Mutant p53 Regulates TNFSF10 in Cancer

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Abstract

Tumor suppressor protein p53 is an essential regulator of cell death. p53 activates genes that cause programmed cell death, or apoptosis, which stops damaged cells from dividing and suppresses cancer initiation and progression. About half of all human cancers carry p53 mutations, which affect the protein's normal function and can promote tumor growth. This study investigates the effects ofp53 mutation on the expression of *TNFSF10*, encoding the pro-apoptotic protein TRAIL. An analysis of transcriptomic data from the DepMap database showed that *TNFSF10* is overexpressed in renal cell carcinoma (RCC) cell lines carrying mutant but not wild type p53. The function of TRAIL in promoting the survival of cancer cells was investigated through a literature review. Finally, analysis of drug sensitivity data from the Cancer Therapeutic Response Portal revealed that RCC cells with high expression of TRAIL show increased sensitivity to the bioactivated compound SNS-032. These data suggest that SNS-032 could be used as a treatment to selectively target mutant p53 RCC tumors characterized by high TRAIL expression.

Research Question

How does mutation of p53 affect expression of apoptotic genes?

Hypothesis

Mutant p53 causes overexpression of genes that inhibit apoptosis to promote survival of RCC cells.

Introduction

The Hallmarks of Cancer

Cancer is a disease caused by uncontrolled cell division (J. S. Brown et al., 2023) Normally, cells grow, divide, and die in a controlled way. In contrast, cancer cells grow and divide abnormally due to mutations in two main types of genes: proto oncogenes and tumor suppressor genes. Proto oncogenes, when mutated, can promote cancer initiation and progression, while tumor suppressor genes inhibit these processes by suppressing cell growth and division or triggering cell death. (G. Brown, 2021). The hallmarks of cancer, the defining traits of cancer cells, can result from mutations that disable tumor suppressor genes or activate proto-oncogenes. Common hallmarks of cancer include abnormal cell division, evasion of cell death, and the ability to spread to other parts of the body, also known as metastasis (Fares et al., 2020). Apoptosis resistance is an important hallmark of cancer and a factor in resistance to treatment because many cancer treatments induce apoptosis to destroy tumor cells (Elmore, 2007).

Apoptosis and Tumor Suppression

Apoptosis is a form of programmed cell death through which aged, damaged, or harmful cells are eliminated in a controlled way to maintain tissue homeostasis (Mustafa et al., 2024). Apoptosis can be initiated through two main pathways: the extrinsic pathway, which is triggered by signals outside the cell, and the intrinsic pathway, which is controlled by proteins inside the

cell that detect damage. Immune cells can also cause apoptosis to kill infected or harmful cells (Elmore, 2007). These pathways activate enzymes called caspases that break down the cell's DNA and other molecular structure, leading to cell death. Apoptosis can prevent damaged cells from turning into cancer cells and metastasizing. When apoptosis fails, these damaged cells might not die but instead divide, which raises the risk of tumor development. Cancer treatments like chemotherapy, radiation, and targeted therapies often work by triggering apoptosis, so when this process is impaired, these treatments become less effective (Mustafa et al., 2024).

The p53 Tumor Suppressor

p53 is a protein produced by the *TP53* gene and is known as the "guardian of the genome." because it protects cells from becoming cancerous (Shen et al., 2023). p53 is a tumor suppressor, which means it stops cell growth to prevent damaged or abnormal cells from dividing uncontrollably (Sammons et al., 2020). Tumor suppressors like p53 help keep cells healthy by repairing DNA damage and preventing cells with damaged DNA from dividing. If DNA damage is detected, p53 initiates cell cycle arrest to enable cells to repair DNA. In cases where DNA damage is too severe to be repaired, p53 may instead trigger apoptosis (Ozaki & Nakagawara, 2011). p53 regulates apoptosis by transactivating pro-apoptotic genes such as *BAX, BAK1, PMAIP1* (encoding NOXA) (Ozaki & Nakagawara, 2011). In this way, p53 ensures that dangerous cells are removed before they become harmful (Sammons et al., 2020).

