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Impact of TP53 Gain-of-Function Mutations on Metabolic Reprogramming in Prostate Cancer Progression

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Authors' contributions

This work was carried out in collaboration among all authors. Authors YAY & SAA conceived and designed the study. Author FJU performed the experiments. Authors FJU and WY analyzed/interpreted the metabolomics data. Authors FJU and DGG wrote the manuscript. All authors contributed to the review and approval of the final version of the manuscript.

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Original Research Article

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ABSTRACT

Despite advancements in the treatment of advanced prostate cancer, including metastatic castration-resistant prostate cancer (mCRPC), the disease remains lethal, often developing resistance to conventional therapies such as androgen deprivation therapy (ADT) and chemotherapy. Therapy resistance poses a significant challenge, particularly in aggressive forms of prostate cancer. Data on TP53 mutations, especially gain-of-function (GOF) mutations, and their specific impact on prostate cancer metabolism remain limited, underscoring the need for a deeper understanding to uncover new therapeutic avenues. This study aimed to explore the metabolic consequences of TP53 GOF mutations (R175H and R273H) in prostate cancer progression using metabolomics analysis. Approximately 300 metabolites were significantly altered across PC3-GFP (KO), PC3-TP53WT (WT), and PC3-TP53 mutant (R175H & R273H) models. Key findings revealed dysregulation of essential metabolites, such as α-ketoglutarate and spermidine, which support cancer cell survival and progression. These insights not only enhance our understanding of the interplay between TP53 mutations and metabolic alterations but also identify potential biomarkers for prognosis and therapeutic targeting in therapy-resistant prostate cancer. In the TP53-R175H mutant, numerous essential metabolites were dysregulated (downregulated: glutamine, GAP/DHAP, and α-ketoglutarate; Upregulated: deoxyuridine, spermidine, and pseudouridine) when compared with the control (KO) thereby causing metabolic shifts that favor cancer cell survival. Conversely, metabolites such as GlaNAC/GlcNAC, adenosine, acetoacetic acid, acetylcholine, octopamine, itaconic acid, lactic acid, and NADP+ are all downregulated in the R273H mutant. While spermine, spermidine, N-acetyl-DL-serine, pseudouridine, deoxyguanosine, sucrose, and AMP/dGMP are upregulated by the R273H mutant but downregulated in GFP (KO). These findings showed that TP53 GOF mutations (R175H & R273H) cause metabolic alterations in numerous metabolites essential for tumor survival and progression. Furthermore, it may provide therapeutic benefits by identifying appropriate biomarkers that could be used as diagnostic and/or prognostic tools for prostate cancer.

Keywords: Prostate cancer; PC-3; TP53Wild-Type; TP53-R175H; TP53-R273H; metabolomics; metabolic alterations.

ABBREVIATIONS

TP53: Tumor suppressor protein 53
PC: Prostate Cancer
CRPC: Castration Resistance Prostate Cancer
KO: Knockout
GFP: Green fluorescent protein
GAP: Glyceraldehyde-3-phosphate
DHAP: Dihydroxy acetone phosphate
WT: Wild-type
ADT: Androgen Deprivation Therapy
HEK293T: Human embryonic kidney 293T
GOF: Gain-of-function

1. INTRODUCTION

Prostate cancer (PC) is one of the most common malignancies affecting men worldwide, and androgen hormone deprivation therapy (ADT) serves as an effective first-line treatment, inducing favorable responses in advanced PC patients (Siegel, 2020). However, a substantial proportion of these individuals eventually progress to a more aggressive stage known as Castration-Resistant Prostate Cancer (CRPC). CRPC, characterized by a loss of responsiveness to ADT, poses significant clinical challenges and compromises patient clinical/survival outcomes (Dong, 2019, Teo, 2019). Advanced lethal PCs often manifest loss or mutation in the tumor suppressor protein 53 (TP53). This aberration plays a pivotal role in the progression of prostate cancer, contributing to heightened resistance to therapeutic modalities and adaptive metabolic alterations (De Laere, et al., 2019, Teroerde, et al., 2021). The emerging data indicates that directing cancer therapy toward TP53 is an appealing and promising

strategy. Tumors harboring TP53 mutations often display accelerated progression, unfavorable predictive outcomes, and resistance to therapeutic interventions (Olivier, 2010, Stiewe, 2018, Zhou, et al., 2019). TP53 mutation has been identified as having pro-metastatic potential in addition to its role in tumorigenesis. However, the precise mechanisms underlying tumorigenesis and disease progression in prostate cancer induced by mutant TP53 remain elusive (Mantovani, et al., 2019).

The upregulation of amino acid synthesis and metabolism heightens PC progression. Notably, emphasis is placed on anaplerosis, rather than energy production, as a key aspect influencing the disease progression (Ahmad, 2021, Strmiska, 2019). Several studies have demonstrated that TP53 mutants modify metabolic pathways regulated by wild-type TP53 to support the provision of metabolites essential for proliferation (Kollareddy, 2015, Zhang, et al., 2013, Zhou, et al., 2014). For instance, the TP53R175H mutant utilizes exogenous pyruvate in cancer cells, enhancing survival during glucose deprivation. Conversely, the R273H mutant has been observed to suppress the expression of phase 2 detoxifying enzymes, thereby promoting cell survival under oxidative stress conditions (Chavez-Perez, et al., 2011, Kalo, et al., 2012). The involvement of TP53 mutations is noteworthy in contributing to enzalutamide resistance in LNCaP cells, which hinders the androgen receptor (AR) signaling pathways (Long, 2021).

