell carcinoma (Poorly        |
|             |              |                                 | differentiated)                        |

Clinical Samples for Co-culture Used in Fig. 4
| Variables | No. of cases | HR (95%CI) | P |
|-----------|--------------|------------|---|
| HNSCC-94  | Male 53      | Right tongue carcinoma (Moderately differentiated) | T2N1M0 |
| HNSCC-95  | Female 79    | Squamous cell carcinoma (Highly differentiated) | T1N1M0 |
| HNSCC-96  | Male 61      | Left maxillary gingival carcinoma (Poorly differentiated) | T3N1M0 |
| HNSCC-97  | Male 60      | Squamous cell carcinoma (Moderately differentiated) | T2N0M0 |
| HNSCC-98  | Male 77      | Total tongue carcinoma (Moderately differentiated) | T4N3M0 |
| HNSCC-99  | Female 60    | Right buccal mucosa carcinoma (Highly differentiated) | T2N0M0 |
| HNSCC-100 | Female 76    | Right tongue carcinoma (Moderately differentiated) | T3N0M0 |
| HNSCC-101 | Male 50      | Right mouth floor carcinoma (Moderately differentiated) | T2N2M0 |
| HNSCC-102 | Male 48      | Left buccal mucosa carcinoma (Moderately differentiated) | T2N0M0 |
| HNSCC-103 | Male 71      | Right tongue carcinoma (Highly differentiated) | T1N0M0 |
| HNSCC-104 | Female 70    | Right tongue carcinoma (Highly differentiated) | T1N0M01 |
| HNSCC-105 | Female 55    | Left tongue carcinoma (Moderately differentiated) | T2N1M0 |
| HNSCC-106 | Male 45      | Bilateral mouth floor carcinoma (Poorly differentiated) | T4N1M0 |
| HNSCC-107 | Male 69      | Left buccal mucosa carcinoma (Moderately differentiated) | T3N0M0 |