When p53 is mutated, it may lose its normal function, allowing cells with damaged DNA to survive and divide abnormally. p53 is one of the most frequently mutated genes in human cancers. Mutations of p53 are found in around 50% of all tumors (Aubrey et al., 2018). These mutations usually occur at "hotspot" sites in the *TP53* gene and can prevent the p53 protein from performing its normal functions. (Liu et al., 2024). Mutant p53 may undergo a loss-of-function and lose the ability to kill damaged cells. However, gain-of-function p53 mutations can enable cells to acquire new functions that help cancer cells survive and grow (Zhang et al., 2024). Therefore, targeting p53 mutations represents an important area for cancer research and, potentially, treatment.

Renal Cell Carcinoma

Renal cell carcinoma (RCC) is a common form of kidney cancer and arises from epithelial cells in the kidney tubules (Padala et al., 2020). The most common subtype of kidney cancer is clear cell RCC (ccRCC), which makes up about 90% of all kidney cancers (Hsieh et al., 2017). RCC is usually caused by the inactivation of the Von Hippel-Lindau (*VHL*) tumor suppressor gene, which activates hypoxia response pathways that help the tumor grow under low oxygen conditions by changing its metabolism, making new blood vessels, and avoiding cell death (Amendolare et al., 2022). RCC makes up 2% to 3% of all cancers globally. Metastatic RCC has worse outcomes, with 5 year survival decreasing to about 12% (Padala et al., 2020). Treatments for RCC include immunotherapy, chemotherapy, radiation, or surgery, as monotherapies or in combination. Late diagnosis and treatment resistance remain major challenges in the treatment of RCC (Padala et al., 2020).

p53 Mutations and Apoptosis Regulation

Even though *TP53* mutations are less common in RCC than in other cancers, they can cause tumors to grow more quickly and respond poorly to treatment (Amendolare et al., 2022). *TP53*

mutations can disrupt the regulation of apoptotic genes, potentially allowing cancer cells to avoid cell death and continue growing. *TNFSF10*, also known as TRAIL, causes cancer cells to undergo apoptosis by binding to death receptors on the surface of cells and triggering caspases (Pimentel et al., 2023). TRAIL is an important part of the extrinsic apoptotic pathway. It attaches itself to the target cell surface death receptors DR4 and DR5, which causes the death-inducing signaling complex (DISC) to assemble and initiate caspase-8 to be activated. This causes a cascade of caspase activation, leading to cell death (Yuan et al., 2018). TRAIL often suppresses tumors by causing cancer cells to undergo apoptosis while leaving healthy cells unaffected. However, paradoxically, TRAIL can potentially accelerate the spread of cancer by inducing non-apoptotic pathways which promote inflammation, survival, and metastasis in some situations, especially when signaling from apoptosis is disrupted (Isono et al., 2018). *TNFSF10* is normally activated by p53, but TRAIL expression may be decreased or disrupted in RCC cells with p53 mutations. If TRAIL is overexpressed in cells carrying mutant p53, it could potentially help the tumor grow by metastasis, avoiding the immune system, or causing inflammation (Guerrache & Micheau, 2024).

TNFSF10 expression varies highly in wild-type p53 cells but is higher on average in mutant p53 cells. Most cell lines do not depend on *TNFSF10* to survive, but some mutant TP53 lines, like 786-O, depend on it. A few wild-type lines also show some dependency, meaning *TNFSF10*'s role can change depending on the cell. CTRP data show that RCC cells with high *TNFSF10* expression respond more strongly to SNS-032, than wild-type TP53 cells. These results suggest SNS-032 can target RCC cells with high TRAIL expression and TP53 mutations.