Furthermore, in the context of prostate cancer patients, the presence of mutant TP53 has been linked to the emergence of resistance to docetaxel (Liu, 2013). Therefore, the presence of TP53 mutations has been proposed as a potential predictive marker for anticipating the efficacy of docetaxel treatment (Teroerde, et al., 2021). Emergence evidence showed that the TP53 mutant alters metabolism to promote tumor development in cancer cells (Mao & Jiang, 2023), as modified metabolism was reported to be one of the hallmark features of cancer cells which supports rapid growth and survival in hostile environments (Yang, et al., 2024, Kim et al., 2024). Another study highlights the significance of asparagine synthetase-mediated metabolic adaptation as a synthetic vulnerability in castration-resistant prostate cancer with TP53 gain of function mutations, providing a rationale for co-targeting intracellular and extracellular asparagine production to treat lethal prostate cancers (Yoo et al., 2023).

Despite the advancements in the treatment of advanced prostate cancer, it persists as a lethal disease resistant to existing therapies & the rapid emergence of resistance presents a formidable obstacle, there is a pressing need for novel therapeutic approaches to enhance patient responsiveness and mitigate drug resistance. To gain insight into the impact of TP53 mutations, particularly gain-of-function mutations, on the metabolic adaptation of prostate cancer cells, this study is carefully prepared to systematically examine how TP53 mutations influence the metabolic dynamics within prostate cancer cells.

2. MATERIALS AND METHODS

2.1 Cell Culture

HEK293T and PC-3 cell lines were obtained from ATCC (American Type Culture Collection, Chicago, IL), cultured & maintained in Dulbecco's modified Eagle's medium (DMEM) and RPMI-1640 medium, respectively. Both media were supplemented with 10% fetal bovine serum (FBS) (Bovogen Biologicals, Australia) and 1% penicillin/streptomycin (100 U/ml) stabilized solution (Sigma-Aldrich, USA). Both cell lines were maintained & incubated at 37°C, 10 % CO_2 incubator. All cells were verified to be mycoplasma-free (Lonza) and genetically authenticated by ATCC.

2.2 DNA Plasmids & Gene Knockout Experiment

Lentiviral constructs targeting TP53 knockdown (KD) were created by incorporating the cDNA into the pFUGW-H1 vector. The pLenti6.3/V5-DEST-GFP plasmid (Addgene_40125) was generously provided by Lynda Chin 91, while the pLenti6/V5-p53_R273H (Addgene_22934) and pLenti6/V5-p53_R175H (Addgene_22936) plasmids were gifts from Bernard Futscher. Lentiviruses were generated through the transfection of HEK293T cells with a Δ8.9 packaging vector and VSVG envelope vector (2:1:1 ratio) in Opti-MEM media (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). PC3 cells were transduced with the virus in the presence of 8 µg/ml polybrene (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, the selection of stably expressing cells was achieved by adding puromycin (1 µg/ml, Invitrogen, Thermo Fisher Scientific, Waltham,

MA, USA), blasticidin (5 µg/ml Thermo Fisher Scientific, Waltham, MA, USA), or zeocin (100 µg/ml, Thermo Fisher Scientific, Waltham, MA, USA).

2.3 Western Blot

Western blot analysis was conducted according to Anker et al., 2018. Whole-cell extraction was performed using RIPA buffer (Thermo Fisher Scientific, Waltham, MA, USA) (50 mM Tris, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, and 1% NaDeoxycholate [pH 7.4]), supplemented with protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 10 µg/ml peptasin A, 10 µg/ml aprotinin, and 5 µg/ml leupeptin). Protein concentrations were quantified using Bio-rad protein assay kits (BioRad, Hercules, CA). Subsequently, protein lysates were separated by SDS-PAGE, transferred onto nitrocellulose membranes, blocked with PBS containing 0.2% Tween 20 and 5% BSA, and probed with primary antibodies. The primary antibodies employed were p53 (FL-393, sc-6243, Santa Cruz; 1:1000) and anti-β-actin (Sigma, 1:5000). The developed blot was visualized using the ECL Prime Western Blot Detection Kit by the manufacturer's protocol (Amersham, United Kingdom).

