Dichloroacetate Reprograms JunD Expression and Enhances Chemosensitivity in Acute Myeloid Leukemia via p53 Status

Sana Belkahla*

Department of Biology, College of Science, King Faisal University, P.O. Box 400 Al-Ahsa, 31982, Saudi Arabia

Abstract: The protein JunD plays an important role in cell proliferation, differentiation and apoptosis. In the context of leukemia, particularly acute myeloid leukemia, JunD overexpression has been implicated in disease progression and cell proliferation. However, JunD can also act as a tumor suppressor in certain cancer cases, thus making its role controversial. Here, we investigated the molecular mechanisms by which dichloroacetate (DCA) modulates JunD expression and its impact on chemosensitivity in acute myeloid leukemia (AML) cells, specifically focusing on the involvement of p53.

The results showed a significant reduction in JUND mRNA levels in OCI-AML3 (wt-P53) cells treated with 1 and 5 mM DCA (48±6.50 and 53±10.17%, respectively; $p< 0.05$) compared to untreated cells. On the other hand, in HL60 a P53-Null cell, a significant increase in JunD expression (132.7±6.02%; $p< 0.05$) was observed when compared to untreated cells. Similar results were observed in OCI-AML3 cells transfected with Si-P53 plasmid to reduce the expression of p53.

Additionally, *JUND* knockdown significantly decreased cell viability (compared to scramble Si-RNA) and further amplified the DCA effect (10.2±1.48%; $p< 0.05$). Similar results were noticed when cells were subjected to doxorubicin at different concentrations. In particular, the combination of DCA and doxorubicin resulted in enhanced anti-leukemic effects, indicating that JunD is essential for modulating the cellular response to doxorubicin.

Overall, we demonstrate for the first time that DCA decreased JunD expression and sensitized leukemic cells to chemotherapy, and these effects may depend on p53 status.

Keywords: Leukemia, Dichloroacetate, doxorubicin, JunD, P53, and chemotherapy.

INTRODUCTION

JunD is one of the key members of the Jun family of transcription factors, which are components of the activator protein-1 (AP-1) complex identified in the 1990s [1, 2]. AP-1 plays a pivotal role in regulating gene expression in response to various physiological and pathological stimuli, such as growth factors, cytokines, stress, bacterial and viral infections, and intracellular PI3K/Akt and MAPK signaling [3]. JunD is encoded by the *JUND* gene, which, like other members of the Jun family (c-JUN and JUNB), influences a wide array of cellular processes, including proliferation, differentiation, transformation and apoptosis [4, 5]. The JunD protein contains a basic leucine zipper domain [6], which allows it to dimerize with other AP-1 family members (such as Fos, ATF or Maf) and bind to DNA at specific AP-1 sites, known as 12-O-tetradecanoylphorbol-13-acetate response elements [7]. These interactions enable JunD to regulate transcription of a variety of target genes. *JUND*, such as c-JUN and JUNB, can positively and negatively influence gene transcription depending on their dimerization partners and cellular context [1, 2, 8].

In cancer biology, *JUND* plays a complex role due to its capacity to function both as an oncogene and as a tumor suppressor depending on the cellular environment [9]. This duality makes *JUND* an important target in cancer therapy. In certain cancer types, including leukemia, dysregulation of JunD can suppress apoptosis, allowing abnormal cells to continue proliferating [10, 11]. JunD's role in leukemia, particularly in acute myeloid leukemia (AML), has attracted notable research interest. In AML, dysregulated expression of JunD alters the balance between cell survival and apoptosis, and it has been shown that overexpression of JunD promotes the regulation of anti-apoptotic genes (such as BCL2) and tumor suppressor genes (such as p53), which may enhance leukemogenesis and block apoptosis in leukemic cells [12, 13]. In addition, JunD is considered a therapeutic target in the treatment of leukemia due to its role in maintaining the stemness of leukemia stem cells, a subset of cells in AML responsible for relapse and chemoresistance [14, 15]. JunD is also implicated in the cellular response to oxidative stress, a factor often elevated in cancer cells, including leukemia [16, 17]. It has been shown that JunD can regulate the expression of genes involved in oxidative stress responses and help cells survive in stressful environments by neutralizing reactive oxygen species (ROS) that would otherwise induce apoptosis or senescence [18].

*Address correspondence to this author at the Department of Biology, College of Science, King Faisal University, P.O.Box 400 Al-Ahsa, 31982, Saudi Arabia; Tel: +966 0135896869; E-mail: Sbelkahla@kfu.edu.sa

ISSN: 1929-2260 / E-ISSN: 1929-2279/26

Currently, some therapeutic strategies based on the inhibition of AP-1 activity to disrupt the dimerization of JunD and other AP-1 family members may be a potential way to reduce cancer proliferation and enhance cell death [19].

The pathogenesis of leukemia frequently results in p53 dysfunction [20]. TP53 mutations typically occur in leukemias, particularly in AML, and are associated with difficult karyotypes, therapy-related illness, and poor outcomes [20, 21]. For example, 10% of AMLs have TP53 mutations while up to 70% of those with a difficult karyotype do [20] TP53 has been altered in approximately 50% of T-ALL cell lines, particularly relapsed T-ALL [22]. These alterations often effected both p53 alleles [22]. TP53 mutations may result in loss of function (LOF) or gain of function (GOF) [23]. LOF mutations affect p53's DNA binding and transcriptional activity that prevents cell cycle arrest, apoptosis, and DNA repair [21, 23]. In contrast, GOF mutations may increase LIC self-renewal and work with other oncogenes or pathways to produce leukemia [20, 23]. In leukemia, mutant p53 activates inflammatory pathways that allow leukemia cells to escape immune-mediated death [24] In addition to direct mutational events affecting TP53, the increased expression of the MDM2 of MDMX proteins serve to suppress p53 transcriptional activity in leukemia [25]. A p53 deficiency is much higher in adult AML than TP53 mutations [26]. Specifically, p53 pathway inactivation is leukemogenic in the development of adult AML [25].

The majority of cancer cells rely on glycolysis for energy production, even in the presence of oxygen, a phenomenon known as the Warburg effect [27]. This is inefficient but helps cancer cells survive and proliferate.

Dichloroacetate (DCA) is a small chemical compound known for its ability to shift cancer cell metabolism by inhibiting pyruvate dehydrogenase kinase (PDK), an enzyme that regulates the conversion of pyruvate into acetyl-coenzyme A [28]. DCA shifts the cell metabolism from glycolysis back to oxidative phosphorylation in the mitochondria. By restoring mitochondrial function, DCA encourages cancer cells to undergo apoptosis [28].

Previous research on DCA's impact has shown that DCA reduces the expression of hypoxia-inducible factor 1α [29-32], a key regulator of metabolic adaptation, which can affect downstream transcription factors (potentially including JunD) by modifying cell survival pathways [33]. Additionally, DCA has been shown to affect oxidative stress and ROS levels [34] through pathways involving Nrf2 [35-37], a regulator of antioxidant responses. Since JunD is part of the AP-1 complex, which can respond to oxidative stress, the hypothesis that DCA's modulation of the redox balance may indirectly alter JunD activity is plausible. Further research is needed to explicitly confirm DCA's direct effects on JunD expression in leukemia and other cancer types. However, given its impact on metabolic and apoptotic pathways, DCA may influence the broader regulatory environment in which JunD operates. Nevertheless, no study has linked DCA-induced JunD expression to p53 dependency in AML. The present study is the first comprehensive report to evaluate the impact of DCA on JunD expression and cell proliferation, and study the effect of JunD downregulation on sensitivity to doxorubicin.

MATERIALS AND METHODS

Cell Lines, and Cell Culture

The leukemic human cell line OCI-AML3 (RRID:CVCL_1844) is a human acute myeloid leukemia (AML) cell line that originated from the peripheral blood of a 57-year-old male subject diagnosed with an acute myeloid leukemia (AML FAB M4). OCI-AML3 possesses a lymphoblastoid shape and features appropriate for suspension culture.