This study aims to understand how mutations in p53 alter the expression of pro-apoptotic genes like TRAIL A better understanding of the relationship between p53 and *TNFSF10* could clarify how p53 mutations promote RCC and help identify new treatments that selectively target cancer cells overexpressing TRAIL.

Methods

The DepMap gene expression database was used to examine the impact of *TP53* mutations on the expression of pro-apoptotic genes in renal cell carcinoma (RCC). RCC cell lines were selected based on expression of wild-type or mutant *TP53*. All DepMap RCC models were analyzed and grouped by *TP53* hotspot mutation status into a wild-type p53 group and a mutant p53 group. These groups were used to compare gene expression and CRISPR results. A scatterplot was used to compare gene expression between these groups, and candidate genes upregulated in mutant p53 cell lines were identified

Besides gene expression, the importance of candidate genes for RCC cell survival was determined by analyzing CRISPR-based dependency data from DepMap. To support the data analysis, a literature review was performed to identify pro-oncogenic functions of candidate genes.

Finally, the relation between drug response and expression of candidate genes was examined using the Cancer Therapeutic Response Portal (CTRP). CTRP was used to find drugs that were predicted to show enhanced efficacy in RCC cells that overexpress *TNFSF10*. The correlation analysis plot was analyzed to identify correlations betweenTNFSF10 expression and drug

response across cancer cell lines. Advanced filters were used to select only kidney cancer cell lines, and the interquartile multiplier was set to 1.0 to highlight the most significant correlations. Drugs near the bottom of the graph for which efficacy was strongly correlated with *TNFSF10* expression were further examined.

Results

Analysis of gene expression data from the DepMap database suggests that *TNFSF10* is overexpressed in RCC cell lines with mutant *TP53* relative to those with wild-type *TP53* (Figure 1). When TNFSF10 expression is plotted across all RCC models grouped by TP53 hotspot mutation status, the mutant p53 group shows higher average expression than the wild-type group, as indicated by points lying above the diagonal line in the scatterplot (Figure 1). Based on these data, *TNFSF10* was selected as a candidate gene for further investigation (Wang et al., 2025).