2.4 Hydrophilic Metabolome of TP53 Mutants

V5-DEST-GFP, V5-p53-wt-p53, V5-p53-R175H, and V5-p53-R273H cell lines were cultured at a density of 2×10^6 cells in triplicate and allowed to adhere overnight in a humidified 5% CO_2 incubator at 37°C for 24 hours. Cells were washed twice with cold PBS, and pellets were resuspended in 80% methanol and then lysed by five freeze-thaw cycles (LN2 freezing followed by thawing at room temperature). Samples were then collected and centrifuged at 20,000 x g for 30 min at four °C, and the supernatant was dried using SpeedVac. 50% acetonitrile was added to the tube for reconstitution, followed by vortexing for 30 sec. The sample solution was then centrifuged for 15 min at 20,000 x g, four °C. The supernatant was collected for liquid-chromatography mass spectrometry (LC-MS) analysis. Samples in triplicates were analyzed by High-Performance Liquid Chromatography, High-Resolution Mass Spectrometry, and Tandem Mass Spectrometry (HPLC-MS/MS). Specifically, the system consisted of a Thermo Q-Exactive in line with an electrospray source and an Ultimate3000 (Thermo) series HPLC consisting of a binary pump, degasser, and auto-sampler outfitted with an Xbridge Amide column (Waters; dimensions of 4.6 mm × 100 mm and a 3.5 µm particle size). The mobile phase A contained 95% (vol/vol) water, 5% (vol/vol) acetonitrile, 20 mM ammonium hydroxide, 20 mM ammonium acetate, pH = 9.0; B was 100% Acetonitrile. The gradient was as follows: 0 min, 15% A; 2.5 min, 30% A; 7 min, 43% A; 16 min, 62% A; 16.1-18 min, 75% A; 18-25 min, 15% A with a flow rate of 400 µL/min. The capillary of the ESI source was set to 275 °C, with sheath gas at 45 arbitrary units, auxiliary gas at five arbitrary units, and the spray voltage at 4.0 kV. In positive/negative polarity switching mode, an m/z scan range from 70 to 850 was chosen, and MS1 data was collected at a resolution of 70,000. The automatic gain control (AGC) target was set at 1 × 106, and the maximum injection time was 200 ms. The top 5 precursor ions were subsequently fragmented in a data-dependent manner, using the higher energy collisional dissociation (HCD) cell set to 30% normalized collision energy in MS2 at a resolution power of 17,500. Besides matching m/z, metabolites are identified by matching either retention time with analytical standards and/or MS2 fragmentation pattern. Data acquisition and analysis were carried out by Xcalibur 4.1 software and Trace finder 4.1 software, respectively (both from Thermo Fisher Scientific).

3. STATISTICAL ANALYSIS

Descriptive results were shown as mean $\pm$ SD. Metabolomics analyses were carried out using MetaboAnalyst. Values of $p<0.05$ were considered statistically significant. All statistical tests were two-tailed, with a significance level of $p<0.05$. All the statistical analyses were performed using licensed GraphPad Prism (v9.2) (Gupta, et al., 2023; Gupta, et al., 2023, Gupta, et al., 2023).

4. RESULTS

4.1 Generation of Stable PC-3 Mutant Cell Lines

To explore the TP53 mutations and their consequences in the progression of prostate cancer, we transduced & generated PC-3 cells (TP53 null) with V5-DEST-GFP (Control), V5-TP53-WT, V5-TP53-R175H (P53 Mutant) and V5-TP53-R273H (P53 Mutant), as shown in Fig. 1.

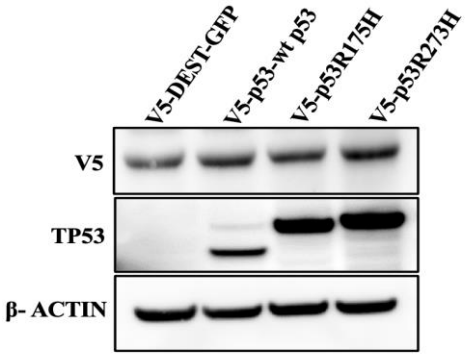

Fig. 1. Western Blot of the transduced PC-3 mutant cell lines indicating respective proteins of the transducers.

4.2 Metabolomics of PC3-GFP (KO), PC-3 TP53 WT and Mutants (R175H & R273H)

To study the expression of the metabolites and pattern of their dysregulation in the wildtype and mutant cells, we conducted metabolomics analysis on triplicates of PC-3 cells with wild-type (WT), PC3-GFP (KO), TP53-R175H, and TP53-R273H mutations. The result of metabolomics identified about 300 significant differentially altered metabolites in the PC3-TP53 proteins using liquid chromatography-mass spectrometry (LC/MS) analysis.

4.2.1 Comparison between pc3-gfp (ko) & pc3-tp53wt (wt)

In comparing GFP (KO) and WT (Fig. 2A), upregulated metabolites are depicted in red, and downregulated ones in blue. Metabolites that are upregulated in GFP (KO) are found to be downregulated in TP53WT, and the reverse is also true. In the GFP (KO) group, where TP53 is absent, there is an increase in metabolites, including lactic acid, propionic acid, acetoacetic acid, epinephrine, histamine, and stearic acid. Conversely, in the WT group, these metabolites are decreased due to the presence of TP53. Additionally, metabolites like deoxyguanosine, spermidine, and sucrose were found to be decreased in GFP (KO) and increased in WT. In the volcano plot (Fig. 2B), guanidine and spermidine are highlighted as the most significantly decreased metabolites, represented by blue dots. Conversely, the most increased considerably metabolites, like epinephrine/normetanephrine, propionic acid, and histamine, are indicated by orange dots (Fig. 2B).

4.2.2 Comparison between pc3-tp53wt & pc3-tp53-r175h

Comparing WT to the R175H mutant, metabolites such as glutamine, taurine, carnitine, GAP/DHAP, N-acetyl-L-glutamine, adenosine, propionic acid, and 2-HG that are upregulated in WT are found to be downregulated in the R175H mutant (Fig. 3A). Conversely, metabolites like alanine, deoxyuridine, aspartic acid, c-di-GMP, and N1/N8-acetylspermidine are downregulated in WT but upregulated in the R175H mutant. The volcano plot identifies GAP/DHAP and 2-HG as the most significantly decreased metabolites. Meanwhile, c-di-GMP, deoxyuridine, and N1/N8-acetylspermidine are the most increased considerably metabolites (Fig. 3B).

