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Original Research

Gain-of-function hot spot mutant p53^{R248Q} regulation of integrin/FAK/ERK signaling in esophageal squamous cell carcinoma

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ABSTRACT

Purpose: *TP53,* encoding the protein p53, is among the most frequently mutated genes in all cancers. A high frequency of 60 – 90% mutations is seen in esophageal squamous cell carcinoma (ESCC) patients. Certain p53 mutants show gain-of-function (GoF) oncogenic features unrelated to its wild type functions.

Methods: This study functionally characterized a panel of p53 mutants in individual ESCC cell lines and assayed for GoF oncogenic properties.

Results: The ESCC cell line with endogenous p53^{R248Q} expression showed suppressed tumor growth in an immunocompromised mouse model and suppressed colony growth in *in vitro* three-dimensional culture, when depleted of the endogenous p53 protein expression. This suppression is accompanied by suppressed cell cycle progression, along with reduced integrin expression and decreased focal adhesion kinase and extracellular-regulated protein kinase signaling and can be compensated by expression of a constitutively active mitogen-activated protein. P53^{R248Q} enhances cell proliferation upon glutamine deprivation, as compared to other non-GoF mutants.

Conclusions: In summary, study of the functional contributions of endogenous p53 mutants identified a novel GoF mechanism through which a specific p53 mutant exerts oncogenic features and contributes to ESCC tumorigenesis.

Introduction

TP53 is one of the most frequently mutated genes reported in all human cancers and encodes the master tumor suppressor protein p53. Loss of wild type (WT) p53 function plays a central role in tumor pathogenesis, affecting crucial processes including cell survival, cell cycle regulation and proliferation, and cell metabolism. *TP53* mutations leading to loss of WT p53 tumor suppressive functions (including stopgain, splicing and frameshift indels) have been characterized and well-studied in cancer biology. Recently, evidence of gain-of-function (GoF) p53 mutations that exert WT function-independent oncogenic effects in cancer biology is emerging [1]. The GoF p53 mutant is increasingly recognized as an important driver of tumor pathogenesis in various cancer types. The acquisition of key GoF p53 mutants is associated with early age onset [2], increased cell growth [3], and enhanced cell invasion and metastasis [4]. Protein expression of such p53 mutants provides tumor cells with a selective growth advantage.

Esophageal squamous cell carcinoma (ESCC), the dominant histologic type of esophageal cancer in developing countries, has one of highest frequencies of *TP53* mutations among all cancer types, reaching 60–90% in ESCC patients [5, 6]. However, little is known about the functional influences of p53 mutants in established ESCC tumors. No functional study has been performed to analyze the functional effects of endogenous p53 mutants in ESCC. Despite the deadly nature of ESCC (5-year survival rate < 5%) [7], few oncogenic drivers and targets for molecular therapies have been identified and examined.

Previously, we showed by whole-exome sequencing of ESCC tissue samples and survival analysis that missense *TP53* mutations are correlated with poorer ESCC patient survival, as compared to loss of function *TP53* mutations [8]. In the current study, we analyzed the functional influences of depletions of endogenous missense p53 mutants in a panel of ESCC cell lines using *in vivo* and *in vitro* models. The hot spot mutant p53^{R248Q} possesses GoF properties by regulating extracellular matrix (ECM)-cell interaction. Functional and mechanistic studies show that p53^{R248Q} regulates integrin/focal adhesion kinase (FAK)/extracellular signal-regulated kinase (ERK) signaling. Overall, these findings highlight a specific and novel oncogenic role of p53^{R248Q} in cancer cells.

Materials and methods

Chemical reagents

All inhibitors used in this study were purchased from Selleckchem (Houston, TX).

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Cell lines

ESCC cell lines including KYSE150 (CVCL_1348), KYSE180 (CVCL_1349), KYSE450 (CVCL_1353), and SLMT (CVCL_E305) were cultured as described [9]. Cell line authentication by short tandem repeat DNA profiling and mycoplasma test by PCR amplification of mycoplasma DNA were performed for all cell lines used.

Plasmids and lentivirus preparation and infection

Oligonucleotides encoding p53- (CTTGTACTCGTCGGTGATCA and GAGCGCTGCTCAGATAGCGA) and p63-targeted sgRNAs (GCTGAGCCGTGAATTCAACG and TGTGTGTTCTGACGAAACGC) were designed using sgRNA Design Tool (https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design) and cloned into lentiCRISPRv2 vector (Addgene plasmid # 52,961; http://n2t.net/addgene:52961; RRID:Addgene_52,961). Non-targeting sgRNA (sequence: GTTCCGCGTTACATAACTTA) was used as a negative control [10]. A plasmid encoding the constitutively active MEK1 mutant (Addgene plasmid # 64,604) was used to express MEK1 in $p53^{R248Q}$-depleted cells. A plasmid encoding Renilla luciferase (Addgene plasmid #74,444) was used to label cells for three-dimensional (3D) live-cell quantification. Lentivirus preparation and infection were performed as described [9].

In vivo *tumorigenicity assay*

Subcutaneous injection of cancer cells in nude mice was performed as described [9]. Tumors were inoculated in both flanks of a mouse. Six mice were used per group. All experimental procedures were approved by the Committee on the Use of Live Animals in Teaching and Research (CULATR; approval reference number 3429-14) and performed in the Laboratory Animal Unit of the University of Hong Kong.

Subcellular fractionation

Subcellular fractionation was performed using the Subcellular Protein Fractionation Kit for Cultured Cells (Thermo Fisher Scientific, Waltham, MA) according to manufacturer's protocol.

Two-dimensional (2D) cell proliferation assay

The proliferation and viability of cells were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as we previously described [11].

Spheroid formation assay

Cells were seeded on ultra-low attachment plates (Thermo Fisher Scientific) and quantified by MTT assay.