HL-60 (RRID: CVCL_0002) is a human promyelocytic leukemia cell line isolated from a 36-year-old female peripheral blood with acute promyelocytic leukemia. HL-60 is a widely used in research and for studying myeloid differentiation, immune responses, and cancer biology.

OCI-AML3 (ACC 582) and HL60 (CCL-240) were purchased from DSMZ and American Type Culture Collection respectively and cultured in RPMI 1640-Glutamax medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS. All cell lines were routinely tested for mycoplasma contamination and authenticated by STR profiling by the suppliers (DSMZ and ATCC).

Transfection Conditions

OCI-AML3 and HL60 cells were transfected with small interfering RNA (siRNAs) at a concentration of 30 nM for 48 h using Lipofectamine RNAi Max (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. siRNAs specific to *JUND* (cat. no. sc-35728) or si-P53 (ON-TARGETplus; SMARTpools, from Dharmacon cat. no. 4390824) were used to silence the target genes. siRNA (cat. no. sc-

37007) and control siRNA (siCtrl; ON-TARGETplus; SMARTpools from Dharmacon cat. no. 4390843), which had no considerable homology to any known human sequence, were transfected as the negative controls.

Reverse Transcription-Quantitative PCR (RT-qPCR)

Total RNA was extracted using NucleoSpin RNA isolation columns (Macherey-Nagel GmbH), and RT was carried out using iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, Inc.). qPCR was performed with KAPA SYBR Green qPCR SuperMix (CliniSciences) and a CFX Connect™ Real-Time qPCR system (Bio-Rad Laboratories, Inc.). The RT-qPCR thermocycling conditions were as follows: An initial step of 10 min at 95˚C, followed by 35 cycles of 15 sec at 94˚C and 60 sec at 60˚C. All samples were normalized to β-actin mRNA levels. The ΔΔCt method was used for relative quantification. The results were expressed as relative to control values arbitrarily set at 100. The primers used were as follows: *JUND* forward 5'-GTGCCCAGGAACTCAGAGAG-3' and reverse 5'-TAAAGGAAAGGCAGGGTTTG-3'; and β-actin forward 5'-GAGGGAAATCGTGCGTGACA-3' and reverse 5'-AATAGTGATGACCTGGCCGT-3'.

Western Blotting

The cells were washed with ice-cold TBS buffer (20 mM Tris, pH 7.5, 150 mM NaCl) and lysed in RIPA buffer (50 mM Tris-HCl, pH 8, 150 mM NaCl, 1% Nonidet P40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM sodium orthovanadate and 1 mM NaF) for 1 h at 4˚C under constant agitation. The cell lysate supernatant was recovered by centrifugation at 16,000 x g for 25 min at 4˚C. The protein concentration was measured with a BCA protein assay kit (MOLEQULE-ON) according to the manufacturer's instructions. Next, supernatant was mixed with an equal volume of 2X Laemmli's buffer (4% SDS, 20% glycerol, 0.004% bromophenol blue and 125 mM Tris-HCl) supplemented with 10% dithiothreitol, followed by heating for 5 min at 95˚C. Subsequently, 50 µg of protein was subject of one-dimensional 10% SDS-PAGE, and the separated proteins were electrophoretically transferred to nitrocellulose membranes (MOLEQULE-ON) for 2 h at 0.8 mA/cm^2 in a semidry blotter. The membranes were blocked in TBS-Tween (TBST) buffer (20 mM Tris, pH 7.5, 150 mM NaCl and 0.1% Tween 20) supplemented with 5% BSA for 1 h at room temperature. The blocked membranes were washed twice with TBST and incubated with primary anti-JunD antibody (1 µg/ml; cat. no. #720035; Thermo Fisher Scientific, Inc.) for 3 h at room temperature and anti-β-actin antibody (1:5,000; C4 clone; Santa Cruz Biotechnology, Inc.) for 1 h, which was used as a loading control for normalization. Target proteins were detected with Immobilon Western Chemiluminescent HRP Substrate (MilliporeSigma; Merck KGaA). A LI-COR detector (LI-COR Biosciences) was used for quantification of the detected bands.

MTT Assay

Cells (1x10^4/per well) were seeded in 96-well tissue culture plates. After overnight incubation, the culture medium was replaced with fresh medium containing 1 or 5 mM DCA. After 7 days of incubation, media was replenished every 48 hours to ensure nutrient availability and stable drug concentrations.

MTT assay was performed by replacing the medium with 50 µl 1 mg/ml MTT solution, and the plates were incubated in the dark for 3 h. The MTT solution was then removed, and the dark blue formazan precipitates were dissolved in 100 µl propan-1-ol. Next, the optical density at 570 nm was measured using a microplate reader. Untreated and DCA/DOX/Si-JUND treated cells as negative and positive controls for viability, respectively.

Statistical Analysis

Statistical analysis was performed using ANOVA followed by Bonferroni's correction for comparisons between multiple groups, while Student's t-test was used for comparisons between two groups, and Tukey post hoc test was applied after ANOVA for multiple comparisons.The experiments were performed at least three times in triplicates. $P<0.05$ was considered to indicate a statistically significant difference. Data are presented as mean ±Standard Error of the Mean (SEM).

Statement: This study was deemed exempt from formal ethics approval by our institutional ethics committee, as it exclusively involved established human cell lines and did not include human subjects, human tissue, or identifiable human data, thus falling outside the scope of research requiring specific approval numbers.

RESULTS

DCA Regulates JunD Expression

The present study examined the impact of DCA on *JUND* gene expression in the OCI-AML3 AML cell line.

In particular, 1 and 5 mM DCA concentrations were used, which are in line with plasma levels detected in patients receiving DCA, which are close to 0.5 mM [38, 39]. After DCA treatment, the present results revealed decrease in *JUND* mRNA levels by 48%±6.5% for 1mM and 53%±10.17% for 5mM. Compared to control cells, *JUND* transcript levels were significantly lower upon exposure to 1 and 5 mM DCA concentrations according to qPCR analysis (Figure **1A**). Notably, the decrease was more marked at the higher dose, suggesting that the effect was dose dependent. Interestingly, an opposite effect was noted in HL60 cell. In fact 1 and 5 mM DCA treatment increases significantly JUND mRNA to 122.6%±3.1% and 131.5%±3.34%, respectively.

In the present study, a specific anti-JunD protein antibody was employed in western blot analysis to validate the results observed at the mRNA level. As shown in Figure **1B**, the results verified that DCA administration significantly decreased the expression of JunD protein in OCI-AML3 and significantly increased in HL60 cells. Densitometric analysis of the protein bands demonstrated a distinct dose-dependent decline/increase in JunD protein levels, which corroborated the present findings on mRNA expression. Both the mRNA and protein levels of *JUND* were moderately reduced at 1 mM DCA, whereas both were significantly reduced at 5 mM DCA.

DCA Reduces JUND Expression through p53

As previously described [40], a particular siRNA targeting p53 (si-P53) was used in the present study to transduce OCI-AML3 cells in order to evaluate the function of p53. Using three distinct cell groups (cells transfected with si-P53, cells transfected with siCtrl and non-transfected cells), the impact of DCA treatment on JunD expression was studied. When exposed to DCA, cells treated with siCtrl showed a marked downregulation in JunD mRNA and protein levels, which was comparable to the results observed in non-transfected cells (Figure **2**). On the other hand, after DCA treatment, si-P53-transduced cells showed a significant increase in JunD expression. These cells exhibit behavior akin to that of cell lines devoid of wild-type p53 expression, which results in an overexpression of JunD. Increased JunD levels in response to DCA appear to be caused by a disruption in p53 signaling leading to altered JunD regulation.