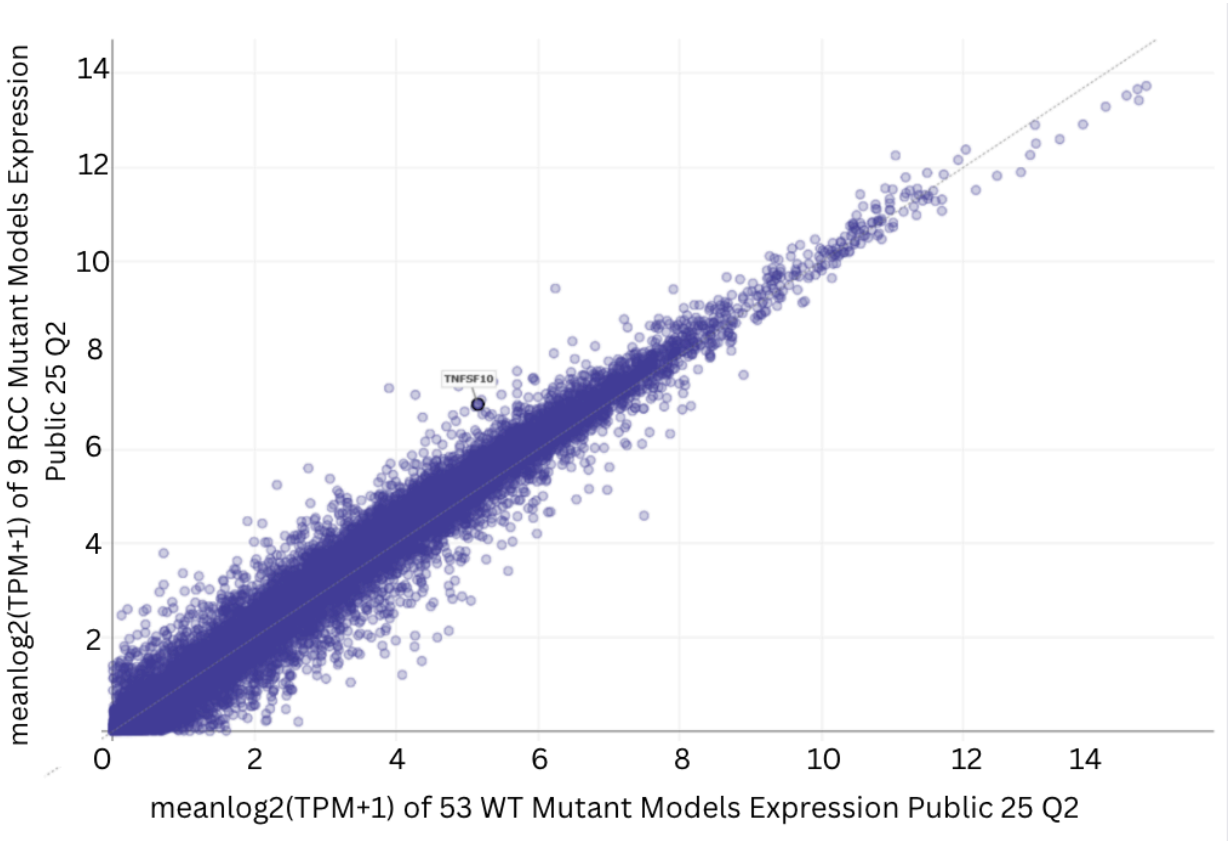

Figure 1. *TNFSF10* Expression in Wild Type vs. Mutant *TP53* RCC Cell Lines. Comparison of *TNFSF10* gene expression between RCC cell lines with wild-type (WT) TP53 (n=53) and mutant TP53 (n=9). Gene expression data from DepMap show that *TNFSF10* is higher in mutant TP53 RCC lines compared to WT TP53 lines, which is indicated by points above the diagonal line that represents equal expression.

Dependency scores from the DepMap CRISPR dataset were used to determine if RCC cells are dependent on *TNFSF10* for survival (Figure 2). *TNFSF10* dependency is higher in RCC cell lines with mutant *TP53* than in wild-type *TP53* cell lines (Figure 2). *TNFSF10* levels vary highly in wild-type TP53 cells, but mutant TP53 cells demonstrate more uniformly elevated levels in TNFSF10 by comparison (Figure 2). Some wild-type TP53 lines also depend on *TNFSF10*, which suggests that the impact of TRAIL on survival may be context dependent.