Clinical Samples for Co-culture Used in Fig. 4
| Variables  | No. of cases | HR (95% CI) | P | 
|------------|--------------|-------------|---|
| HNSCC-108  | Female 75    | Left maxillary gingival carcinoma (Highly differentiated) | T2N0M0 |
| HNSCC-109  | Male 70      | Right tongue carcinoma (Highly differentiated) | T1N0M0 |
| NSCLC-33   | Male 53      | Right lung non small cell lung cancer (Moderately differentiated) | T3N1M0 |
| NSCLC-34   | Female 64    | Right lung non small cell lung cancer (Poorly differentiated) | T4N0M0 |
| NSCLC-35   | Female 40    | Left lung non small cell lung cancer (Poorly differentiated) | T4N2M0 |
| NSCLC-36   | Female 52    | Left lung non small cell lung cancer (Magnocellular carcinoma of lung) | T4N1M0 |
| NSCLC-37   | Male 78      | Right lung non small cell lung cancer with brain metastasis (Poorly differentiated) | T4N2M1 |
| NSCLC-38   | Male 57      | Left lung non small cell lung cancer (Poorly differentiated) | T3N2M0 |
| NSCLC-39   | Female 52    | Right lung non small cell lung cancer (Moderately differentiated) | T4N0M0 |
| NSCLC-40   | Female 60    | Right lung non small cell lung cancer (Moderately differentiated) | T3N1M0 |
| NSCLC-41   | Male 71      | Right lung non small cell lung cancer with brain metastasis (Poorly differentiated) | T4N2M1 |
| NSCLC-42   | Female 70    | Right lung non small cell lung cancer (Moderately differentiated) | T4N0M0 |
| NSCLC-43   | Female 57    | Left lung non small cell lung cancer (Poorly differentiated) | T3N0M0 |
| NSCLC-44   | Female 62    | Left lung non small cell lung cancer (Moderately differentiated) | T4N1M0 |
| NSCLC-45   | Male 45      | Right lung non small cell lung cancer (Moderately differentiated) | T4N2M0 |
| Variables | No. of cases | HR (95%CI) | P                  |
|-----------|-------------|------------|--------------------|
| NSCLC-46  | Male        | 70         | Right lung non small cell lung cancer | Squamous cell carcinoma (Moderately differentiated) | T4N0M0 |
| NSCLC-47  | Female      | 65         | Right lung non small cell lung cancer with multiple bone metastases | Squamous cell carcinoma (Poorly differentiated) | T4N2M1 |
| NSCLC-48  | Female      | 50         | Right lung non small cell lung cancer | Adenocarcinoma (Moderately differentiated) | T3N1M0 |
| NSCLC-49  | Male        | 55         | Left lung non small cell lung cancer | Magnocellular carcinoma of lung | T3N0M0 |
| NSCLC-50  | Female      | 59         | Right lung non small cell lung cancer | Squamous cell carcinoma (Moderately differentiated) | T2N0M0 |
| NSCLC-51  | Female      | 73         | Right lung non small cell lung cancer | Squamous cell carcinoma (Poorly differentiated) | T4N1M0 |
| NSCLC-52  | Male        | 70         | Left lung non small cell lung cancer | Adenocarcinoma (Moderately differentiated) | T3N0M0 |
| NSCLC-53  | Male        | 53         | Left lung non small cell lung cancer | Squamous cell carcinoma (Moderately differentiated) | T4N0M0 |
| NSCLC-54  | Male        | 60         | Right lung non small cell lung cancer | Adenocarcinoma (Moderately differentiated) | T4N1M0 |
| NSCLC-55  | Male        | 62         | Left lung non small cell lung cancer | Adenocarcinoma (Poorly differentiated) | T3N1M0 |
| NSCLC-56  | Male        | 58         | Right lung non small cell lung cancer | Squamous cell carcinoma (Poorly differentiated) | T4N1M0 |
| NSCLC-57  | Female      | 50         | Left lung non small cell lung cancer | Adenocarcinoma (Moderately differentiated) | T3N1M0 |
| NSCLC-58  | Female      | 47         | Left lung non small cell lung cancer | Adenocarcinoma (Poorly differentiated) | T4N0M0 |
| NSCLC-59  | Male        | 57         | Right lung non small cell lung cancer with multiple bone metastases | Squamous cell carcinoma (Moderately differentiated) | T3N1M1 |
| NACLC-60  | Female      | 66         | Right lung non small cell lung cancer | Adenocarcinoma (Moderately differentiated) | T4N2M0 |