3D matrigel-embedding colony formation assay

Luciferase-labeled cells were embedded in Matrigel (Corning Inc., Corning, NY) and quantified by bioluminescence-based live-cell imaging using PE IVIS Spectrum *in vivo* imaging system (PerkinElmer, Inc., Waltham, MA). Enduren (Promega Corporation, Madison, WI) was used as the substrate for Renilla luciferase.

Bromodeoxyuridine (BrdU) incorporation and cell cycle analysis

Matrigel-embedded colonies were labeled by BrdU before dissociation into single cells and fixation by ethanol. Cells were then incubated with APC-conjugated anti-BrdU antibody (#17-5071-42, Thermo Fisher Scientific) and DyeCycle green DNA dye (Thermo Fisher Scientific) before subjecting to flow cytometry analysis as described [9] using NovoCyte Quanteon flow cytometer (ACEA Biosciences, San Diego, CA).

Western blotting analysis

Western blotting analysis was performed as previously described [9]. P84 protein expression was used as a loading control. Antibodies targeting p53 (#2527), integrin α_V (#4711), integrin β_3 (#13166), phospho-FAK (#8556), total-FAK (#3285), phospho-ERK (#4370), total-ERK (#4695), and total-MEK (#4694) were purchased from Cell Signaling Technology (Danvers, MA). Antibody targeting p84 (#GTX70220) was purchased from GeneTex (Irvine, CA).

Statistical analysis

The error bars shown in the figures represent the 95% confidence interval. Independent sample *t*-test was applied. All tests of significance were 2-sided. A *p*-value less than 0.05 is considered statistically significant. For multiple-test comparisons, the *p*-value was adjusted as described [9]. An adjusted *p*-value less than 0.05 is considered significant.

Results

$p53^{R248Q}$ exerts a GoF role in vivo tumor growth

To functionally characterize various p53 mutants in ESCC development, we utilized CRISPR-mediated protein functional knockout (fKO) [9] to deplete p53 protein expression in a panel of four ESCC cell lines endogenously expressing different p53 mutants (Fig. 1a) and subjected them to *in vivo* subcutaneous nude mouse tumorigenesis assay. We confirmed by Sanger sequencing that all the cell lines tested routinely express a homogenous population of mutated *TP53* mRNA; no wildtype *TP53* mRNA expression was detected (Supplementary Fig. 1). The $p53^{R248Q}$ mutant in the KYSE150 cell line, one of the hot spot p53 mutants, is the only p53 mutant tested that exerts a GoF oncogenic role and its depletion significantly suppresses tumor growth as compared to other ESCC cell lines harboring different p53 mutations (Fig. 1b). Depletion of p53 protein expression in KYSE180 harboring $p53^{I195T}$, KYSE450 $p53^{H179R}$, and SLMT $p53^{R273L}$ results in no difference in tumor growth as compared to control groups, suggesting that these p53 mutants do not possess oncogenic GoF.

Subcellular localization and stability of $p53^{R248Q}$

Given the specific GoF role of $p53^{R248Q}$, its distinct molecular features were compared to other p53 mutants showing non-GoF roles. Subcellular localization plays a critical role in mediating functions of WT and mutant p53 proteins [12]. Cell fractionation was used to examine subcellular localization of the p53 mutants in ESCC cell lines. All non-GoF mutants localize to the cytoplasm and nucleus, consistent with previous reports (Fig. 2a). Interestingly, $p53^{R248Q}$ exhibits concentrated subcellular localization to the membrane fraction as compared to non-GoF p53 mutants (Fig. 2a). Protein stability also plays an essential role in p53 biology. We determined the protein stability of the p53 mutants by treatment with inhibitors of known mutant p53 stabilizers, heat shock protein 90 (HSP90) [13] and histone deacetylase (HDAC) [14]. The $p53^{R248Q}$ is resistant to HSP90 or HDAC inhibition-induced protein degradation as compared to other mutants (Fig. 2b). These data suggest $p53^{R248Q}$ possesses unique features of subcellular localization profile and enhanced protein stability, which may contribute to its GoF roles.

Depletion of $p53^{R248Q}$ suppresses in vitro 3D colony formation in an ECM-dependent manner

To dissect the GoF oncogenic features of $p53^{R248Q}$, the proliferation characteristics of $p53^{R248Q}$-fKO cells in vitro were examined. Surprisingly, when cultured under the routine 2D cell culture model, there