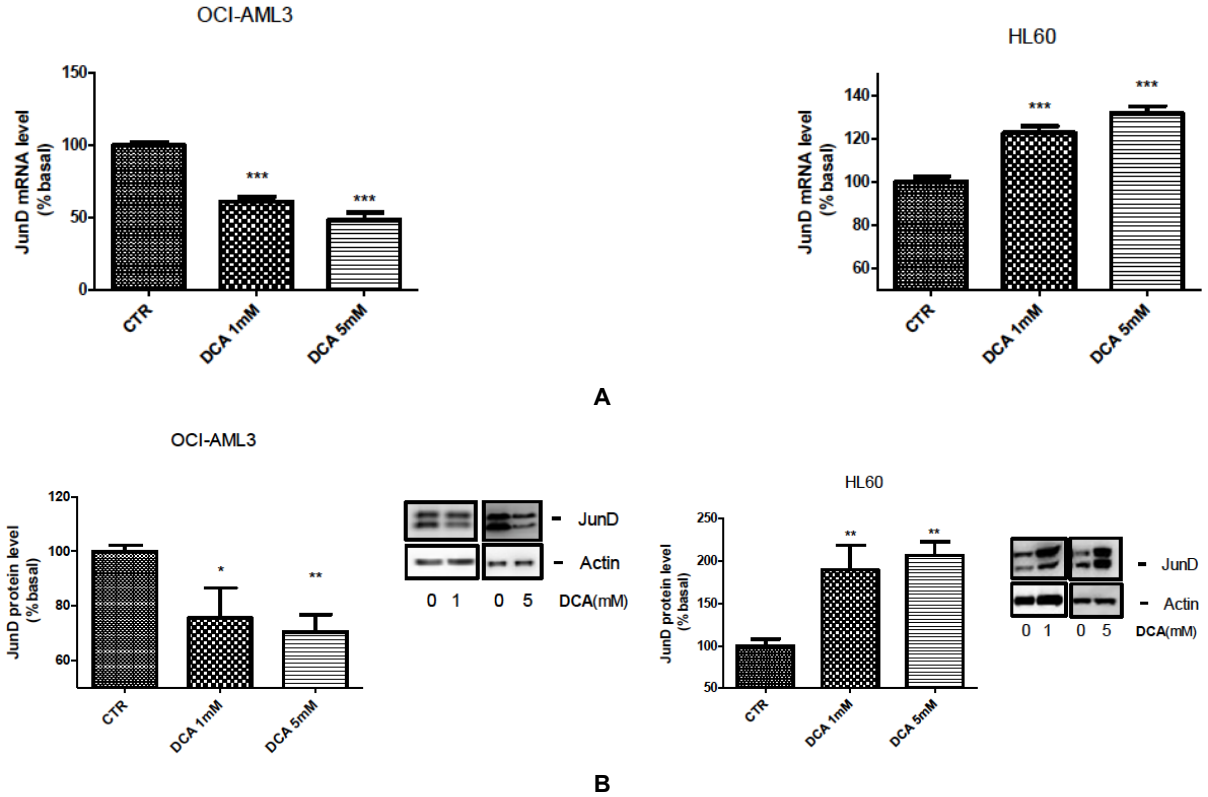

Figure 1: DCA-induced JUND expression in leukemic cells.

Different hematopoietic cell lines, including (**A**) OCI-AML3 wild-type p53 and (**B**) HL-60 null p53, were treated with 1 and 5 mM DCA for 1 week. The RNA levels and protein levels were analyzed by quantitative PCR and western blotting, respectively. The bar graphs represent the mean ± SEM of three independent experiments performed in triplicate. *P<0.05, **P<0.01, ***P<0.001 compared to untreated cells. DCA, dichloroacetate.

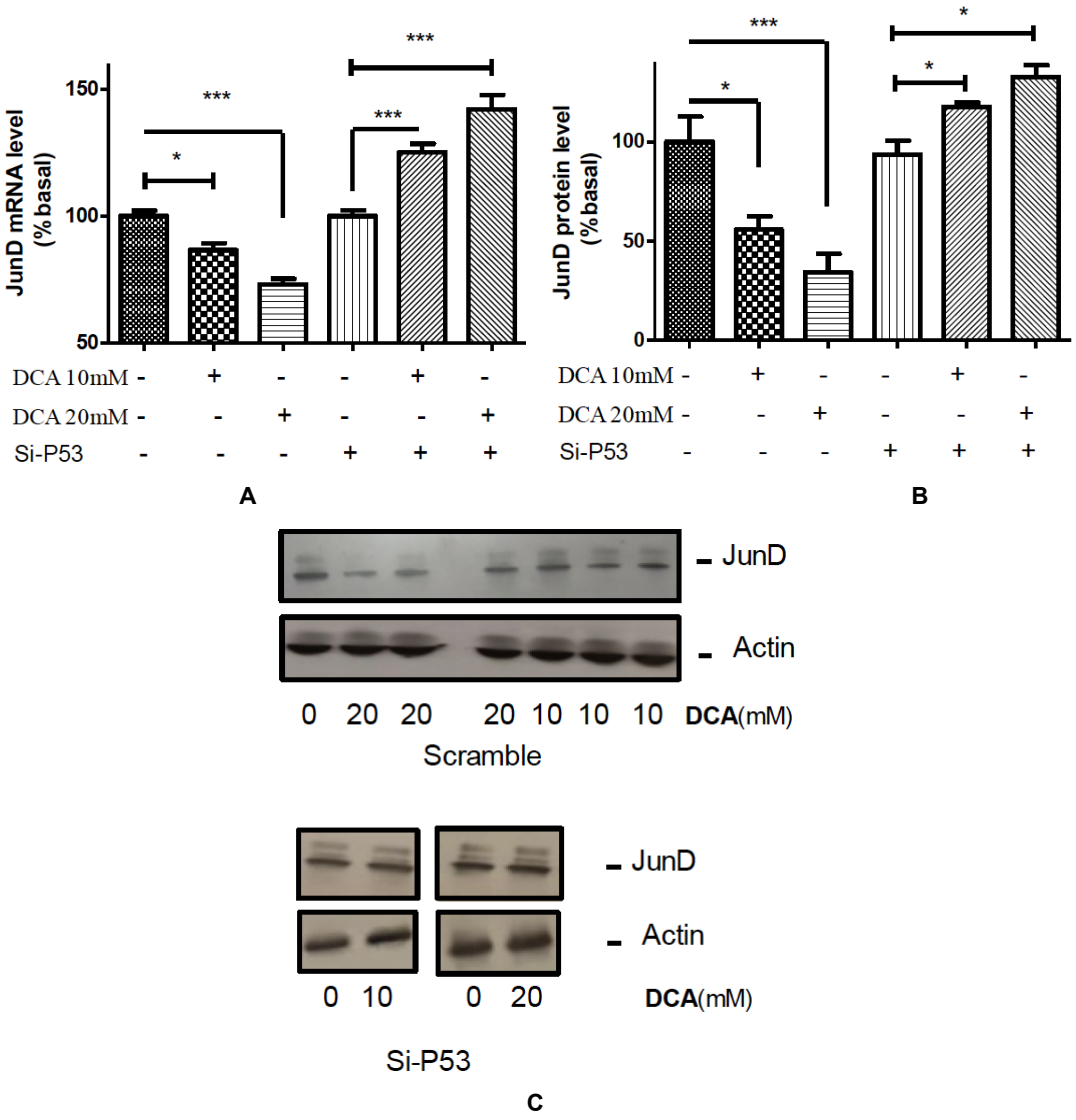

Figure 2: P53 knockdown prevents DCA-induced JUND upregulation.

OCI-AML3 cells were transfected with siRNA for p53 or control (scramble siRNA, and 24 h later were treated with 10 and 20 mM DCA for 24 h. The protein and mRNA levels were quantified as described in Figure 1. The bar graphs represent the mean ± SEM of three independent experiments performed in triplicate. *P<0.05, **P<0.01, ***P<0.001 compared to control cells (cells transfected with scramble Si-RNA). DCA, dichloroacetate; siRNA, small interfering RNA.

JunD Inhibition Reduces Cancer Cell Viability

It was hypothesized that DCA may decrease the expression of JunD and reduce cell proliferation. To test this hypothesis, DCA was administered at doses of 1 and 5 mM for 1 week to the AML cell line OCI-AML3. The findings showed that treatment with 1 and 5 mM of DCA decreased cell viability by 10%±0.70% and 13.75% ±0.47% respectively (Figure **3A**).