**Primers Used in Present Study**

| Gene | Direction | Primer Sequence |
|------|-----------|-----------------|

**Clinical Samples for Co-culture Used in Fig. 4**
| Variables | No. of cases | HR (95%CI) | P |
|-----------|-------------|-------------|---|
| HLA-A     | Forward     | 5'-ACCCTCGTCTGCTACTCTC-3' |   |
|           | Reverse     | 5'-CTGTCTCTCTGCTCCAATACT-3' |   |
| HLA-B     | Forward     | 5'-CAGTTCTGAGGTTGACAG-3' |   |
|           | Reverse     | 5'-CAGCCGTACATGCTCTGGA-3' |   |
| HLA-C     | Forward     | 5'-CCATGAGGTATTTGAGGACG-3' |   |
|           | Reverse     | 5'-TCTCGGAACCTCTCGTGCG-3' |   |
| B2M       | Forward     | 5'-GAGGCTATCCAGCTACTCCA-3' |   |
|           | Reverse     | 5'-GGGCAGGCTACTCATCTTTT-3' |   |
| XBP-1     | Forward     | 5'-CTGAGTCGGCAGGACGTG-3' |   |
|           | Reverse     | 5'-GGCTGGTAGAAGACTGAGGTC-3' |   |
| DRP-1     | Forward     | 5'-CTGCTCTTACATGCTAGTG-3' |   |
|           | Reverse     | 5'-GAGGCTCTCCGGGTGACAATTC-3' |   |
| TPP2      | Forward     | 5'-CCGCTACCCGGAGATGAGT-3' |   |
|           | Reverse     | 5'-GCCCTGAGGATTTTGTCCAGC-3' |   |
| THBS2     | Forward     | 5'-GACACGGCTGGATCTACCTAC-3' |   |
|           | Reverse     | 5'-GAAGCTGTCTATGGAGTCGCA-3' |   |
| TNFRSF21  | Forward     | 5'-ATTGGCACATACGCGGATTT-3' |   |
|           | Reverse     | 5'-GGCTTTGTGTTGTAATGCT-3' |   |
| NLRP1     | Forward     | 5'-GCAGTGCTATGGCCCTGGA-3' |   |
|           | Reverse     | 5'-GAGCTTGTTAGAGAAGGTCAG-3' |   |
| DCN       | Forward     | 5'-ATGAAAGGCCACATCTCTCC-3' |   |
|           | Reverse     | 5'-GTCGCCGTCATACGGAACCT-3' |   |
| IL18RAP   | Forward     | 5'-ATGCTCTTGTGGGGCTGGATA-3' |   |
|           | Reverse     | 5'-GTGAGAGCTGGATTGCTGGC-3' |   |
| 4-1BB     | Forward     | 5'-AGCTGTTACACATAGTAGCCAC-3' |   |
|           | Reverse     | 5'-GGACAGGACTGCAAATCAGT-3' |   |
| FGF2      | Forward     | 5'-AGAAGAGCGACCTCACATCA-3' |   |
|           | Reverse     | 5'-CGGTAGCAGCAACTCCTTGG-3' |   |
| CUL3      | Forward     | 5'-TGAGAAGAGCTGCTACATTTG-3' |   |
|           | Reverse     | 5'-GCCTCTCTGCTCACTCAGT-3' |   |
| CCND1     | Forward     | 5'-GCTGCGAAGTGGAAACCATC-3' |   |
|           | Reverse     | 5'-CTCTCTTCTGCAATTTTTGGA-3' |   |
| MRC2      | Forward     | 5'-CCGAAACCAGGCTATTTCAAC-3' |   |
|           | Reverse     | 5'-CGGTCACTACATAGTCACATGCCC-3' |   |
| CTSF      | Forward     | 5'-AGCCTATGCTACATTTGACACG-3' |   |
|           | Reverse     | 5'-CGCACCAGCTATTGGACAAAGAC-3' |   |