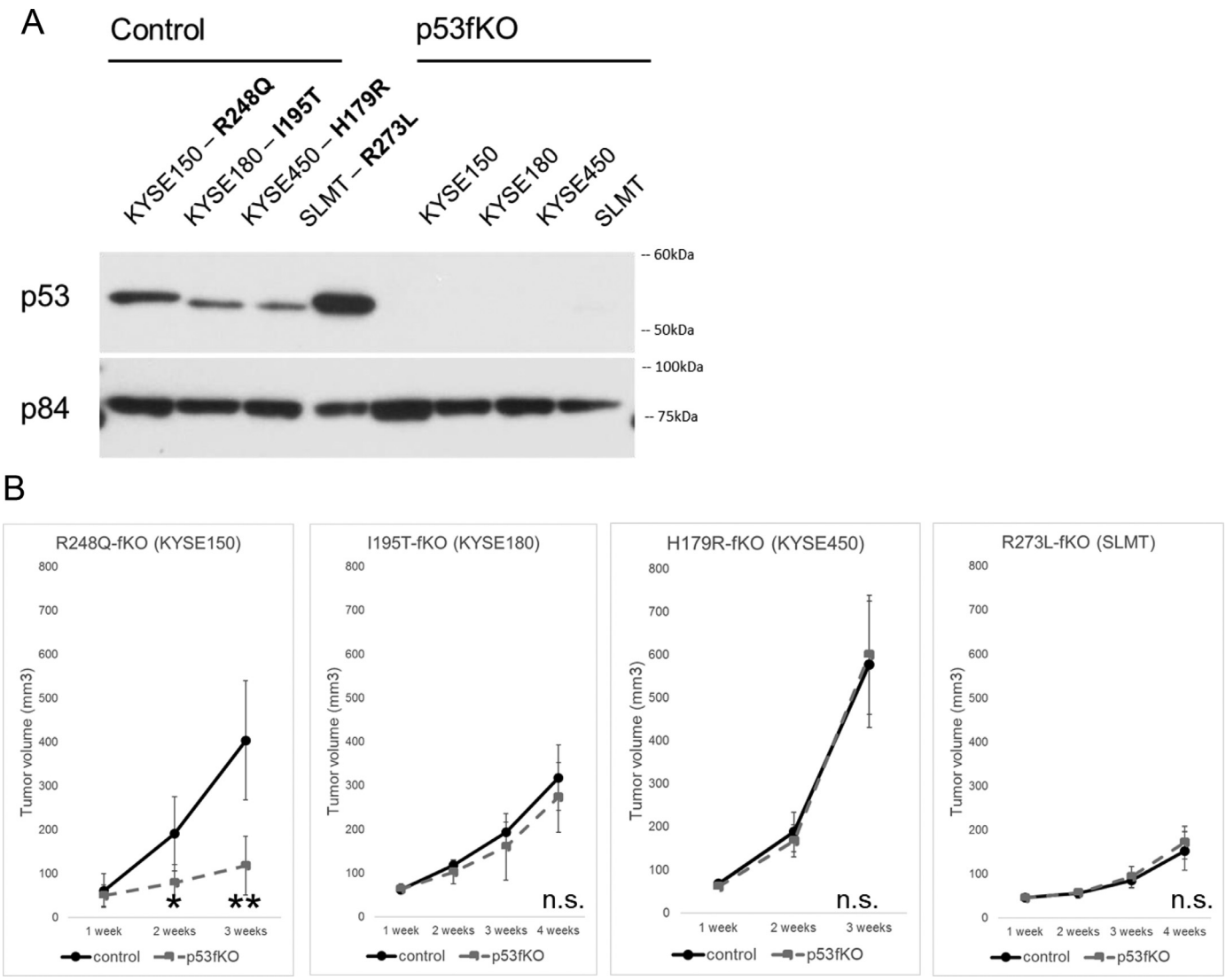

Fig. 1. *In vivo* functional analysis of p53 mutants. a) Western blotting analysis shows endogenous protein expression of p53 mutants (Control) and CRISPR-mediated p53 protein depletion (p53fKO) in a panel of ESCC cell lines. b) Subcutaneous tumorigenicity assay in nude mice shows $p53^{R248Q}$ depletion suppressed tumor growth in KYSE150 as compared to control. *: *p*-value<0.05; **: *p*-value<0.01; n.s.: *p*-value>0.05.

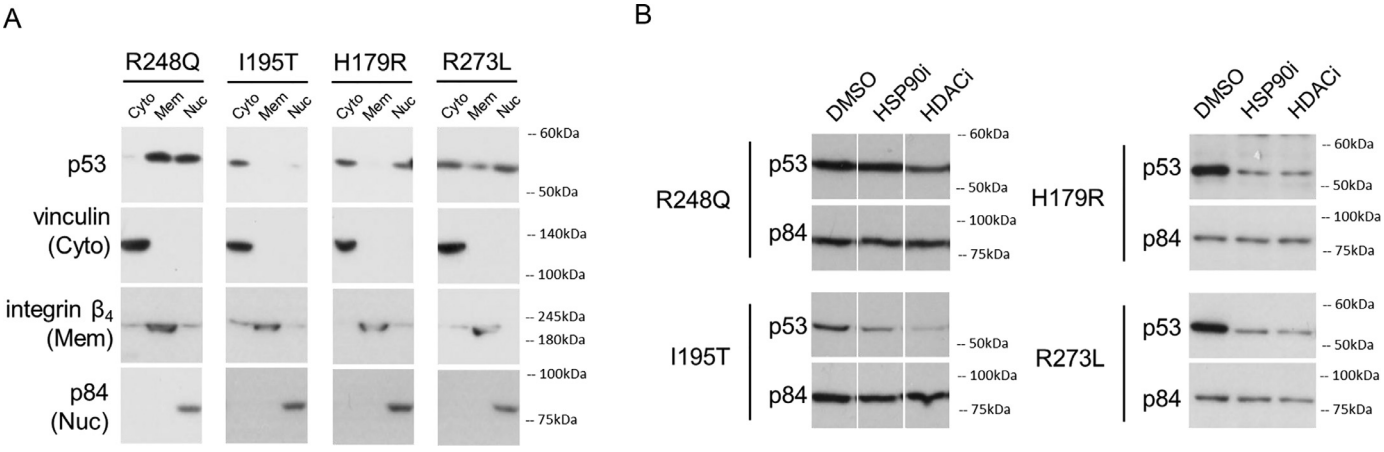

Fig. 2. Potential GoF p53 mutant-specific characteristics. a) Subcellular localization assay shows concentrated localization of $p53^{R248Q}$ in the membrane (Mem) fraction, as compared to other mutants. Cyto: cytoplasmic fraction; Nuc: nuclear fraction. b) Inhibitor assay shows differential response to HSP90 inhibition (HSP90i) and HDAC inhibition (HDACi) of cell lines bearing different p53 mutants. Cells were treated with 5 μM 17-DMAG for HSP90i and 5 μM suberoylanilide hydroxamic acid for HDACi for a period of 24 h before lysate collection.