Additionally, knockdown experiments were conducted to examine the function of JunD. It was found that the cancer cells' viability significantly decreased when JunD expression was inhibited. When cells were treated with DCA, this impact was further amplified, suggesting that DCA therapy and JunD suppression possibly work in concert. According to these findings, JunD targeting may enhance DCA's anticancer effects, making it a strong option for improving treatment efficacy in AML. Together, the present results demonstrate that DCA has a marked anti-proliferative effect that is enhanced by JunD knockdown in addition to reducing JunD expression.

Downregulation of JUND may Affect Chemoresistance and Enhance Cell Death

In the present study, siRNA targeting *JUND* was used to effectively downregulate its expression in cancer cells. Cells were then subjected to doxorubicin

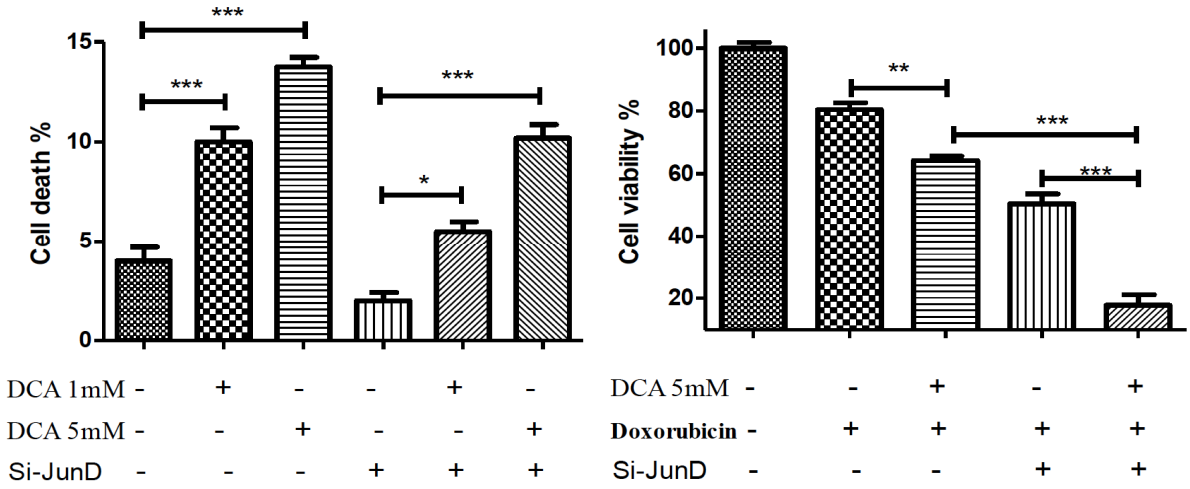

Figure 3: DCA reduces JUND expression through p53.

OCI-AML3 cells were treated with 1 or 5 mM DCA for 7 days, and then the cells were transfected with a small interfering RNA specific for JUND 48 h before treatment. Cells were then incubated with 5 μM doxorubicin. The bar graphs represent the mean ± SEM of three independent experiments performed in triplicate; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to control cells. DCA, dichloroacetate.

treatment at different concentrations. Compared to cells treated with doxorubicin alone (80%±2.29%), cells with downregulated *JUND* showed a significant reduction in viability by 50.4%±3.12% ($p<0.05$) when treated with doxorubicin (Figure **3B**).

DISCUSSION

Leukemia is a type of cancer that affects the blood and bone morrow. It typically develops from the uncontrolled proliferation of white blood cells due to various factors such as inflammation, oxidative stress, DNA damage and genetic mutations that disrupt normal gene regulation and the cell cycle [41]. The exact molecular mechanisms behind the initiation and progression of leukemia are still being uncovered.

In a previous study, our group highlighted the critical role of DCA in the proliferation of blood cancer cells and its regulation of numerous genes that are necessary for cell cycle progression and chemoresistance [40]. DCA is a small molecule that has shown a potential role in modifying the expression of various proteins, and plays a critical role in cellular apoptosis by inhibiting the enzyme PDK, which leads to the activation of pyruvate dehydrogenase. This metabolic change from anaerobic glycolysis (the Warburg effect) back to oxidative phosphorylation leads to a reduction in lactic acid production and may modify various signaling pathways, including those related to ROS production, mitochondrial function and apoptosis, all of which may indirectly impact the expression of transcription factors, which can in turn regulate the expression of target genes involved in cell proliferation, differentiation and programmed cell death, thus enhancing carcinogenesis [42]. Our group previously confirmed that DCA increased ROS generation and stimulated p53 activation [43]. This metabolic drug can activate stress-response pathways, which can affect the activity of several transcription factors. In cancer cells, DCA-induced metabolic stress can lead to altered gene expression profiles, where JunD upregulated or modulated as part of the cellular response to oxidative stress.

Members of the AP-1 transcription factor family play a crucial role as cancer oncogenes [44-46]. AP-1 is a complex formed by JUN and FOS family members, which are well known for its ability to control almost all-cellular process from cell proliferation, differentiation to apoptosis [46-49]. The dimerization of AP-1 family members can result in the transcription of various genes, ultimately activating multiple biological functions [49-52]. Previous studies indicated that activation of JUN family members enhanced cellular proliferation and tumor formation by stimulating the expression of cell cycle control genes such as cyclins D1, A and E [45,52,53]. Similarly, depending on the cell's context, the inhibition of multiple AP-1 family members leads to a decrease in cyclins expression and inhibition of cell proliferation [52-54]. Increased expression of JUN family members has been observed in various aggressive cancer types, including breast cancer [55,56], lymphoma [57], colorectal adenocarcinoma [58] and prostate cancer [51,59]. To understand the role of JunD as suppressor or promoter of leukemia, cell viability was analyzed in the present study using an AML cell line treated or not with a *JUND*-silencing

vector. The results indicated that JunD expression is associated with cell proliferation, and its inhibition reduces the proliferation of leukemic cells by enhancing cell death. These results are in agreement with those from previous studies that have highlighted the complex role of JunD in leukemia, particularly in myeloblastic leukemia [60], adult T-cell leukemia/lymphoma and cutaneous T-cell lymphoma (CTCL), which suggest that JunD dysregulation enhances leukemogenesis and is linked to aggressive forms of leukemia.

It was previously shown that JunD influenced the transcription of genes involved in apoptosis and cell cycle regulation, which are crucial in leukemia progression. For example, JunD can regulate genes such as p53 (a tumor suppressor) and BCL2 (anti-apoptotic), which play an important role in leukemia cell survival. Disruption of these pathways due to dysregulated JunD expression can lead to the uncontrolled proliferation of leukemic cells [61]. JunD is also involved in upregulating genes such as CCR4, MYB, MDM2 and BCL6, which are crucial in the proliferation of leukemic cells. Knockdown of *JUND* via siRNA in certain leukemia and CTCL cell lines has been shown to significantly reduce cell proliferation, thus highlighting its role as a promoter of tumorigenesis in these cancer types [62]. These observations make JunD a potential therapeutic target, especially in aggressive forms of leukemia and lymphoma. Conversely, in certain hematological malignancies, JunD has been reported to act as a tumor suppressor. For example, in T-cell acute lymphoblastic leukemia, decreased JunD expression has been linked to increased leukemogenesis, suggesting that JunD may inhibit the progression of leukemia in certain circumstances by regulating pathways involved in cell cycle arrest and apoptosis. A study published by Weitzman *et al.* [63] in 2000 found that JunD can suppress cell transformation and proliferation in this type of leukemia, functioning as a counterbalance to oncogenic signaling.

In another context, it was shown that AP-1 proteins are implicated in cellular transformation and are associated with poor clinical outcomes in prostate cancer [51,53]. Previous research suggests that inhibition of JunD reduces prostate cancer cell proliferation [60, 64] due to the downregulation of genes involved in cell cycle arrest. In addition, it was confirmed that JunD is overexpressed in pancreatic cancer and enhances pancreatic β cell survival during metabolic stress [65].