Clinical Samples for Co-culture Used in Fig. 4
| Variables   | No. of cases | HR (95%CI)          | P   |
|------------|-------------|---------------------|-----|
| VTA1       | Forward     | 5’-CTCCCGCACAGTCTCAAGAG-3’ |     |
|            | Reverse     | 5’-AACGACAGTAATAGGACCAC-3’ |     |
| CTSO       | Forward     | 5’-GCCTTCCGGGAAATTCTTTAATAG-3’ |     |
|            | Reverse     | 5’-TCCAGTCAAATCTTAAAGGCAA-3’ |     |
| NEDD4L     | Forward     | 5’-GACATGGAGCATGAGGGAA-3’ |     |
|            | Reverse     | 5’-GTTGCGCTTAAATTTTGCACT-3’ |     |
| TNFRSF6B   | Forward     | 5’-GTACGGAGGTGAGGAAA-3’ |     |
|            | Reverse     | 5’-CAGGGCGAGCTTAGCTCA-3’ |     |
| WNT4       | Forward     | 5’-AGGAGGAGGTGCGAGAAA-3’ |     |
|            | Reverse     | 5’-CGAGTCCATGACTTCCAGGT-3’ |     |
| AKT2       | Forward     | 5’-ACCACAGTCATCGAGGACC-3’ |     |
|            | Reverse     | 5’-GGAGCCACACTGTGATCCA-3’ |     |
| ATG5       | Forward     | 5’-AAAGATGTGCTTGAGATGCTG-3’ |     |
|            | Reverse     | 5’-CACTTGTGCTAGTTCAACGTCA-3’ |     |
| ATG7       | Forward     | 5’-CAGTTGTGCCCTTTTATGATGTC-3’ |     |
|            | Reverse     | 5’-CCAGCCGATATCCTCGAGC-3’ |     |
| CDC23      | Forward     | 5’-CTGTCTGTCCATAAAAGCCG-3’ |     |
|            | Reverse     | 5’-GTAAGGCCGAAATAGCAGC-3’ |     |
| PDCD6IP    | Forward     | 5’-ATGGCCAGATTTGTTGCTG-3’ |     |
|            | Reverse     | 5’-CGCTTGGTAAAACTCGTCTGG-3’ |     |
| MDM2       | Forward     | 5’-GAATCATCGGACTCAGGTATC-3’ |     |
|            | Reverse     | 5’-TCTGTCTCAATTGCTTCCTC-3’ |     |
| WNT3A      | Forward     | 5’-AGCTACCCGATCTGCTGTC-3’ |     |
|            | Reverse     | 5’-CAAACTCAGTGCTTCGACT-3’ |     |
| UBE2R2     | Forward     | 5’-GGACGAGTCCGACCTCTAAA-3’ |     |
|            | Reverse     | 5’-GGTGTTGGAATGGGTAAGTCA-3’ |     |
| PARD6A     | Forward     | 5’-AGCATCGTCAGGAGTGAGA-3’ |     |
|            | Reverse     | 5’-GTATAAGCAGATGTACAGTCC-3’ |     |
| ERAP1      | Forward     | 5’-CCCCTCAAATGGTGCCCTTGC-3’ |     |
|            | Reverse     | 5’-GAGATGCTTCAFCTGCTGAC-3’ |     |
| GAPDH      | Forward     | 5’-CGCTGAGTCAGTCGGAGTC-3’ |     |
|            | Reverse     | 5’-GCTGATGATCTTGGAGCTGTC-3’ |     |
| ChIP-qPCR  | Forward     | 5’-TTGGAGATCGGACCATCCA-3’ |     |
| (TPP2-1)   | Reverse     | 5’-CTGGAGGCGGAGCAGAC-3’ |     |
| ChIP-qPCR  | Forward     | 5’-ATGTCCTCAGACTCTCCC-3’ |     |
| (TPP2-2)   | Reverse     | 5’-CAGGATGGAGGAGGAGAAG-3’ |     |