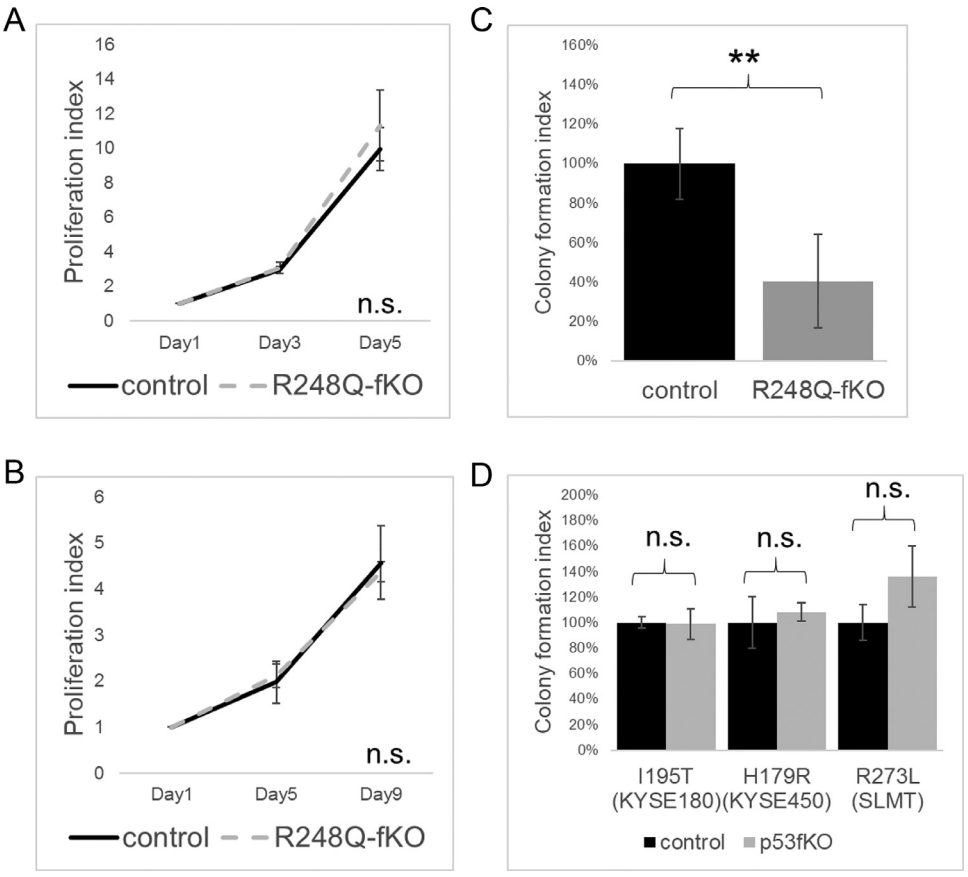

Fig. 3. *In vitro* functional analysis of p53 mutants. a-c) Depletion of p53 in $p53^{R248Q}$-bearing cells (R248Q-fKO) does not suppress proliferation in 2D (a) and 3D ECM-free cultures (b) but suppress colony formation in Matrigel-embedded cultsures (c), as compared to control cells. d) Depletions of non-GoF p53 mutants in other cell lines result in no significant changes of colony formation. Colony formation index in (C) and (D) was calculated on Day 9; n.s.: *p*-value>0.05; **: *p*-value<0.01.

was no difference of cell proliferation between $p53^{R248Q}$-fKO cells compared to control cells (Fig. 3a). ECM-free spheroid culture on ultralow attachment culture surface also failed to reveal functional differences of $p53^{R248Q}$-fKO cells compared to control cells (Fig. 3b). To better recapitulate and analyze the GoF roles of $p53^{R248Q}$ in vitro, an ECM-rich Matrigel-embedding 3D culture in which ESCC cell lines with modified p53 protein expression are embedded in a thick layer of Matrigel followed by live-cell monitoring of colony formation, was used. Significant colony formation was suppressed with $p53^{R248Q}$ depletion, concordant with the *in vivo* tumor suppression data (Fig. 3c). Consistently, depletion of $p53^{I195T}$, $p53^{H179R}$, or $p53^{R273L}$ results in no colony formation changes (Fig. 3d). These results suggest ECM-rich culture serves as a reliable in vitro model in ESCC and that interaction with ECM is essential for $p53^{R248Q}$-mediated oncogenic function.

Depletion of $p53^{R248Q}$ suppresses G2/M transition

The cellular mechanisms of suppressed colony formation by $p53^{R248Q}$ depletion were examined. Increased regulated cell death may contribute to suppressed colony formation. However, a variety of specific cell death inhibitors targeting different regulated cell death pathways does not diminish the differences of colony formation between $p53^{R248Q}$-fKO and control cells (Fig. 4a). Investigation of the cell cycle progression profile of $p53^{R248Q}$-fKO cells showed the proportion of cells in G2/M transition is significantly increased in $p53^{R248Q}$-fKO cells, as compared to control cells (Fig. 4b). These data suggest that cell cycle progression, rather than cell viability, contributes to the suppressed colony formation by $p53^{R248Q}$ depletion.

$p53^{R248Q}$ regulates integrin/FAK/ERK signaling

Oncogenic GoF of $p53^{R248Q}$ is ECM-dependent. Integrins are transmembrane receptors that mediate cell-ECM interaction. The protein expressions of the known oncogenic α_V/β_3 integrins are down-regulated in $p53^{R248Q}$-fKO cells as compared to control cells (Fig. 4c). Along with down-regulated integrins, phosphorylation of FAK, the key mediator of intracellular signaling by integrins, and phosphorylation of ERK, a key effector downstream of FAK, and a crucial regulator of G2/M transition, are also suppressed.