JunD has been evaluated as a crucial player in metabolic diseases [66-69]. Angel and Karin [66] suggested that JunD modulates apoptosis and oxidative stress in pancreatic β cells. Due to its role in the control of lipids metabolism in hepatocytes and cardiomyocytes, JunD was reported to be a fundamental player in lipid metabolism and insulin secretion. By silencing JunD expression, these authors showed altered lipid accumulation and confirmed the regulatory role of JunD in pancreatic β cells. In addition, Hilfiker-Kleiner *et al.* [70] suggested that knockout of JunD *in vivo* enhanced the mortality rate in mice, and induced the apoptosis of cardiomyocytes and fibrosis. It was also confirmed that inhibition of JunD expression could upregulate hypoxia-inducible factor-1α, vascular endothelial growth factor p53 and BAX protein, and downregulate the expression of BCL2 protein .In addition, it was shown that JunD protected the pressure-overloaded heart from cardiac apoptosis [69].

It was confirmed that several biological processes, including cell cycle control/regulation, cell survival, cell morphology and cellular motility, were found to be altered in JunD-deficient cells in both transcriptomic and proteomic analyses [71]. By shifting the metabolic balance towards apoptosis (via oxidative stress or mitochondrial dysfunction), DCA may modulate JunD's role in this pathway, either by downregulating its expression or altering its functional role in cell survival. This makes DCA a compound of interest in cancer research, particularly in targeting metabolic pathways that sustain tumor growth. To explore the role of DCA and its efficiency as an anticancer treatment, and elucidate by which pathways DCA can enhance apoptosis, the present study analyzed the mRNA and protein levels in leukemic cells treated with DCA, and it was shown that DCA reduced JunD expression in leukemic cell lines (OCI-AML3) with wild-type p53, which led to decreased cell proliferation and enhanced apoptosis. An opposite effect was shown with HL60 cells, which is a null p53. To understand if the effect of DCA as a downregulator of JunD depends on p53 status, OCI-AML3 cells were transfected with a plasmid to reduce the expression of p53. The results revealed an opposite effect similar to the present results confirmed in null p53 HL60 cell lines.

The current results are in agreement with those of Weitzman *et al.* [72], who confirmed that primary fibroblasts lacking JunD displayed p53-dependent proliferation arrest, enhanced expression of p19 (Arf) and premature senescence. By contrast, JunD-

deficiency in immortalized cell lines induced cell proliferation and could upregulate cyclinD1 levels. Furthermore, the authors confirmed that UV irradiation of fibroblasts with *JUND* (-/-) upregulated p53 expression, which enhanced cell apoptosis. The anti-apoptotic role of JunD was confirmed *in vitro* and in an *in vivo* model of hepatitis. Those findings suggest that JunD protects tumor cells from cell death and senescence due to oxidative stress by acting as a gene regulator of the signaling pathways that link Ras to p53 [72]. These beneficial effects of JunD inhibition make it a promising therapeutic target to reduce tumor growth and activate apoptosis pathways.

In previous studies, our group has confirmed that the effect of DCA depends on p53 status. It was demonstrated that wild-type p53-negative cells were more sensitive to the combination of chemotherapeutic and metabolic drugs [73-75]. These findings indicate that the tumor's p53 status influences the effectiveness of co-treatments involving these therapies. Of note, p53 status is routinely assessed in patients with cancer at most hospitals, which may help clinicians select the most appropriate treatment. The present study used DCA concentration of 1 mM similar to those used in the clinic [76,77], the 5 mM dose was used to establish the mechanism.

To better understand the critical role of JunD in chemotherapy, the effect of doxorubicin, a chemotherapeutic molecule used in the clinic to treat leukemia, was evaluated. The present results showed that leukemic cells with higher JunD expression tended to be more resistant to chemotherapeutic drugs such as doxorubicin. However, after downregulation of JunD expression, leukemic cells tended to be more sensitive to doxorubicin, and the rate of apoptosis increase depended on JunD expression. It was also shown that a combined treatment based on the use of metabolic drugs (DCA) and chemotherapy could be more efficient in killing leukemic cells. A previous study by Ishikawa *et al.* [78] explored the role of JunD in leukemia and chemoresistance. The authors found that JunD expression was associated with increased resistance to chemotherapy in AML. In particular, the study highlighted that overexpression of JunD in leukemic cells led to resistance to cytotoxic drugs such as doxorubicin and etoposide, which suggests that JunD could be involved in the survival mechanisms of leukemic cells, contributing to poor responses to conventional chemotherapy. Previous studies demonstrated that DCA, when combined with other drugs such as PX-478, not only targets metabolic pathways but also enhances apoptosis and reduces cellular proliferation. In ovarian cancer models, DCA has shown the ability to reverse resistance to chemotherapy drugs such as cisplatin by affecting gene expression networks.

Since overexpression of PDK was confirmed to be associated with tumor chemoresistance, co-treatment based on the use of DCA and traditional chemotherapy has been evaluated in several cancer types. It could be hypothesized that PDK inhibition may be a way to overcome drug resistance for cancer treatment. PDK2 isoform has been associated with the development of paclitaxel resistance in non-small-cell lung cancer (NSCLC). Notably, DCA plus paclitaxel were able to kill resistant cells better more efficiently than either DCA or paclitaxel alone [72]. Similarly to the findings in NSCLC, an *in vivo* study on bladder cancer revealed higher expression of the PDK4 isoform in high-grade tumors compared to those of lower grade. Additionally, the combined treatment of DCA and cisplatin significantly reduced tumor volumes compared to either treatment alone [73]. Another recent study further supported DCA's ability to reverse PDK4-related chemoresistance in human hepatocellular carcinoma.

Similar, a treatment based on the use of DCA with doxorubicin against hepatocarcinoma suggested that DCA alters the antioxidant defense and activates apoptosis [79]. The synergic effect of DCA and doxorubucin forces tumor cells to better response to the treatment, and makes them chemosensitive to chemotherapeutics drugs. For example, DCA used in combination with the antibiotic salinomycin, which has been demonstrated to be a potential anticancer agent, have been evaluated together in colorectal cancer cells. The two drugs appear to work synergistically, boosting their effectiveness by suppressing proteins that contribute to drug resistance in cancer cells [80].

Our results indicate that patients can be classified for treatment effectiveness based on their p53 status. Because p53 is routinely evaluated in AML patients and has been proven to impact survival and the response to treatment [20] [21], we suggest that p53 could be a potentially valuable marker to predict who will respond to DCA-based therapies. Our results indicate that patients with wild-type p53 AML may be a particularly well-suited group for treatment with DCA because the use of DCA successfully decreased JunD protein expression and increased chemosensitivity in this group. Therefore, a combination of DCA with standard chemotherapeutic agents (e.g., doxorubicin)

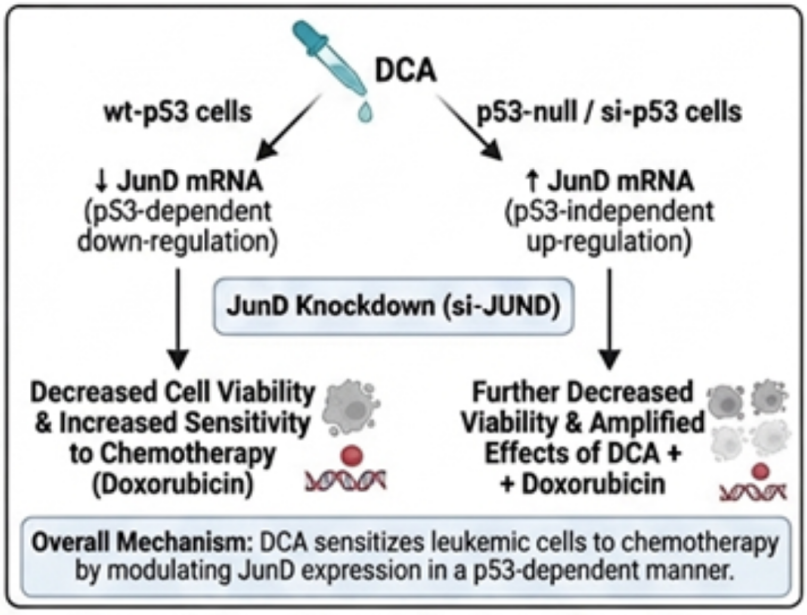

Figure 4: Schematic diagram illustrating the proposed DCA–p53–JunD axis.

could improve the effectiveness of these agents by simultaneously targeting metabolic functions as well as cellular pathways that promote cell survival. In contrast, we recommend an alternative or modified treatment strategy for AML patients with p53 mutations or p53 null). For this sub-group, DCA actually resulted in a compensatory increase in JunD expression; thus, future studies should be conducted that test DCA in conjunction with agents that inhibit JunD as well as agents that target other survival pathways activated by the absence of functional p53. Therefore, stratifying AML patients based on their p53 status represents a promising opportunity in the field of personalized medicine to help overcome therapeutic resistance; which currently remains a major barrier to successful treatment of AML patients.