Clinical Samples for Co-culture Used in Fig. 4
| Variables | No. of cases | HR (95%CI)       | P     |
|-----------|-------------|------------------|-------|
| ChIP-qPCR (TPP2 distal) | Forward 5'-AAAGTACATCTCCCATCTCTGG-3' |       |       |
|           | Reverse 5'-CCCAGAAGCTATACGAAGTTGAA-3' |       |       |
| pGL4-TPP2 | Forward 5'- AACGGTACCACACTCCAGCTGGGCCACA-3' |       |       |
|           | Reverse 5'- ATCTCGAGTACTCCGGGTTAGCGGCAGA-3' |       |       |

Clinical Samples for Co-culture Used in Fig. 4

**Declarations**

**Conflict of Interest Disclosure Statement**

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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Figures

Low pSer616 DRP-1 correlates with high MHC-I and indicates better prognosis in cancer patients. A. Representative immunofluorescence images for MHC-I and pSer616 DRP-1 of HNSCC, NSCLC and melanoma patients with different postoperative survival time (MHC-I: green, pSer616 DRP-1: red). DAPI, nuclear staining. Scale bars, 10 μm. B, C. Representative immunochemistry images and scores for MHC-I and pSer616 DRP-1 of HNSCC patients with different postoperative survival time. Magnification, 200× and 400×. D. Spearman order correlation analysis of MHC-I and pSer616 DRP-1 association in HNSCC, NSCLC and melanoma based on IHC score. E. Kaplan-Meier survival curves for HNSCC, NSCLC and melanoma patients are plotted for pSer616 DRP-1 and MHC-I expression. Survival differences were analyzed using log-rank test.
Mdivi-1 mediates MHC-I expression in syngeneic tumor models. A. Tumor volume measurements after subcutaneous implantation, arrow indicates the time of Mdivi-1 or DMSO treatment (mean ± s.e.m; n = 6, **, p < 0.001). B. Weights of harvested tumors (n = 6, **, p < 0.001). C. Photograph of harvested tumors. D. Membrane expression of H-2Kb and SIINFEKL-H-2Kb complex of isolated primary cancer cells from harvested tumors by flow cytometry (mean ± s.e.m; n = 5; **, p<0.001). E. IFN-γ-producing CTLs as quantified by ELISpot (mean ± s.e.m; n = 6; **, p<0.001). F. The isolated tumor-infiltrated CD8+ T cells were stained with anti-OVA-H-2Kb tetramer and anti-CD8a, and the percentages of infiltrated OVA-specific CTLs were quantified by flow cytometry (mean ± s.e.m; n = 3; *, p<0.01). G. Biodistribution of cancer cells 12 days and 25 days after inoculation (n = 6). Color scales represent photon intensities. H. Kaplan-Meier survival curve was plotted for tail vein tumor inoculation model, and survival difference was analyzed using log-rank test (n = 6).
Mdivi-1 improves the adoptive T cell therapy (ACT) in PDX tumor models by upregulating MHC-I. A. Scheme of ACT therapy through tumor-specific CTLs transferred into NOD/SCID mice transplanted with autologous HNSCC or NSCLC PDXs. B. Tumor volume measurements were monitored weekly after ACT for five consecutive weeks (mean ± s.e.m; n = 3 per PDX group; **, p<0.001). C. Weights of harvested HNSCC or NSCLC PDXs (mean ± s.e.m; n = 3 per PDX group; *, p<0.01, **, p<0.001). D. Apoptosis of cancer cells as determined by EpCAM and TUNEL immunostaining (mean ± s.e.m; n = 9, 3 sections per PDX; **, p<0.001). E. Flow cytometric analysis of MHC-I membrane expression in isolated HNSCC and NSCLC primary cancer cells. F indicates the fold change of MFI (mean fluorescence intensity) normalized to DMSO group (mean ± s.e.m; n = 3 per PDX group; **, p<0.001 compared with ACT accompanied by DMSO treatment or DMSO alone). F. Representative immunofluorescence images for EpCAM and CD8 of harvested PDXs (EpCAM: red; CD8: green). DAPI, nuclear staining. Scale bars, 25 μm. G. Number of IFN-γ-producing CTLs as quantified by ELISpot (mean ± s.e.m; n = 3 per PDX group; *, p<0.01, **, p<0.001). H-I. Evaluation of intracellular markers in association with cytotoxic function by flow cytometry (mean ± s.e.m; n = 3 per PDX group; **, p<0.001 compared with ACT accompanied by DMSO treatment).
Figure 4

Mdivi-1 improves the cytotoxic function of CTLs targeting autologous cancer cells in vitro. A. Scheme of tumor-specific CTLs generation and in vitro co-culture. B. 24 hours later, Th1 cytokines production was quantified by specific ELISAs (mean ± s.e.m; n = 5; **, p<0.001 compared with non-coculture group). C. Residual E/T ratio was evaluated by flow cytometry after 3 days based on CD8 and EpCAM expression (mean ± s.e.m; n = 3; *, p<0.01, **, p<0.001 compared with E/T ration=1:1 group). D. Cell viability of primary cancer cells and PI uptake of tumor-specific CTLs after sequential treatment by MTS and flow cytometry. E. Evaluation of MHC-I membrane expression in HNSCC and NSCLC primary cancer cells by flow cytometry. F indicates the fold change of MFI normalized to mock (mean ± s.e.m; n = 7; #, p<0.05, **, p<0.001 compared with mock). F. 12 hours after co-culture, EpCAM+ cancer cells were harvested and cell death was examined with flow cytometry based on the uptake of PI and Annexin V. E/T ratio was 5:1 (mean ± s.e.m; n = 4; **, p<0.001 compared with mock). G. Th1 cytokines production was quantified by specific ELISAs after 24 hours. E/T ratio was 5:1 (mean ± s.e.m; n = 5; **, p<0.001 compared with mock). H. Evaluation of residual E/T ratio by flow cytometry based on CD8 and EpCAM expression respectively following 3 days. E/T ratio was 5:1 (mean ± s.e.m; n = 3; *, p<0.01, **, p<0.001 compared with mock). E indicates effector cells, namely T cells. T indicates targeted cells, namely cancer cells.
Figure 5