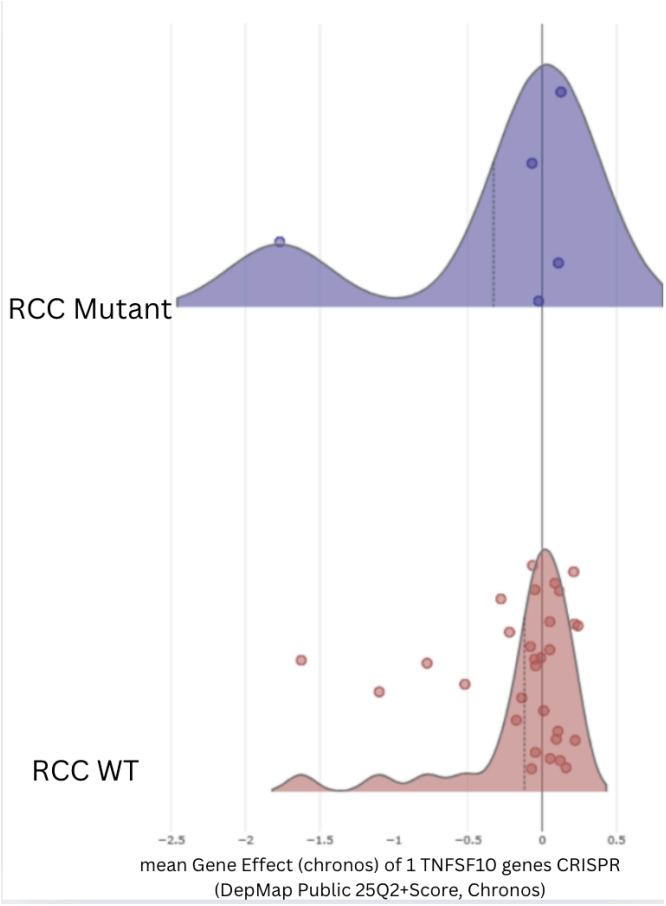

Figure 2. Dependency of RCC Cell Lines on *TNFSF10.* Density plots show *TNFSF10* gene expression and CRISPR dependency scores in kidney cancer cell lines grouped by p53 mutational status. 53 RCC cell lines with WT p53 and 9 cell lines with mutant p53 were plotted.

These data suggest that , despite its canonical role inducing extrinsic apoptosis, TRAIL could exert paradoxical oncogenic function in cells with mutant p53. Corroborating this notion, prior studies have reported that,under certain conditions, TRAIL can promote tumor progression in p53 mutant cancers, including RCC. In these contexts, TRAIL signaling may activate non-apoptotic pathways that support cell survival, proliferation, and metastasis when p53 function is lost (Wang et al., 2025).

Identifying Drugs for Selective Targeting

To identify drugs that could selectively target cancer cells with mutant p53 and high *TNFSF10* expression, we examined data from the CTRP. This database includes comparisons of gene expression and response to a large library of bioactive drugs and chemotherapy drugs in thousands of cancer cell lines.

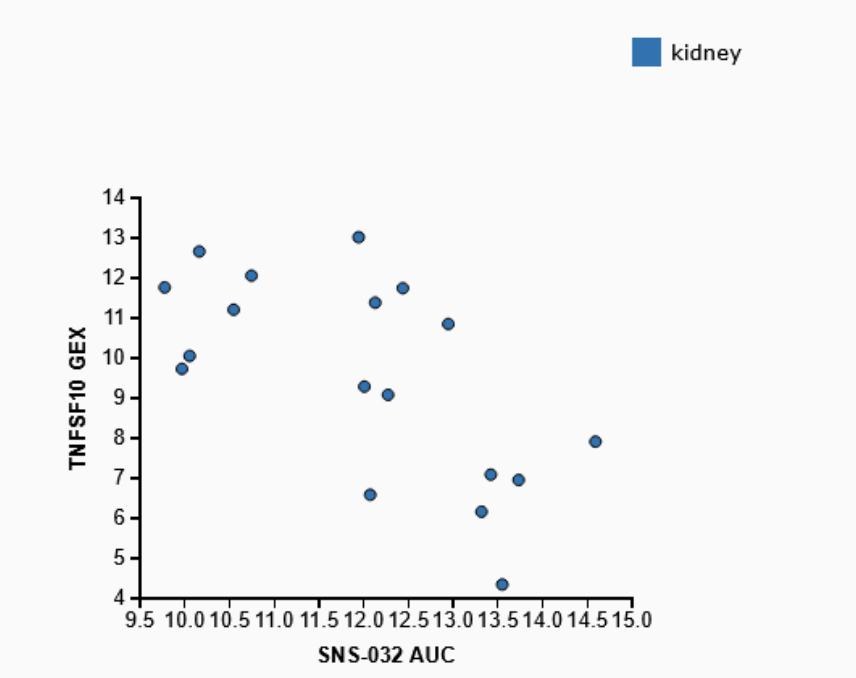

Figure 3. Impact of TRAIL Overexpression on SNS-032 Response in Renal Cancer. Correlation between *TNFSF10* expression and response to SNS-032 in RCC cell lines.