ERK activation is critical for gain-of-function of $p53^{R248Q}$

To functionally validate the critical role of ERK signaling, a constitutively active construct of mitogen-activated protein kinase (MEK1), the upstream kinase of ERK phosphorylation, in $p53^{R248Q}$-fKO cells was used to reactivate suppressed ERK signaling (Fig. 5a). Introduction of constitutively active MEK1 in $p53^{R248Q}$-fKO cells completely compensates for the suppressed tumor growth (Fig. 5b) and 3D colony formation (Fig. 5c), as compared to control cells, indicating ERK signaling is the main effector downstream of $p53^{R248Q}$ for its oncogenic GoF.

ERK regulation by $p53^{R248Q}$ enhances cell proliferation upon glutamine (Gln) deprivation

Gln metabolism has been shown to play a critical role in the survival and proliferation of cancer cells and may act as a new therapeutic target [15]. Interestingly, a recent study highlighted Gln addiction as a molecular basis for ESCC therapy [16]. Furthermore, another recent study showed that Gln deprivation leads to activation of ERK signaling in cancer cells [17]. We examined the functional influence of ERK regulation by $p53^{R248Q}$ upon Gln deprivation in ESCC. Gln deprivation significantly reduces cell proliferation in cells harboring non-GoF p53 mutants, but not in cells with $p53^{R248Q}$ (Fig. 6a). Consistent with the proliferation results, phosphorylation of ERK increases only in $p53^{R248Q}$ harboring cells upon Gln deprivation (Fig. 6b). Depletion of $p53^{R248Q}$ renders cells vul-

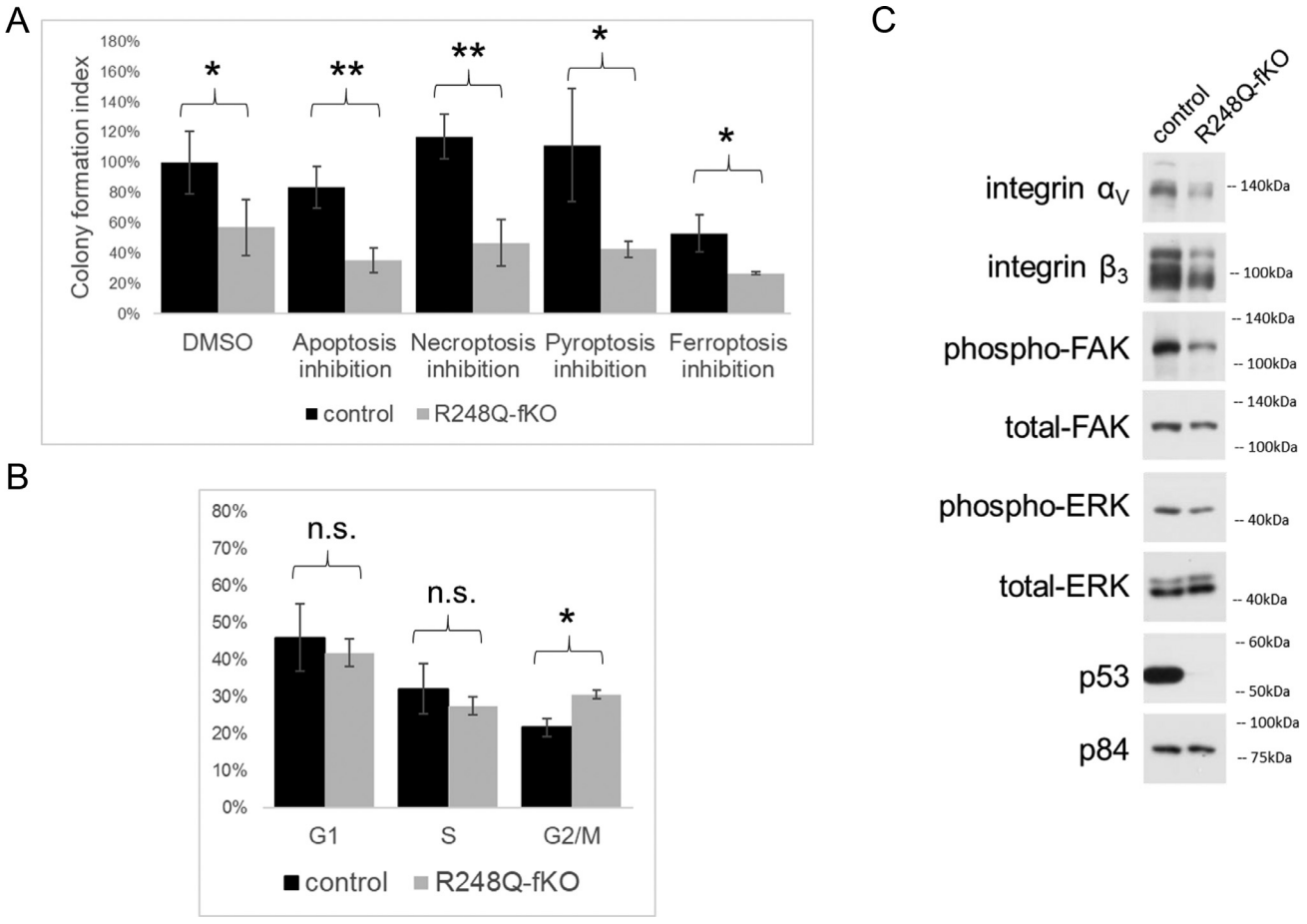

Fig. 4. Mechanistic analysis of $p53^{R248Q}$. a) Multiple regulated cell death inhibition treatments do not diminish the suppressed colony formation in $p53^{R248Q}$-fKO cells. Specific inhibitors for apoptosis (10 μM Z-VAD-FMK), necroptosis (50 μM Necrostatin-1 s), pyroptosis (5 μM Z-YVAD-FMK), and ferroptosis (10 μM Ferrostatin) were added on Day 1. Colony formation index was calculated on Day 9. b) Flow cytometry-based cell cycle analysis shows depletion of $p53^{R248Q}$ results in increased G2/M population as compared to control cells. c) Western blotting analysis shows deregulated integrin-FAK-ERK signaling in $p53^{R248Q}$-depleted cells. *: p-value<0.05; **: p-value<0.01; n.s.: p-value>0.05.