4.2.3 Comparing pc3-tp53wt to pc3-tp53-r273h

In the WT sample, metabolites like acetoacetic acid, inosine, epinephrine/normetanephrine, adenosine, propionic acid, a-KG, 2-HG, and GAP/DHAP are upregulated, but these are downregulated in the R273H mutant (Fig. 4A). Conversely, metabolites such as spermine, deoxyguanosine, thymidine, deoxyuridine, sucrose, and N-acetyl-DL-serine are downregulated in WT but upregulated in the R273H mutant (Fig. 4A). In the volcano plot, the most significantly decreased metabolites, including GAP/DHAP, itaconic acid, GalNAC/GlcNAC, -KG, and 2-HG, are indicated by blue dots. Meanwhile, the most significantly increased metabolites, such as spermine, N-acetyl-DL-serine, deoxyuridine, and thymidine, are highlighted with orange dots (Fig. 4B). These metabolites were selected because they have a p-value $<$of 0.05 and a cutoff $>$of 2 FC.

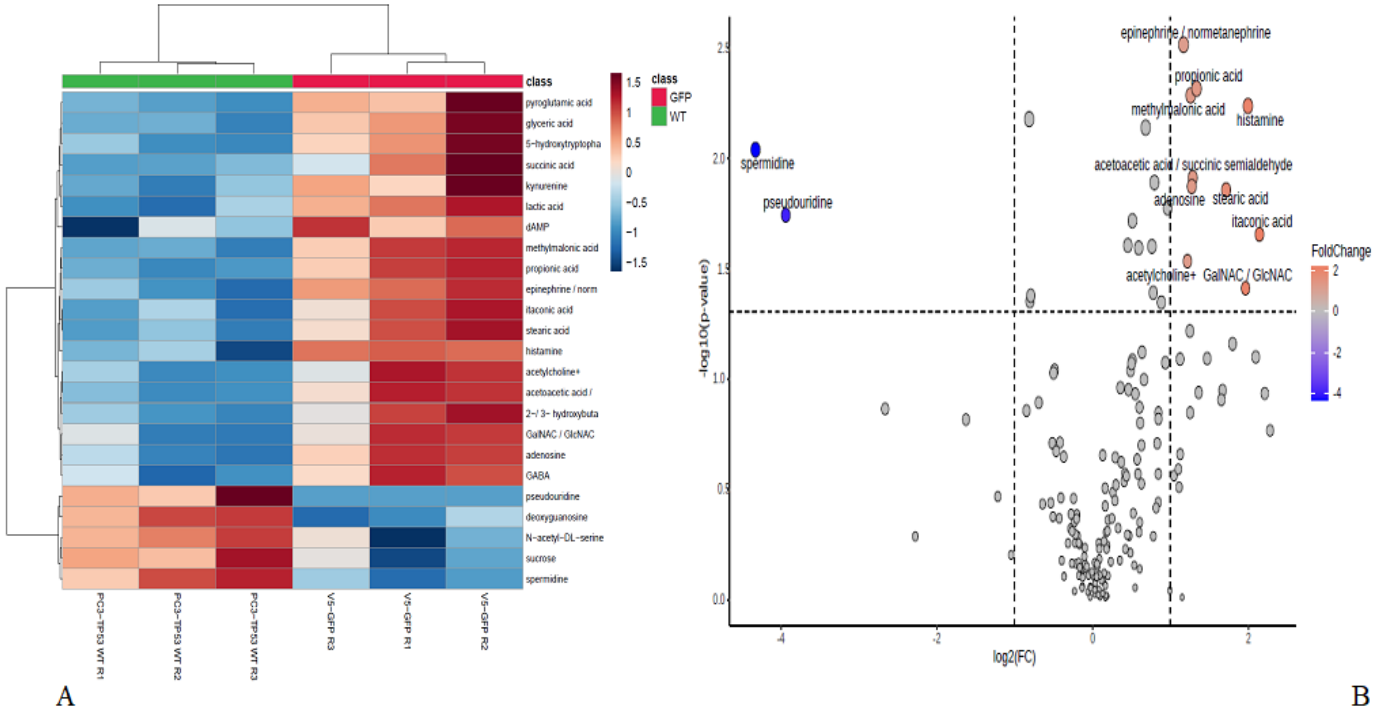

Fig. 2. Expression of numerous metabolites in the PC3-GFP (KO) & PC3-TP53WT (WT). (A) Heatmap depicting the expression of most dysregulated metabolites. Upregulated metabolites are shown in red, while the downregulated metabolites are in blue (B) Volcano plot comparing the altered metabolites