These data revealed that in RCC cells, high expression of *TNFSF10* correlates with increased sensitivity to the bioactive compound SNS-032, which is annotated as an inhibitor of cyclin-dependent kinases (CDKs). According to the CTRP findings, RCC cells with high TRAIL levels are more sensitive to cell death induced by SNS-032, compared to cells expressing wild-type *TP53* (Figure 3). This suggests that SNS-032 could be effective in selectively eliminating RCC cells with high TRAIL expression and *TP53* mutations (Conroy et al., 2009).

Discussion

This study suggests that *TP53* mutations can alter the behavior of genes involved in cell death, including *TNFSF10.* As a part of the extrinsic apoptotic pathway, TRAIL helps eliminate damaged or abnormal cells by inducing apoptosis in healthy cells with functional p53. (Kuribayashi et al., 2008). However, TRAIL acts differently in RCC cells that carry a p53 mutation (Isono et al., 2018). This study found TNFSF10 is overexpressed in some RCC cell lines with *TP53* mutations. Prior studies suggest that TRAIL overexpression in RCC cells may not cause cell death, despite its canonical role in activating extrinsic apoptosis. By helping cancer cells survive, proliferate, and resist treatment, TRAIL may exert paradoxical pro-tumor effects in the context of p53 mutations (Guerrache & Micheau, 2024). These data suggest that

mutant p53 could alter the function of genes that regulate extrinsic apoptosis, turning a signal for cell death into one that promotes the growth of tumors (Ozaki & Nakagawara, 2011).

The finding that RCC cell lines with mutant *TP53* are dependent on *TNFSF10* for survival compared to wild-type cells is supported by gene dependency data. This is a surprising finding because *TNFSF10*, usually induces apoptosis. In cancer cells with mutant TP53, *TNFSF10* can activate other signaling pathways that promote cell survival and growth. The switch in function explains why RCC cells with mutant TP53 rely on *TNFSF10* for survival (Guerrache & Micheau, 2024).

This study also identified a bioactive compound, SNS-032, that may show selective lethality towards RCC cells overexpressing TRAIL. Prior literature suggests that RCC cell lines with high TRAIL expression and *TP53* mutations respond better to SNS-032. The study found that SNS-032 blocks important proteins called cyclin-dependent kinases (CDKs) 2, 7, and 9. They showed that only 6 hours of treatment was enough to stop cancer cells from growing and make them die. This effect was also observed in blood cells from patients in clinical trials, showing that SNS-032 works in people. Although the study looked at blood cancers, this drug might also kill RCC cells that need CDKs, especially ones with mutant TP53 and high TRAIL (Conroy et al., 2009).

These results combined suggest that the function of TRAIL in cancer is cell-type and context specific and can differ based on p53 mutational status and function (Pimentel et al., 2023). New treatment options for RCC and other cancers with *TP53* mutations could become possible if scientists understand how mutant p53 affects the production and functioning of apoptotic genes like *TNFSF10* (Yuan et al., 2018).

Impact and Significance

These data and analyses suggest a link between *TNFSF10*, p53 mutational status, and cancer cell death and survival. *TNFSF10* has been shown to support tumor growth and survival in cells with p53 mutations, while TRAIL is known to induce apoptosis in cancer cells. (Pimentel et al., 2023) (Guerrache & Micheau, 2024). Cancers carrying *TP53* mutant cancers may therefore not respond well to treatments that activate TRAIL, which could worsen the cancer's progression by promoting metastasis or treatment resistance (Yuan et al., 2018). These findings suggest the need for stratification of RCC patients based on their p53 mutation status and the potential benefit of treatments with selective lethality towards p53 mutant cancers. When *TP53* is altered in cancers like RCC, *TNFSF10* expression may exert more oncogenic than tumor suppressive effects. For better results, future studies should focus on understanding how to stop *TNFSF10*'s oncogenic behavior in cells carrying mutant p53. By focusing on *TNFSF10*'s altered pathway, RCC and other tumors with *TP53* mutations might respond better to therapy.

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