Inhibition of mitochondrial fission alleviates oxidative stress and UPR in cancer cells. A. Mitochondrial morphology of TSCCs was visualized by fluorescent dye MitoTracker Red CMXRos (Mito: red). B. Oxidation of cardiolipin in TSCC was assessed by fluorescent dye NAO (NAO: green). DAPI, nuclear staining. Scale bars, 2 μm. C. Assessment of intracellular ROS by flow cytometry in TSCCs (mean ± s.e.m; n = 9; #, p<0.05, *, p<0.01). D. Immunoblotting for associated ER stress markers in TSCCs. MW indicates molecular weight. GAPDH, loading control. E. Flow cytometric analysis of MHC-I membrane expression after depicted treatments in TSCCs (mean ± s.e.m; n = 4; *, p<0.01, **, p<0.001, DN IRE1α compared with vector 1 and IRE1α+XBP-1s, XBP-1s compared with vector 2 and IRE1α+XBP-1s). F. Flow cytometric analysis of MHC-I membrane expression after depicted treatments in TSCCs (mean ± s.e.m; n = 5; *, p<0.01, **, p<0.001, siDRP-1 compared with NC and siDRP-1+XBP-1s, XBP-1s compared with vector and siDRP-1+XBP-1s). M indicates mean fluorescence intensity. For experiments that were conducted at different time points, the isotype tubes were adjusted to ensure that their fluorescence intensity was at similar levels. NC indicates negative control.
Bioinformatic analysis based on mRNA profile indicated TPP2 as the potential target of XBP-1s. A. Scatter plot of relative mRNA expression in CAL-27 treated with shXBP-1s compared with negative control. Genes in green and red are differentially expressed at significant levels (n = 3 per group). B. Volcano plot of differentially expressed genes on the basis of fold change and p value. Genes to be focused on were labeled. C. GSEA (KEGG, REACTOME and BIOCARTA) pathway distribution for shXBP-1s versus negative control of CAL-27. Horizontal line denotes FDR significance cutoff of 0.05. Immune-, metabolism-, post-translation- and cancer-related gene sets were demarcated by dots in indicated colors respectively. D. Gene sets upregulated in shXBP-1s CAL-27 compared to negative control (FDR<0.05 and NES>1.5). Color gradation is based on GSEA NES. Gene sets demarcated in Panel C were specified. E. Gene sets downregulated in shXBP-1s compared to negative control of CAL-27 (FDR<0.05 and NES<-1.5). Color gradation is based on GSEA NES. Gene sets demarcated in Panel C were specified. Gene sets containing TPP2 were highlighted. NES indicates normalized enrichment score. F. Downregulated genes from the two gene sets. Color gradation is representative of log2 fold change over negative control. Relevant genes were labeled and TPP2 was labeled in red. shCtrl indicates negative control. G. Diagrammatic drawing of associated genes divided into four groups based on respective functions that are recognized in cancer biology. H. Distribution of XBP-1 occupancy frequencies in TPP2 promoter in three different cancer cell lines based on ChIP-seq database respectively. The most enriched peaks are highlighted. I. Motif analysis (motif-counter) showed the enriched XBP-1 motif in TPP2 promoter, the arrow indicates that the highest score binding sites consistently located in the forward strand, which was highlighted in panel H. J. ChIP-qPCR analysis of the XBP-1 genomic occupancy in the TPP2 promoter in SCC-9 and CAL-27 as indicated. Immunoprecipitated DNA was measured by qRT-PCR with primers to amplify the TPP2 promoter region, including the distal site (mean ± s.e.m; n = 4; **, p<0.001 by one-way ANOVA followed by Dunnett’s tests for multiple
comparisons). K. Luciferase assay demonstrated that knockdown of XBP-1 inhibited TPP2 promoter's activity in SCC-9 and CAL-27. SCC-9 and CAL-27 cells with stable expression of wild type (wt) or mutant (mut) TPP2 promoter delivered pGL4.20-Basic vectors were co-transfected with shXBP-1 or shCtrl (mean ± s.e.m; n = 5; **, p<0.001 by one-way ANOVA followed by Dunnett's tests for multiple comparisons). L. Luciferase reporter assay demonstrated that XBP-1 activated TPP2 promoter (mean ± s.e.m; n = 3; **, p<0.001 by one-way ANOVA followed by Dunnett's tests for multiple comparisons). M. qRT-PCR verified the downregulation of TPP2 from microarrays in TSCCs after XBP-1s knockdown (mean ± s.e.m; n = 4; **, p<0.001). N. Immunoblotting further verified the downregulation of TPP2 in TSCCs after XBP-1s knockdown. MW indicates molecular weight. β-actin, loading control. O. Overall survival of cancer patients with different levels of TPP2 using The Cancer Genome Atlas (TCGA) database. HNSC: Head and neck squamous cell carcinoma. LUSC: Lung squamous cell carcinoma. SARC: sarcoma.