In summary, the present study has demonstrate direct association between p53, JunD and DCA in the context of AML. The capacity of DCA to suppress JunD expression offers a potentially effective treatment approach, especially when combined with methods that target additional cancer cell survival pathways (Figure **4**).

The current study present some limitations that should be taken into account. First, the results are limited in their generalizability since they rely on two leukemic cell lines and mostly use *in vitro* models, which might not adequately represent the intricate interactions *in vivo*. The *in vivo* validation of our findings is necessary. This will require comprehensive studies in relevant animal models of AML to assess the efficacy, as well as the pharmacokinetics and safety profile of the DCA-based combination therapy. Furthermore, the study lacks deep mechanistic understanding of the ways in which DCA influences signaling pathways, more research is required, especially with regard to the specific downstream and upstream targets of *JUND* in connection with DCA and Dox therapy. Our study shows that there is a p53 dependent regulation of JunD due to DCA treatment, but we have not yet identified the exact mechanisms involved. We do not know at this time if p53 directly regulates JUND transcription, or if the effects of p53 on JUND transcription are due to other signalling pathways. We believe that DCA can create metabolic stress in p53-wild type cells, and p53 can be activated by the subsequent increase in reactive oxygen species or changes to mitochondrial function from the DCA treatment. Once activated by metabolic stress, p53 may regulate the transcription of JUND directly by binding to specific motifs in the JUND promoter, or alternatively, p53 may indirectly have an effect on JUND transcription by modulating upstream signalling pathways that regulate AP-1 activity. In p53-null cells, there may be alternative stress response pathways up-regulating JunD to allow cell survival when undergoing metabolic changes from DCA treatment due to the absence of the p53-mediated regulatory axis. Use of chromatin immunoprecipitation to determine direct binding of p53 to the JUND promoter and closely monitoring increases in reactive oxygen species, as well as signalling pathways activated by the DCA treatment, will be necessary to determine the molecular mechanisms involved.

The clinical translation will necessitate a focus on optimizing dosing strategies to achieve effective concentrations (likely closer to 1 mM) while rigorously monitoring for and minimizing adverse effects.

In conclusion, the present findings open the door for further research into DCA and JunD-targeted treatments, which may greatly improve patient outcomes in such a difficult disease as AML.

DECLARATION OF CONFLICTING INTERESTS

Authors declare not conflict of interest.

FUNDING

This work is not funded.

ACKNOWLEDGEMENTS

Authors acknowledge the laboratory of molecular genetics and genome, college of veterinary medicine, King Faisal University for the use of their facility.

DATA AVAILABILITY STATEMENT

Data are available upon request from corresponding author.

REFERENCES

[1] Jafri Z, Li Y, Zhang J, *et al*. Jun, an Oncological Foe or Friend? Int J Mol Sci 2025; 26(2): 555. https://doi.org/10.3390/ijms26020555

[2] Milde-Langosch K. The Fos family of transcription factors and their role in tumourigenesis. Eur J Cancer 2005; 41(16): 2449-61. https://doi.org/10.1016/j.ejca.2005.08.008

[3] Selvaraj N, Budka JA, Ferris MW, *et al*. Extracellular signal-regulated kinase signaling regulates the opposing roles of JUN family transcription factors at ETS/AP-1 sites and in cell migration. Mol Cell Biol 2015; 35: 88-100. https://doi.org/10.1128/MCB.00982-14

[4] Millena AC, Vo BT, Khan SA. JunD Is Required for Proliferation of Prostate Cancer Cells and Plays a Role in Transforming Growth Factor-beta (TGF-beta)-induced Inhibition of Cell Proliferation. J Biol Chem 2016; 291: 17964-76. https://doi.org/10.1074/jbc.M116.714899

[5] Barrett CS, Millena AC, Khan SA. TGF-beta Effects on Prostate Cancer Cell Migration and Invasion Require FosB. Prostate 2017; 77: 72-81. https://doi.org/10.1002/pros.23250

[6] Shaulian E. AP-1-The jun proteins: oncogenes or tumor suppressors in disguise? Cell Signal 2010; 22(6): 894-9. https://doi.org/10.1016/j.cellsig.2009.12.008

[7] Kappelmann M, Bosserhoff A, Kuphal S. AP-1/c-Jun transcription factors: regulation and function in malignant melanoma. Eur J Cell Biol 2014; 93(1-2): 76-81. https://doi.org/10.1016/j.ejcb.2013.10.003

[8] Hernandez JM, Floyd DH, Weilbaecher KN, *et al*. Multiple facets of junD gene expression are atypical among AP-1 family members. Oncogene 2008; 27: 4757-67. https://doi.org/10.1038/onc.2008.120

[9] Shaulian E. AP-1: the Jun proteins: oncogenes or tumor suppressors in disguise? Cell Signal 2010; 22: 894-9. https://doi.org/10.1016/j.cellsig.2009.12.008

[10] Weitzman JB, Fiette L, Matsuo K, *et al*. JunD Protects Cells from p53-Dependent Senescence and Apoptosis. Mol Cell 2000; 6(5): 1109-19. https://doi.org/10.1016/S1097-2765(00)00109-X

[11] Nakayama T, Higuchi T, Oiso N, *et al*. Expression and function of FRA2/JUND in cutaneous T-cell lymphomas. Anticancer Res 2012; 32(4): 1367-73.

[12] Lamb JA, Ventura JJ, Hess P, *et al*. JunD Mediates Survival Signaling by the JNK Signal Transduction Pathway. Mol Cell 2003; 12: 1075-85. https://doi.org/10.1016/S1097-2765(03)00203-X

[13] Hemann MT, Lowe SW. The p53-Bcl-2 connection. Cell Death Differ 2006; 13: 1256-9. https://doi.org/10.1038/sj.cdd.4401962

[14] Li T, Dai W, Lu L. Ultraviolet-induced junD Activation and Apoptosis In Myeloblastic Leukemia ML-1 Cells. Mech Signal Transduct 2002; 277(36): 32668-76. https://doi.org/10.1074/jbc.M203519200

[15] Peng, Chen Y, Chen S, *et al*. JUND-dependent up-regulation of HMOX1 is associated with cisplatin resistance in muscle-invasive bladder cancer. J Biochem 2020; 168(1): 73-82. https://doi.org/10.1093/jb/mvaa027

[16] Mori N, *et al*. Constitutive activation of transcription factor AP-1 in primary adult T-cell leukemia cells. Blood 2000; 95: 3915-21.