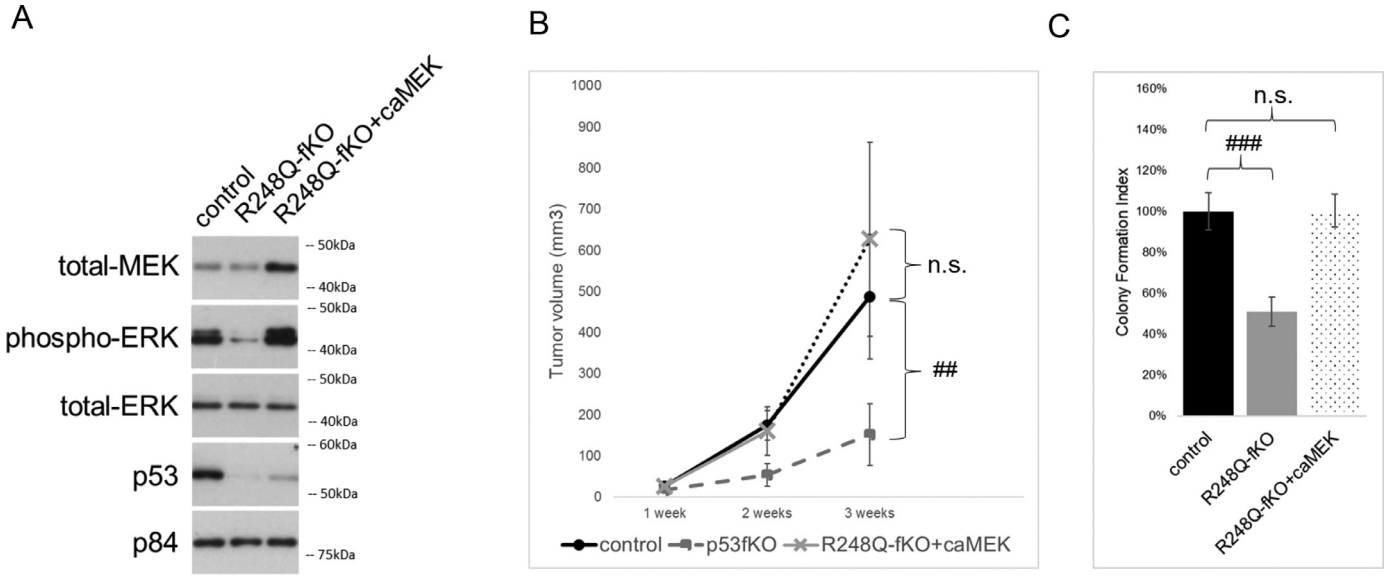

Fig. 5. Crucial contribution of ERK regulation in $p53^{R248Q}$ GoF. a) Introduction of constitutively active MEK construct (caMEK) restores the suppressed ERK phosphorylation in $p53^{R248Q}$-depleted cells. b) Introduction of caMEK restores suppressed *in vivo* tumor growth by $p53^{R248Q}$ depletion. c) Introduction of caMEK restores suppressed colony formation potential in $p53^{R248Q}$-depleted cells. n.s.: p-value>0.05; ##: adjusted p-value<0.01; ###: adjusted p-value<0.001.

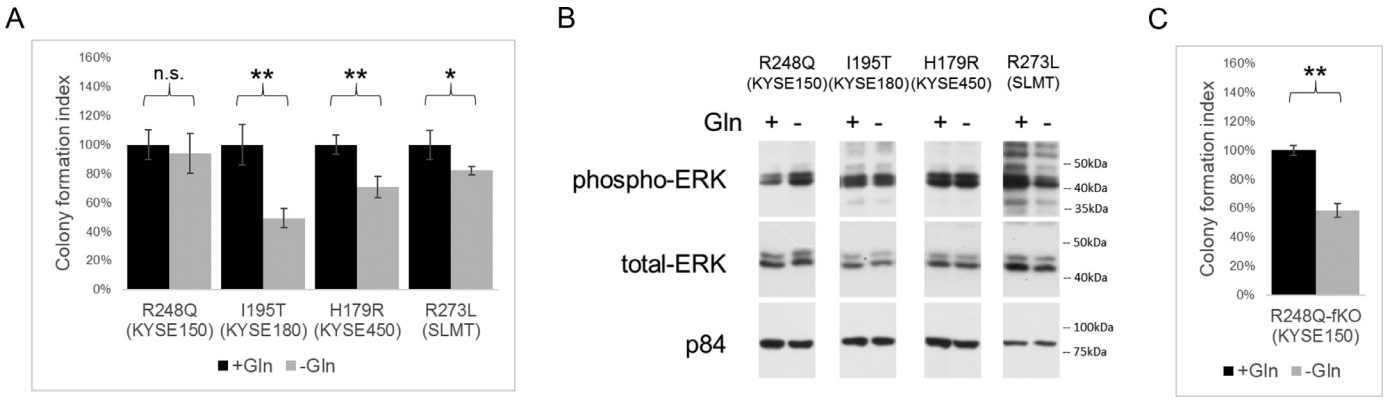

Fig. 6. Gln deprivation and ERK regulation in ESCC cell lines. a) Gln deprivation significantly suppresses proliferation only in cells harboring non-GoF p53 mutants, but not in cells with $p53^{R248Q}$. b) Phosphorylation of ERK increased in $p53^{R248Q}$-harboring cells upon Gln deprivation, but not in other cells harboring non-GoF p53 mutants. c) Depletion of $p53^{R248Q}$ protein expression renders the cells vulnerable to Gln deprivation-induced proliferation suppression. *: p-value<0.05; **: p-value<0.01; n.s.: p-value>0.05.