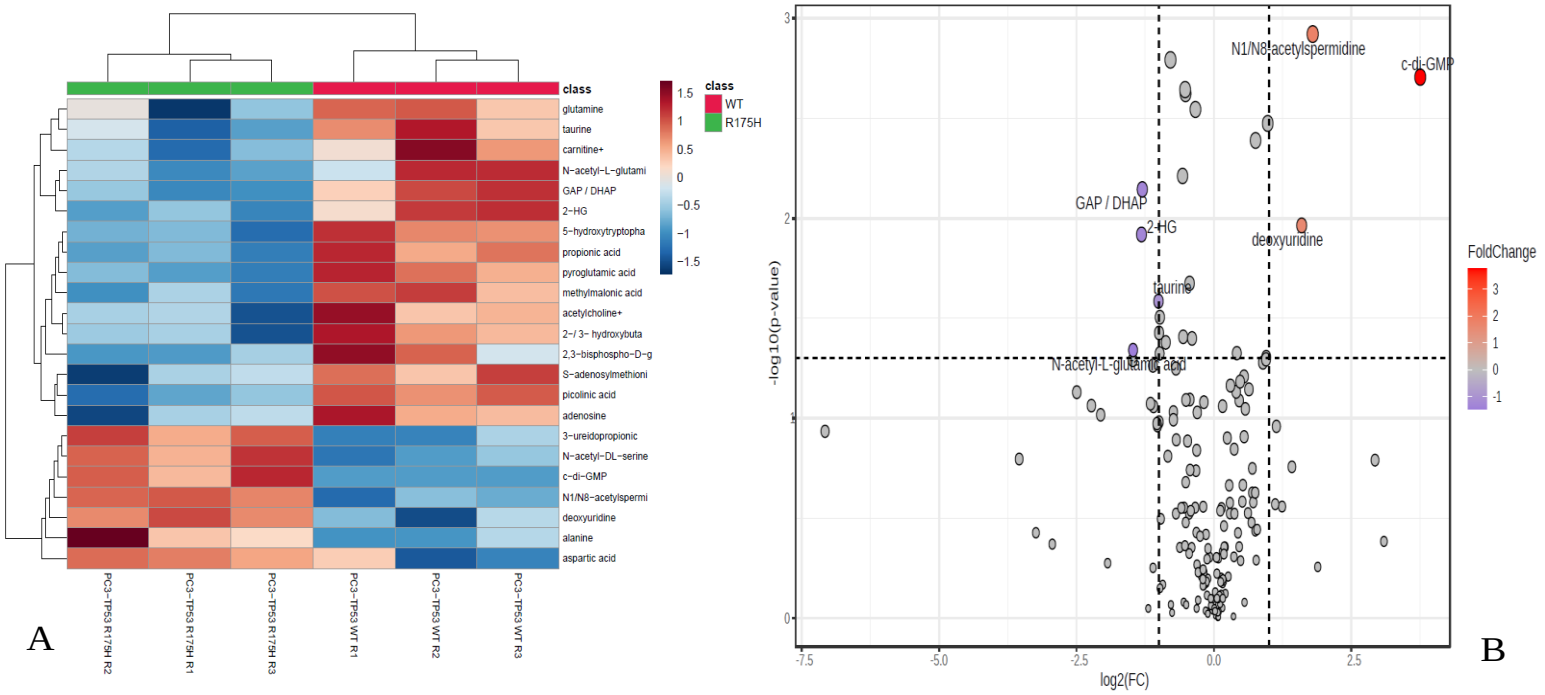

Fig. 3. Expression of numerous metabolites in the PC3-TP53-WT & PC3-TP53-R175H. (A) Heatmap depicting the expression of most dysregulated metabolites. Upregulated metabolites are shown in red, while the downregulated metabolites are in blue (B) Volcano plot comparing the altered metabolites