[17] Murata K, *et al*. A novel alternative splicing isoform of human T-cell leukemia virus type 1 bZIP factor (HBZ-SI) targets distinct subnuclear localization. J Virol 2006; 80: 2495-505. https://doi.org/10.1128/JVI.80.5.2495-2505.2006

[18] Ugbode C, Garnham N, Fort-Aznar L, *et al*. JNK signalling regulates antioxidant responses in neurons. Redox Biol 2020; 37: 101712. https://doi.org/10.1016/j.redox.2020.101712

[19] Fan F, Podar K. The Role of AP-1 Transcription Factors in Plasma Cell Biology and Multiple Myeloma Pathophysiology. Cancers 2020; 12(10): 2830. https://doi.org/10.3390/cancers13102326

[20] Nabinger SC, Chen S, Gao R, *et al*. Mutant p53 enhances leukemia-initiating cell self-renewal to promote leukemia development. Leukemia 2019; 33(6): 1535-9. https://doi.org/10.1038/s41375-019-0377-0

[21] Shin D-Y. TP53 Mutation in Acute Myeloid Leukemia: An Old Foe Revisited. Cancers 2023; 15: 4816. https://doi.org/10.3390/cancers15194816

[22] Eargin J, Cheng J, Haas M. Role of the p53 tumor suppressor gene in the pathogenesis and in the suppression of acute lymphoblastic T-cell leukemia. Leukemia 1992; 6 Suppl 3: 85S-91S.

[23] Prokocimer M, Molchadsky A, Rotter V. Dysfunctional diversity of p53 proteins in adult acute myeloid leukemia: projections on diagnostic workup and therapy. Blood 2017; 130(6): 699-712. https://doi.org/10.1182/blood-2017-02-763086

[24] Chomczyk M, Gazzola L, Dash S, *et al*. Impact of p53-associated acute myeloid leukemia hallmarks on metabolism and the immune environment. Front Pharmacol 2024; 15: 1409210. https://doi.org/10.3389/fphar.2024.1409210

[25] Abramowitz J, Neuman T, Perlman R, *et al*. The P53 Pathway Is Inactive in Acute Myeloid Leukemia. Blood 2012; 120(21): 5122. https://doi.org/10.1182/blood.V120.21.5122.5122

[26] Yang M, Pan Z, Huang K, *et al*. A unique role of p53 haploinsufficiency or loss in the development of acute myeloid leukemia with FLT3-ITD mutation. Leukemia 2022; 36(3): 675-86. https://doi.org/10.1038/s41375-021-01452-6

[27] Warburg O. Über den Stoffwechsel der Carcinomzelle. Naturwissenschaften 1924; 12: 1131-7. https://doi.org/10.1007/BF01504608

[28] Tataranni T, Piccoli C. Dichloroacetate (DCA) and Cancer: An Overview towards Clinical Applications. Oxid Med Cell Longev 2019; 2019: 8201079. https://doi.org/10.1155/2019/8201079

[29] James MO, Jahn SC, Zhong G, *et al*. Therapeutic applications of dichloroacetate and the role of glutathione transferase zeta-1. Pharmacol Ther 2017; 170: 166-80. https://doi.org/10.1016/j.pharmthera.2016.10.018

[30] Ruggieri V, Agriesti F, Scrima R, *et al*. Dichloroacetate, a selective mitochondria-targeting drug for oral squamous cell carcinoma: a metabolic perspective of treatment. Oncotarget 2015; 6(2): 1217-30. https://doi.org/10.18632/oncotarget.2721

[31] Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 1992; 12: 5447-54. https://doi.org/10.1128/mcb.12.12.5447-5454.1992

[32] Semenza GL, Nejfelt MK, Chi SM, *et al*. Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene. Proc Natl Acad Sci U S A 1991; 88: 5680-4. https://doi.org/10.1073/pnas.88.13.5680

[33] Wykoff CC, Pugh CW, Maher ER, *et al*. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 1999; 399: 271-5. https://doi.org/10.1038/20459

[34] Wang GL, Jiang BH, Rue EA, *et al*. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A 1995; 92: 5510-4. https://doi.org/10.1073/pnas.92.12.5510

[35] Kankotia S, Stacpool PW. Dichloroacetate and cancer: New home for an orphan drug? Biochim Biophys Acta Rev Cancer 2014; 1846(2): 617-29. https://doi.org/10.1016/j.bbcan.2014.08.005

[36] Zhao X, Li S, Mo Y, *et al*. DCA Protects against Oxidation Injury Attributed to Cerebral Ischemia-Reperfusion by Regulating Glycolysis through PDK2-PDH-Nrf2 Axis. Oxid Med Cell Longev 2021; 2021: 5173035. https://doi.org/10.1155/2021/5173035

[37] Zhao H, Mao J, Yuan Y, *et al*. Sodium Dichloroacetate Stimulates Angiogenesis by Improving Endothelial Precursor Cell Function in an AKT/GSK-3β/Nrf2 Dependent Pathway in Vascular Dementia Rats. Front Pharmacol 2019; 10: 555. https://doi.org/10.3389/fphar.2019.00523

[38] Stacpoole PW, *et al*. Evaluation of long-term treatment of children with congenital lactic acidosis with dichloroacetate. Pediatrics 2008; 121: e1223-e1228. https://doi.org/10.1542/peds.2007-2062

[39] Michelakis ED, *et al*. Metabolic modulation of glioblastoma with dichloroacetate. Sci Transl Med 2010; 2: 31-4. https://doi.org/10.1126/scitranslmed.3000677

[40] Belkahla S, *et al*. Changes in metabolism afect expression of ABC transporters through ERK5 and depending on p53 status. Oncotarget 2017; 9: 1114-29. https://doi.org/10.18632/oncotarget.23305

[41] Wachter F, Pikman Y. Pathophysiology of Acute Myeloid Leukemia. Acta Haematol 2024; 147(2): 229-46. https://doi.org/10.1159/000536152

[42] Vial E, Sahai E, Marshall CJ. ERK-MAPK signaling coordinately regulates activity of Rac1 and RhoA for tumor cell motility. Cancer Cell 2003; 4(1): 67-79. https://doi.org/10.1016/S1535-6108(03)00162-4

[43] Allende-Vega N, Villalba M. Metabolic stress controls mutant p53 R248Q stability in acute myeloid leukemia cells. Sci Rep 2019; 9: 5637. Allende-Vega N, *et al*. Te presence of wild type p53 in hematological cancers improves the efcacy of combinational therapy targeting metabolism. Oncotarget 2015; 6: 19228-45. https://doi.org/10.18632/oncotarget.4653

[44] Selvaraj N, Budka JA, Ferris MW, *et al*. Extracellular signal-regulated kinase signaling regulates the opposing roles of JUN family transcription factors at ETS/AP-1 sites and in cell migration. Mol Cell Biol 2015; 35: 88-100. https://doi.org/10.1128/MCB.00982-14

[45] Milde-Langosch K, Roder H, Andritzky B, *et al*. The role of the AP-1 transcription factors c-Fos, FosB, Fra-1 and Fra-2 in the invasion process of mammary carcinomas. Breast Cancer Res Treat 2004; 86: 139-52. https://doi.org/10.1023/B:BREA.0000032982.49024.71

[46] Millena AC, Vo BT, Khan SA. JunD Is Required for Proliferation of Prostate Cancer Cells and Plays a Role in Transforming Growth Factor-beta (TGF-beta)-induced Inhibition of Cell Proliferation. J Biol Chem 2016; 291: 17964-76. https://doi.org/10.1074/jbc.M116.714899

[47] Eferl R, Wagner EF. AP-1: a double-edged sword in tumorigenesis. Nat Rev Cancer 2003; 3: 859-68. https://doi.org/10.1038/nrc1209

[48] Lopez-Bergami P, Lau E, Ronai Z. Emerging roles of ATF2 and the dynamic AP1 network in cancer. Nat Rev Cancer 2010; 10: 65-76. https://doi.org/10.1038/nrc2681

[49] Shaulian E, Karin M. AP-1 in cell proliferation and survival. Oncogene 2001; 20: 2390-400. https://doi.org/10.1038/sj.onc.1204383

[50] Zerbini LF, de Vasconcellos JF, Czibere A, *et al*. JunD-mediated repression of GADD45alpha and gamma regulates escape from cell death in prostate cancer. Cell Cycle 2011; 10: 2583-91. https://doi.org/10.4161/cc.10.15.16057

[51] Ouyang X, Jessen WJ, Al-Ahmadie H, *et al*. Activator protein-1 transcription factors are associated with progression and recurrence of prostate cancer. Cancer Res 2008; 68: 2132-44. https://doi.org/10.1158/0008-5472.CAN-07-6055