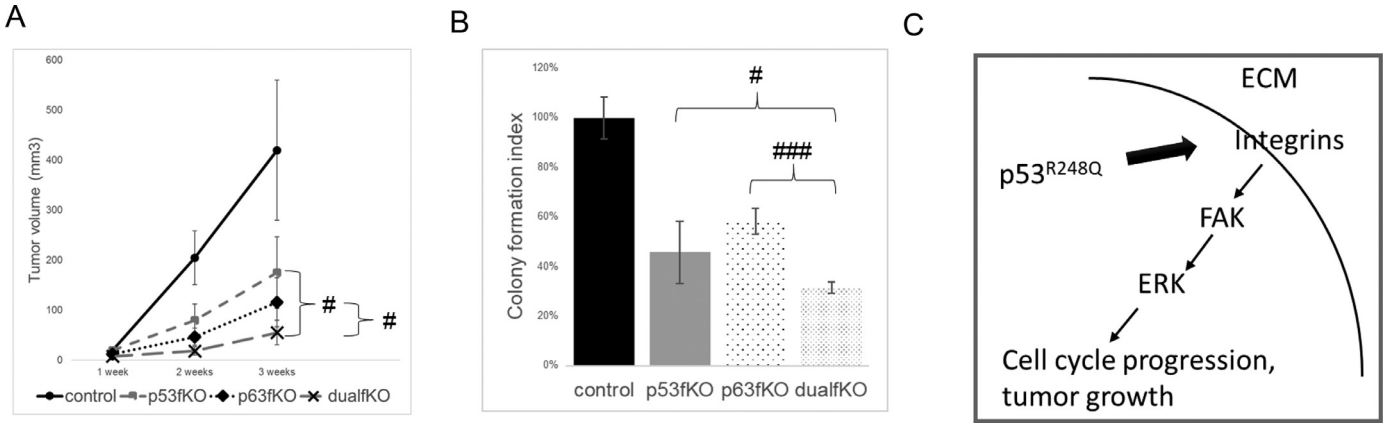

Fig. 7. Interaction between p53R248Q and ΔNp63α. a) Co-depletion of p53R248Q and ΔNp63α shows non-synergistic, non-antagonistic independent effects *in vivo* tumor growth. b) Consistently, co-depletion of p53R248Q and ΔNp63α shows independent effects *in vitro* colony formation. c) Proposed model illustrating the mechanism of oncogenic GoF of $p53^{R248Q}$ in ESCC. #: adjusted p-value<0.05; ###: adjusted p-value<0.001.

nerable to Gln deprivation-induced proliferation suppression (Fig. 6c). All these data suggest $p53^{R248Q}$ contributes to cell proliferation upon Gln deprivation.

Co-depletion of $p53^{R248Q}$ and ΔNp63α shows enhanced tumor suppression

TP63, encoding the p63 protein, is a member of the *TP53* gene family, showing structural and sequence homology with *TP53* and *TP73*. One p63 isoform, ΔNp63α, is highly expressed in squamous cell carcinoma and plays a critical oncogenic role [18-20]. Studies showed that p53 mutants cooperate with ΔNp63α in mediating oncogenic features [21, 22]. We examined the functional influence of co-depletion of $p53^{R248Q}$ and ΔNp63α on tumor growth and 3D colony formation. Depletion of either $p53^{R248Q}$ or ΔNp63α protein expression results suppressed *in vivo* tumor growth and in vitro colony formation. Depletion of both shows moderately enhanced suppressive effects on *in vivo* tumor growth (Fig. 7a) and in vitro colony formation (Fig. 7b) as compared to individual depletion, likely reflecting a non-synergistic interaction between these two oncogenic players.

Discussion

Various GoF p53 mutants have been characterized in different cancer types [1]. However, different GoF p53 mutants likely play cancer type-specific roles, resulting in a limited general understanding. Among the frequent p53 mutants, one of the hot spot mutants, $p53^{R248Q}$, plays a more generalized GoF role in a variety of cancer models. $P53^{R248Q}$ knock-in mice show earlier tumor onset and reduced lifespan compared to p53 null controls; Li-Fraumeni patients with $p53^{R248Q}$ mutations have earlier age of cancer onset compared to patients with other p53 mutations [2]. Experiments on cancer cell lines also showed the GoF property of $p53^{R248Q}$. A study on endometrial cancer showed that $p53^{R248Q}$ promotes cancer progression by regulating REGγ [23]. $P53^{R248Q}$ was shown to regulate PARP1 [24] and HER2 [25] to exert GoF effects in breast cancer. Ablation of $p53^{R248Q}$ was shown to suppress colorectal cancer through regulation of the STAT3 pathway [26]. Our data provide the first functional proof of $p53^{R248Q}$ GoF in ESCC and show a novel associated oncogenic signaling pathway consistent with previous studies. Depletion of $p53^{R248Q}$ protein expression results in suppressed tumor growth and 3D colony formation due to reduced cell cycle progression caused by deregulation of integrin/FAK/ERK signaling, as schematically depicted in Fig. 7c. All these data from the previous and our studies reveal the complexity of tissue/cancer-specific effects related to mutant p53, the most frequently mutated gene in cancer.