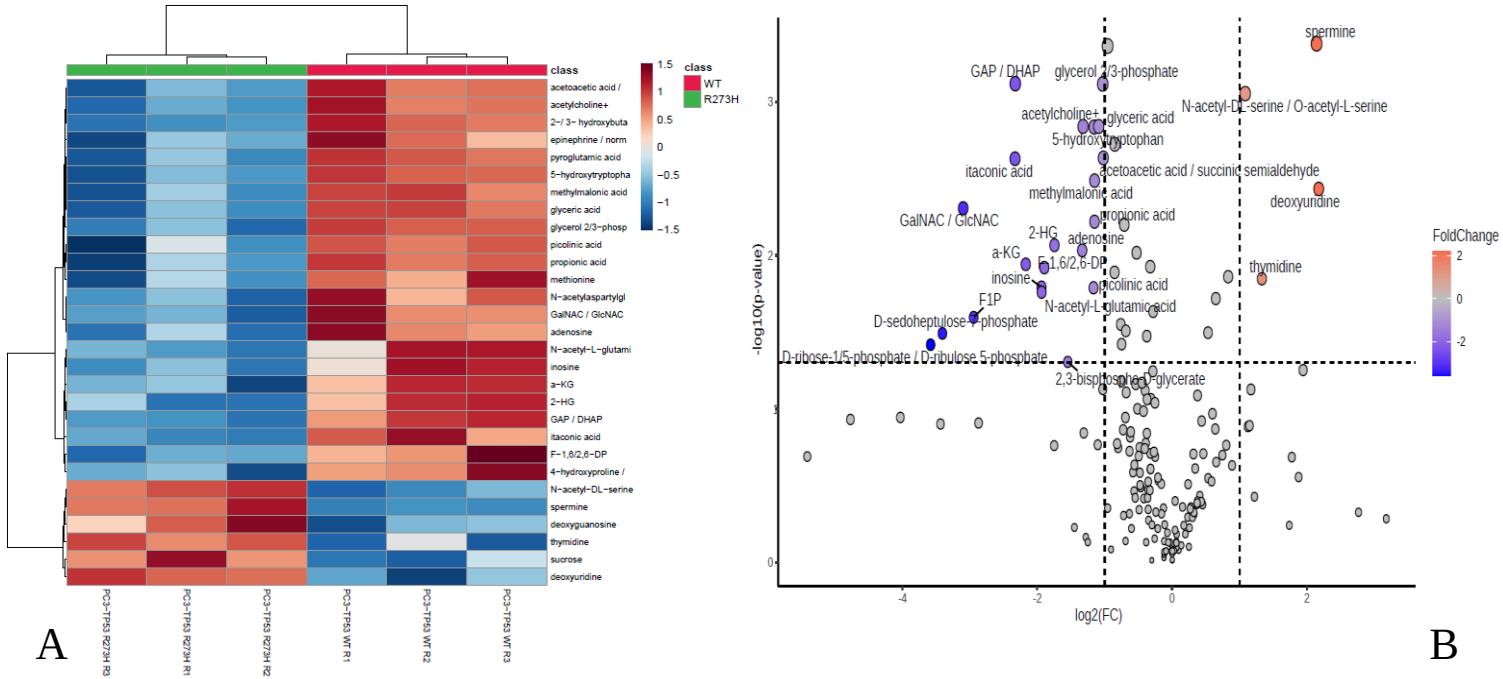

Fig. 4. Expression of numerous metabolites in the PC3-TP53-WT & PC3-TP53-R273H. (A) Heatmap depicting the expression of most dysregulated metabolites. Upregulated metabolites are shown in red, while the downregulated metabolites are in blue (B) Volcano plot comparing the altered metabolites

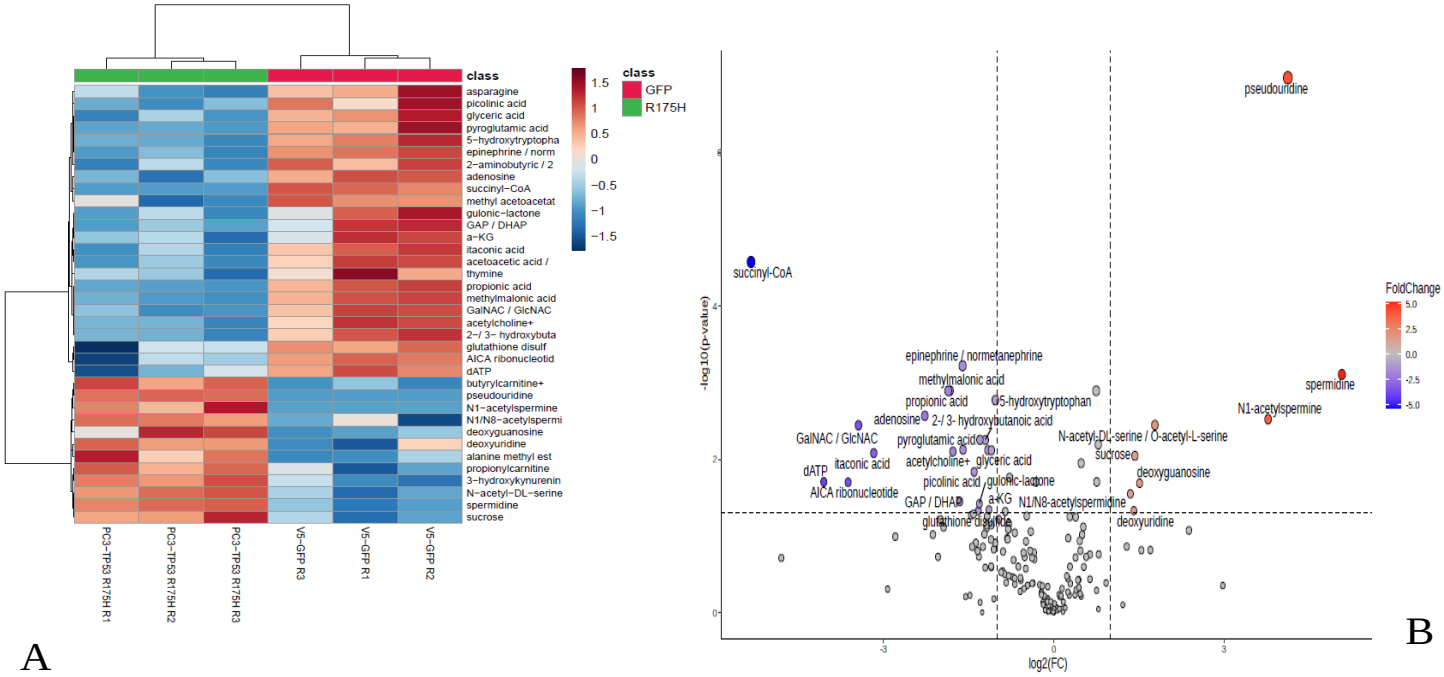

Fig. 5. Expression of numerous metabolites in the PC3-GFP (KO) & PC3-TP53-R175H. (A) Heatmap depicting the expression of most dysregulated metabolites. Upregulated metabolites are shown in red, while the downregulated metabolites are in blue (B) Volcano plot comparing the altered metabolites