**Figure 7**

Bioinformatic analysis based on mRNA profile indicated TPP2 as the potential target of XBP-1s. A. Scatter plot of relative mRNA expression in CAL-27 treated with shXBP-1s compared with negative control. Genes in green and red are differentially expressed at significant levels (n = 3 per group). B. Volcano plot of differentially expressed genes on the basis of fold change and p value. Genes to be focused on were labeled. C. GSEA (KEGG, REACTOME and BIOCARTA) pathway distribution for shXBP-1s versus negative control of CAL-27. Horizontal line denotes FDR significance cutoff of 0.05. Immune-, metabolism-, post-translation- and cancer-related gene sets were demarcated by dots in indicated colors respectively. D. Gene sets upregulated in shXBP-1s CAL-27 compared to negative control (FDR<0.05 and NES>1.5). Color gradation is based on GSEA NES. Gene sets demarcated in Panel C were specified. E. Gene sets downregulated in shXBP-1s compared to negative control of CAL-27 (FDR<0.05 and NES<-1.5). Color gradation is based on GSEA NES. Gene sets
demarcated in Panel C were specified. Gene sets containing TPP2 were highlighted. NES indicates normalized enrichment score. F. Downregulated genes from the two gene sets. Color gradation is representative of log2 fold change over negative control. Relevant genes were labeled and TPP2 was labeled in red. shCtrl indicates negative control. G. Diagrammatic drawing of associated genes divided into four groups based on respective functions that are recognized in cancer biology. H. Distribution of XBP-1 occupancy frequencies in TPP2 promoter in three different cancer cell lines based on ChIP-seq database respectively. The most enriched peaks are highlighted. I. Motif analysis (motif-counter) showed the enriched XBP-1 motif in TPP2 promoter, the arrow indicates that the highest score binding sites consistently located in the forward strand, which was highlighted in panel H. J. ChIP-qPCR analysis of the XBP-1 genomic occupancy in the TPP2 promoter in SCC-9 and CAL-27 as indicated. Immunoprecipitated DNA was measured by qRT-PCR with primers to amplify the TPP2 promoter region, including the distal site (mean ± s.e.m; n = 4; **, p<0.001 by one-way ANOVA followed by Dunnett’s tests for multiple comparisons). K. Luciferase assay demonstrated that knockdown of XBP-1 inhibited TPP2 promoter’s activity in SCC-9 and CAL-27. SCC-9 and CAL-27 cells with stable expression of wild type (wt) or mutant (mut) TPP2 promoter delivered pGL4.20-Basic vectors were co-transfected with shXBP-1 or shCtrl (mean ± s.e.m; n = 5; **, p<0.001 by one-way ANOVA followed by Dunnett’s tests for multiple comparisons). L. Luciferase reporter assay demonstrated that XBP-1 activated TPP2 promoter (mean ± s.e.m; n = 3; **, p<0.001 by one-way ANOVA followed by Dunnett’s tests for multiple comparisons). M. qRT-PCR verified the downregulation of TPP2 from microarrays in TSCCs after XBP-1s knockdown (mean ± s.e.m; n = 4; **, p<0.001). N. Immunoblotting further verified the downregulation of TPP2 in TSCCs after XBP-1s knockdown. MW indicates molecular weight. β-actin, loading control. O. Overall survival of cancer patients with different levels of TPP2 using The Cancer Genome Atlas (TCGA) database. HNSC: Head and neck squamous cell carcinoma. LUSC: Lung squamous cell carcinoma. SARC: sarcoma.

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