[52] Bakiri L, Lallemand D, Bossy-Wetzel E, *et al*. Cell cycle-dependent variations in c-Jun and JunB phosphorylation: a role in the control of cyclin D1 expression. EMBO J 2000; 19: 2056-68. https://doi.org/10.1093/emboj/19.9.2056

[53] Katabami M, Donninger H, Hommura F, *et al*. Cyclin A is a c-Jun target gene and is necessary for c-Jun-induced anchorage-independent growth in RAT1a cells. J Biol Chem 2005; 280: 16728-38. https://doi.org/10.1074/jbc.M413892200

[54] Kajanne R, Miettinen P, Tenhunen M, *et al*. Transcription factor AP-1 promotes growth and radioresistance in prostate cancer cells. Int J Oncol 2009; 35: 1175-82. https://doi.org/10.3892/ijo_00000434

[55] Xu L, Ning H, Gu L, *et al*. Tristetraprolin induces cell cycle arrest in breast tumor cells through targeting AP-1/c-Jun and NF-kappaB pathway. Oncotarget 2015; 6: 41679-91. https://doi.org/10.18632/oncotarget.6149

[56] Kharman-Biz A, Gao H, Ghiasvand R, *et al*. Expression of activator protein-1 (AP-1) family members in breast cancer. BMC Cancer 2013; 13: 441. https://doi.org/10.1186/1471-2407-13-441

[57] Langer S, Singer CF, Hudelist G, *et al*. Jun and Fos family protein expression in human breast cancer: correlation of protein expression and clinicopathological parameters. Eur J Gynaecol Oncol 2006; 27: 345-52.

[58] Mao X, Orchard G. Abnormal AP-1 protein expression in primary cutaneous B-cell lymphomas. Br J Dermatol 2008; 159: 145-51. https://doi.org/10.1111/j.1365-2133.2008.08579.x

[59] Wang H, Birkenbach M, Hart J. Expression of Jun family members in human colorectal adenocarcinoma. Carcinogenesis 2000; 21: 1313-7. https://doi.org/10.1093/carcin/21.7.1313

[60] Lopez-Bergami P, Lau E, Ronai Z. Emerging roles of ATF2 and the dynamic AP1 network in cancer. Nat Rev Cancer 2010; 10: 65-76. https://doi.org/10.1038/nrc2681

[61] Li T, Dai W, Lu L. Ultraviolet-induced junD Activation and Apoptosis In Myeloblastic Leukemia ML-1 Cells. Mech Signal Transduct 2002; 277(36): 32668-7. https://doi.org/10.1074/jbc.M203519200

[62] Song D, Lian Y, Zhang L. The potential of activator protein 1 (AP-1) in cancer targeted therapy. Front Immunol 2023; 14: 1224892. https://doi.org/10.3389/fimmu.2023.1224892

[63] Weitzman JB, Fiette L, Matsuo K, *et al*. JunD protects cells from p53-dependent senescence and apoptosis. Mol Cell 2000; 6(5): 1109-19. https://doi.org/10.1016/S1097-2765(00)00109-X

[64] Elliott B, Millena AC, Matyunina L, *et al*. Essential role of JunD in cell proliferation is mediated via MYC signaling in prostate cancer cells. Cancer Lett 2019; 448: 155-67. https://doi.org/10.1016/j.canlet.2019.02.005

[65] Angel P, Karin M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochim Biophys Acta 1991; 1072: 129-57. https://doi.org/10.1016/0304-419X(91)90011-9

[66] Wang K, Cui Y, Lin P, *et al*. JunD Regulates Pancreatic β-Cells Function by Altering Lipid Accumulation. Front Endocrinol (Lausanne) 2021; 12: 689845. https://doi.org/10.3389/fendo.2021.689845

[67] Laurent G, Solari F, Mateescu B, *et al*. Oxidative Stress Contributes to Aging by Enhancing Pancreatic Angiogenesis and Insulin Signaling. Cell Metab 2008; 7: 113-24. https://doi.org/10.1016/j.cmet.2007.12.010

[68] Hasenfuss SC, Bakiri L, Thomsen MK, *et al*. Regulation of Steatohepatitis and Pparγ Signaling by Distinct AP-1 Dimers. Cell Metab 2014; 19: 84-95. https://doi.org/10.1016/j.cmet.2013.11.018

[69] S, Khan AW, Akhmedov A, *et al*. Hyperglycemia Induces Myocardial Dysfunction via Epigenetic Regulation of JunD. Circ Res 2020; 127: 1261-73. https://doi.org/10.1161/CIRCRESAHA.120.317132

[70] Hilfiker-Kleiner D, Hilfiker A, Kaminski K, *et al*. Lack of JunD promotes pressure overload-induced apoptosis, hypertrophic growth, and angiogenesis in the heart. Circulation 2005; 112(10): 1470-7. https://doi.org/10.1161/CIRCULATIONAHA.104.518472

[71] Elliott B, Millena AC, Matyunina L, *et al*. Essential role of JunD in cell proliferation is mediated via MYC signaling in prostate cancer cells. Cancer Lett 2019; 448: 155-67. https://doi.org/10.1016/j.canlet.2019.02.005

[72] Weitzman JB, Fiette L, Matsuo K, *et al*. JunD protects cells from p53-dependent senescence and apoptosis. Mol Cell 2000; 6(5): 1109-19. https://doi.org/10.1016/S1097-2765(00)00109-X

[73] Sturm I, Bosanquet AG, Hermann S, *et al*. Mutation of p53 and consecutive selective drug resistance in B-CLL occurs as a consequence of prior DNA-damaging chemotherapy. Cell Death Differ 2003; 10: 477-84. https://doi.org/10.1038/sj.cdd.4401194

[74] Lopez-Royuela N, Rathore MG, Allende-Vega N, *et al*. Extracellular-signal-regulated kinase 5 modulates the antioxidant response by transcriptionally controlling Sirtuin 1 expression in leukemic cells. Int J Biochem Cell Biol 2014; 53: 253-61. https://doi.org/10.1016/j.biocel.2014.05.026

[75] Khan AU, Rathore MG, Allende-Vega N, *et al*. Human leukemic cells performing oxidative phosphorylation (OXPHOS) generate an antioxidant response independently of reactive oxygen species (ROS) production. EBioMedicine 2016; 3: 43-53. https://doi.org/10.1016/j.ebiom.2015.11.045

[76] Chu QS, Sangha R, Spratlin J, *et al*. A phase I open-labeled, single-arm, dose-escalation, study of dichloroacetate (DCA) in patients with advanced solid tumors. Invest New Drugs 2015; 33(3): 603-10. https://doi.org/10.1007/s10637-015-0221-y

[77] Koltai T, Fliegel L. Dichloroacetate for Cancer Treatment: Some Facts and Many Doubts. Pharmaceuticals (Basel) 2024; 17(6): 744. https://doi.org/10.3390/ph17060744

[78] Ishikawa C, Senba M, Mori N. Butein inhibits NF-kappaB, AP-1 and Akt activation in adult T-cell leukemia/lymphoma. Int J Oncol 2017; 51(2): 633-43. https://doi.org/10.3892/ijo.2017.4026

[79] Dai Y, Xiong X, Huang G, *et al*. Dichloroacetate Enhances Adriamycin-Induced Hepatoma Cell Toxicity *In vitro* and *In vivo* by Increasing Reactive Oxygen Species Levels. PLoS ONE 2014; 9(4): e92962. https://doi.org/10.1371/journal.pone.0092962

[80] Skeberdytė A, Sarapinienė I, Aleksander-Krasko J, *et al*. Dichloroacetate and Salinomycin Exert a Synergistic Cytotoxic Effect in Colorectal Cancer Cell Lines. Sci Rep 2018; 8: 17744. https://doi.org/10.1038/s41598-018-35815-4

Received on 22-03-2026 Accepted on 25-04-2026 Published on 19-05-2026

https://doi.org/10.30683/1929-2279.2026.15.09