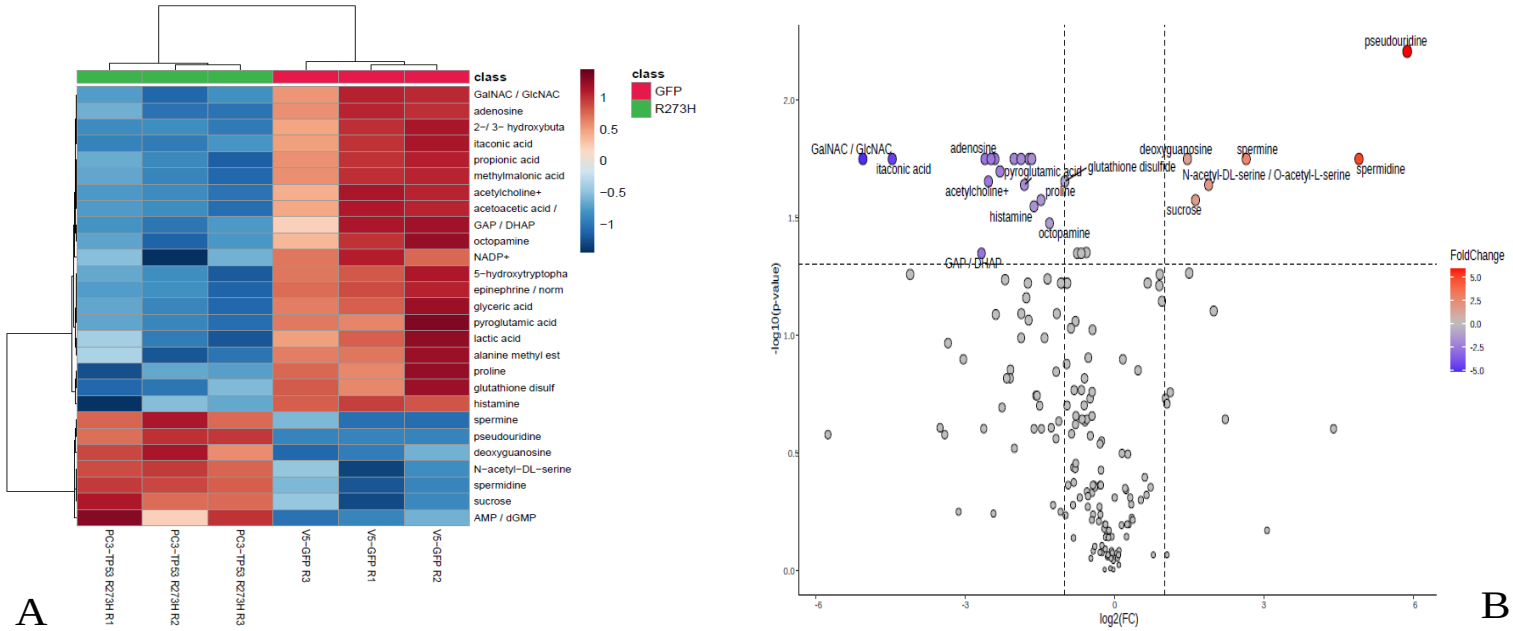

Fig. 6. Expression of numerous metabolites in the PC3-GFP (KO) & PC3-TP53-R273H. (A) Heatmap depicting the expression of most dysregulated metabolites. Upregulated metabolites are shown in red, while the downregulated metabolites are in blue (B) Volcano plot comparing the altered metabolites

4.2.4 Comparison between pc3-gfp (ko) & pc3-tp53-r175h

Metabolites that are upregulated in GFP are found to be downregulated in TP53-R175H, and the reverse is also true. The TP53 mutant (R175H) presence resulted in the downregulation of key TCA cycle and glycolysis intermediates, including α-ketoglutarate/succinyl CoA and GAP/DHAP, respectively. These intermediates are upregulated in GFP. Conversely, metabolites like deoxyguanosine, spermidine, sucrose, and pseudouridine are upregulated in the R175H mutant and downregulated in GFP (Fig. 5A). In the volcano plot, succinyl CoA is the most significantly decreased metabolite, while pseudouridine is the most increased (Fig. 5B).

4.2.5 Comparison between pc3-gfp (ko) & pc3-tp53-r273h

Comparing GFP (KO) to the R273H mutant, metabolites like GalNAC/GlcNAC, adenosine, acetoacetic acid, acetylcholine, octopamine, lactic acid, and NADP+ are downregulated in the R273H mutant but upregulated in GFP. Conversely, spermidine, N-acetyl-DL-serine, pseudouridine, deoxyguanosine, sucrose, and AMP/dGMP are upregulated in the R273H mutant but downregulated in GFP (Fig. 6A). In the volcano plot, pseudouridine and spermidine are the most significantly increased metabolites, while GalNAC/GlcNAC and itaconic acid are the most decreased (Fig. 6B).