The majority of the *TP53* point mutations (3 out of 4 in this study) in ESCC exert a relatively neutral functional role in cancer development and are not required for tumor maintenance (Fig. 1c). This seemingly contradicts with 1) ours and others' immunohistochemical analysis showing moderate to high mutant p53 protein expression in the majority of ESCC patient tissue samples [27, 28] and 2) our previous study showing that *TP53* missense mutations, which usually result in p53 mutant protein expression, are associated with unfavorable patient

survival and compare to loss-of-function (LoF) *TP53* mutations that result in no protein expression [8]. All these data suggest that the majority of *TP53* mutations resulting in significant p53 mutant protein expressions provide the cells with certain selective advantages and are favored. This current study employed the xenograft tumorigenicity model on immunocompromised nude mice that lacks mature functional T lymphocytes, which may fail to reveal critical functional impacts of p53 mutants on oncoimmunology. Further functional analysis comparing p53 missense and LoF mutants using immunocompetent models such as transgenic/syngeneic models and humanized mouse model may be useful.

The $p53^{R248Q}$ was identified as a GoF p53 mutant through a variety of *in vivo* and *in vitro* functional assays. However, besides labor-intensive and time-consuming experimental genetic manipulations and functional assays, there is currently a lack of a GoF-specific molecular/biochemical indicator for efficient GoF p53 mutant identification. In order to promote identification of other GoF p53 mutants, we aimed to determine mutant p53 GoF-specific molecular characteristics in ESCC. Subcellular localization plays a critical role in executing proper functions of the protein and is well-known to be frequently altered by mutation. Besides the nuclear localization for its canonical tumor suppressive role, WT p53 is known to localize in the cytoplasm [29] and endoplasmic reticulum / mitochondria-associated membranes [30]. Subcellular localizations of p53 mutants have not been well categorized and are likely to be mutation- and cancer type-specific [31]. The $p53^{R248Q}$ displays a distinct concentrated localization to the membranous structures, as compared to other non-GoF mutants in ESCC cells. Such subcellular localization may be crucial for the GoF. Protein stability is another key regulator of protein functionality. Protein expression of WT p53 is the result of a sophisticated balance between protein translation and degradation, from which most p53 missense mutants escape. Heat shock protein 90 (HSP90) [13] and histone deacetylase [14] are well-known stabilizers of p53 mutants. However, we found that $p53^{R248Q}$ is resistant to HSP90/HDAC inhibition-induced protein degradation in contrast to other non-GoF p53 mutants. This suggests that $p53^{R248Q}$ achieves enhanced protein stabilization, which may also contribute to its GoF status. These data support the hypothesis that GoF p53 mutant possesses unique molecular/biochemical characteristics that may aid in identification of other GoF mutants.

The current study strongly indicates the importance of incorporating ECM *in vitro* cell culture models for functional analysis in ESCC. ECM is the 3D non-cellular structure present in all tissues. It not only provides physical support for the cellular machinery, but also is involved in regulation of tissue homeostasis in a highly dynamic manner [32]. Components of the ECM constantly interact with cells by serving as ligands for cell receptors (*e.g.* integrins) and regulating key processes such as adhesion, migration, proliferation, and cell survival; the ECM also contributes as reservoirs of growth factors and other signaling molecules. Dysregulated ECM is frequently observed in various pathological conditions including cancer [33]. Therefore, the classic 2D cell and ECM-free spheroid culture models fail to reveal functional influences of critical ECM-cell interaction that contributes substantially to cancer development. Utilizing an *in vitro* ECM-rich culture model based on using Matrigel [34] helped to identify a crucial role of $p53^{R248Q}$ in regulating integrin protein expression and mediating the ECM-dependent outside-in signaling transduction leading to cell proliferation that recapitulates the situation *in vivo*. Integrins, including α_V and β_3, commonly activate FAK [35-38], which in turn activate the MEK-ERK axis [39, 40]. The ERK signaling plays essential roles of integrating external mitogenic signals into critical intracellular events regulating cell proliferation [41, 42]. The p53R248Q mutant regulates phosphorylation of ERK, resulting in suppressed tumor growth and colony formation, which can be fully compensated by introduction of constitutively active MEK. These data suggest that protein expression of $p53^{R248Q}$ leads to activation of ERK signaling and provides selective growth advantages, which may open up unique therapeutic opportunities.

Cancer metabolism has been considered as a promising field in cancer therapeutics [43]. Besides a high demand for glucose as the main energy source, a subset of cancer cells is found to be addicted to Gln, a conditional essential amino acid involved in various key cellular processes including nitrogenous compound synthesis and reactive oxygen species homeostasis [44]. Deprivation of Gln results in reduced proliferation of addicted cells. Gln metabolism-targeted strategy has been implicated in treating numerous types of cancer [45]. Recently, Qie and colleagues analyzed Gln addiction in ESCC and revealed a molecular basis for therapy by targeting glutaminolysis and mitochondrial respiration [16]. WT p53 is known to promote adaptation to Gln deprivation by regulating membrane transport proteins and increasing aspartate [46] or arginine [47] uptake. Interestingly, we now show that as compared to cells harboring non-GoF p53 mutants, cells with $p53^{R248Q}$ maintain proliferation upon Gln deprivation with increased phosphorylation of ERK, while depletion of $p53^{R248Q}$ renders cells vulnerable to Gln deprivation. These data suggest a novel mechanism through which ESCC cells respond to metabolic changes.

Conclusions

The current study functionally analyzes the p53 mutants in ESCC. We demonstrate that $p53^{R248Q}$ exerts a specific GoF role in ESCC and provides new insights into the regulation of ECM-cell interaction. The p53 mutant plays a unique oncogenic role in ESCC development.

Declaration of Competing Interest

The authors declare no conflict of interest.

Author contributions

Conceptualization, VZ Yu and ML Lung; Methodology, VZ Yu and SS So; Validation, VZ Yu and SS So; Writing—original draft preparation, VZ Yu; Writing—review and editing, ML Lung; Supervision, ML Lung; Project administration, VZ Yu and ML Lung; Funding acquisition, ML Lung. All authors have read and agreed to the published version of the manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2020.100982.

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