5. DISCUSSION

In this study, the generation of stable PC-3 cell lines transduced with vector control (V5-DEST-GFP), V5-TP53-wildtype, V5-TP53-R175H, and V5-TP53-R273H, was confirmed from the result of western blot (Fig. 1). This result agrees with the findings of Yoo et al., which showed a western blot with the indicated proteins in PC-3 cells (TP53 null) expressing vector control (V5-DEST-GFP), TP53-R175H, and TP53-R273H (Yoo, 2023). This study supports the idea that targeting a metabolic vulnerability present in TP53-altered tumors could be a promising therapeutic strategy. We demonstrated that the presence of the PC3-TP53-R175H mutant through its gain-of-function mutation reduces α-ketoglutarate/succinyl COA concentration, thereby promoting prostate cancer progression. This is consistent with a study that showed that P53 wild-type triggers an increase in α-KG/succinate ratio, resulting in specific tumor suppressive effects, while loss of p53 inhibits the accumulation of α-ketoglutarate which leads to the development of more aggressive and less differentiated carcinomas, marked by reduced α-ketoglutarate-dependent activity (Morris, et al., 2019). Therefore, TP53 mutants were shown to downregulate the expression of TCA cycle enzymes, leading to decreased α-KG levels in cancer cells (Morris, et al., 2019). p53 rewires glucose and glutamine metabolism to favor the accumulation of α-KG at the expense of succinate. TP53 mutation may lead to alterations in α-KG levels and its downstream metabolic pathways. The change in succinyl-CoA concentration was reported to affect the development of tumors by regulating tumor metabolism or by acting as a substrate to regulate the level of succinyl modification (Tong, 2021). In this study, N-acetyl-DL-serine was upregulated by both mutants (PC3-TP53-R175H & R273H). This result is in line with the findings of Tombari et al*.,* which showed that missense mutant p53 oncoproteins stimulate de novo serine/glycine synthesis and essential amino acid intake, promoting breast cancer growth (Tombari, et al., 2023). PC3-TP53-R273H mutant downregulates an important metabolite, NADP+, which is crucial for redox balance and cellular defense mechanisms, potentially leading to increased oxidative stress in cancer cells (Hayes, et al., 2020). Consistent with our finding, a study showed that TP53 mutation downregulates the expression of enzymes in NADP+ metabolism, such as glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase (Ju, et al., 2020). This dysregulation can further disrupt the NADP+/NADPH balance and affect various cellular processes, including DNA repair, cell cycle control, and apoptosis. Another study showed that TP53 mutants can lead to an altered NADP+/NADPH ratio, resulting in increased oxidative stress and impaired cellular redox homeostasis (Cordani, et al., 2020). According to our findings, PC3-TP53-R175H & PC3-TP53-R273H mutants downregulate GAP/DHAP. This corresponds with a study that showed that TP53 mutant upregulates glyceraldehyde-3-phosphate dehydrogenase (an enzyme that catalyzed the conversion of GAP to 1,3-bisphosphoglycerate) in breast cancer (Harami-Papp, et al., 2016). Growing evidence showed that the TP53 mutant suppressed the activity of TP53-induced glycolysis and apoptosis regulator (TIGAR), a protein that inhibits glycolysis and promotes the pentose phosphate pathway (Wanka, et al., 2012). This led to a shift in resources away from GAP/ DHAP production. The lowered levels of

GAP/DHAP are reflective of the broader metabolic derangements caused by the p53 mutant in the glycolytic pathway. The two mutants (R175H & R273H) also upregulate spermidine, while TP53-R273H upregulates spermine. This agrees with a finding that TP53 mutants regulate ornithine decarboxylase activity (an enzyme that catalyzes the first step in polyamine synthesis), resulting in increased spermidine production (Xuan, et al., 2023). Other studies discovered a decrease in spermine concentration in prostate cancer patients, suggesting spermine to be an endogenous inhibitor of prostate cancer growth (Bentrad, et al., 2019, Maksymiuk, et al., 2018).

In this study, PC3-TP53-R273H mutant decreased adenosine levels, which corresponds with a finding showing that adenosine inhibits cell growth and induces apoptosis in human prostate cancer cells (Aghaei, et al., 2011). We found that PC3-TP53-R175H downregulates glutamine. This is consistent with the findings of Tran *et al.,* which demonstrated that TP53 mutants promote cancer cell survival upon glutamine deprivation through p21 induction both in vitro and in vivo (Tran, et al., 2017). Pseudouridine level was upregulated by the two mutants (PC3-TP53-R175H & R273H). This agrees with the study which reported that an elevated level of pseudouridine is associated with the incidence and progression of prostate cancer, as well as a novel biomarker in prostate cancer progression to advanced disease (Stockert, et al., 2021). Pseudouridine was shown to enhance the stability and translation of mRNA by modifying RNA structure. This leads to more efficient protein synthesis, which can promote the production of oncogenes (cancer-promoting genes) that drive the uncontrolled proliferation of prostate cancer cells (Stockert, et al., 2021). Further research should be carried out to show how each TP53 GOF mutant contributes to the cells' ability to adapt metabolic alterations to support their growth and survival. Also, some of the metabolites identified, such as pseudouridine and spermidine, could serve as biomarkers for prostate cancer progression and treatment efficacy, especially in patients with TP53 mutations.

6. CONCLUSION

This study investigated the significantly altered key metabolites involved in the major metabolic pathways contributing to tumor progression in prostate cancer cells. Presenting insights into the identification of metabolic alterations that result from TP53 GOF mutation (R175H & R273H) to target prostate cancer therapeutically. These mutants downregulate metabolites such as GAP/DHAP involved in glycolysis and α-ketoglutarate in the TCA cycle, which may promote cancer cell survival. However, the upregulation of metabolites such as spermidine and pseudouridine by these mutants highlights their roles in promoting prostate cancer. In conclusion, this study showed the metabolic alterations driven by TP53-R175H & R273H in prostate cancer cells, which could provide therapeutic benefits. Therefore, the changes in molecules (primarily critical enzymes) responsible for metabolic changes in cells with or without TP53 GOF mutations will further be considered, and the correlation between their expression and TP53 GOF mutations in prostate cancer patients will be examined.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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