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# mRNA Expression of Cell Cycle Regulatory Genes and *Eef1a1* in Thioacetamide-Induced Liver Injury and Fibrosis in Mice

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**ABSTRACT**

Liver fibrosis develops in response to persistent liver injury, but the hepatic expression patterns of cell cycle regulators during early thioacetamide (TAA)-induced fibrogenesis remain incompletely characterized. This study characterized liver injury, collagen deposition, and the expression of selected cell cycle regulatory genes and the translation elongation factor gene *Eef1a1* following a two-week TAA administration. Female Swiss albino mice were assigned to untreated healthy (n = 3), vehicle control (n = 6), and TAA (n = 6) groups. TAA (150 mg/kg) or vehicle (0.9% saline) was administered intraperitoneally three times weekly for two weeks, and samples were collected 72 h after the final injection. Liver index, serum alanine and aspartate aminotransferase activities, liver histology, and hepatic gene expression were assessed. Compared with vehicle controls, TAA-treated mice exhibited an increased liver index, elevated serum aminotransferase activities, hepatocellular degeneration, and increased collagen deposition. The collagen-positive area increased 3.5-fold, accompanied by 3.7- and 10.6-fold increases in *Acta2* and *Col1a1* mRNA levels, respectively. Hepatic *Ccnd1*, *Ccna2,* and *Eef1a1* expression increased 10.9-, 17.5-, and 5.0-fold, respectively (all $p < 0.05$), whereas *E2f1* and *Ccne1* expression showed no statistically significant differences. These findings demonstrate that a two-week TAA regimen induces liver injury and fibrogenic responses accompanied by selective increases in *Ccnd1*, *Ccna2,* and *Eef1a1* expression. These findings provide a basis for clarifying the specific cellular sources and functional contributions of these upregulated genes during hepatic fibrogenesis.

**Key words:** cell cycle; cyclins; E2F1; eEF1A1; thioacetamide; liver fibrosis.

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## 1. Introduction

Liver disease constitutes a significant worldwide health challenge [1]. Liver fibrosis is a healing response to chronic damage, characterized by excessive buildup of extracellular matrix (ECM), notably fibrillar collagens. Activated hepatic stellate cells (HSCs) are a major source of ECM-producing myofibroblasts and acquire a proliferative, α-smooth muscle actin (α-SMA)-positive phenotype during fibrogenesis [2]. Characterizing the molecular changes accompanying these responses may help identify processes involved in liver fibrosis development.

Liver injury and fibrogenesis involve proliferative responses in several hepatic cell populations. Hepatocyte depletion can trigger compensatory proliferation in the surviving hepatocyte population [3, 4], whereas activated hepatic stellate cells proliferate in areas of damage [2, 5]. The entry into and progression through the cell cycle are modulated by cyclin-dependent kinases (CDKs), their cyclin partners, and transcription factors from the E2F family. Cyclin D1 contributes to G1 progression, E2F1 regulates genes associated with cell cycle progression and DNA replication, and cyclins E1 and A2 facilitate the transition from G1 to S phase and progression through S phase [6-8]. Experimental studies have also implicated these regulators in fibrogenesis. Global or HSC-specific deletion of cyclin E1 attenuates experimental liver fibrosis [5, 9], while E2F1 has been implicated in fibrogenic responses to cholestatic liver injury [10]. These findings provide a rationale for examining selected cell cycle regulatory genes in the context of liver injury and collagen deposition.

Alongside cell cycle regulation, translation-associated factors may contribute to the cellular responses accompanying liver injury. Eukaryotic translation elongation factor 1A1 (eEF1A1) delivers aminoacyl-tRNAs to the ribosome and also participates in non-canonical cellular functions [11]. In hepatocellular carcinoma, eEF1A1 has been reported to promote G1-phase progression through a STAT1–cyclin D1 pathway [12], supporting investigation of its expression alongside cell cycle regulators. We previously observed dynamic regulation of *E2f1* and *Eef1a1* during lipopolysaccharide-induced activation of primary mouse HSCs [13]. These observations prompted us to examine their hepatic expression, together with selected cyclins, in an in vivo model of chemically induced liver injury and fibrogenesis.

Thioacetamide (TAA) is widely used to induce experimental liver injury and fibrosis [14, 15]. Its metabolic activation generates reactive metabolites that cause hepatocellular injury, and repeated exposure can lead to fibrosis and cirrhosis [14, 15]. Previous studies have examined cell cycle regulators in TAA-treated rats, including G1 and post-G1 regulators and responses to partial hepatectomy in cirrhotic liver [16, 17]. Studies in mice have also investigated proliferation-associated genes such as *Mki67*, *Cdkn3*, and *Trp53* [18]. However, the hepatic expression patterns of selected G1/S- and S-phase regulators alongside *Eef1a1* during short-term TAA exposure remain incompletely characterized. The present study therefore aimed to characterize liver injury and collagen deposition following a two-week TAA regimen in female Swiss albino mice and to assess the accompanying hepatic mRNA expression of *Ccnd1*, *Ccne1*, *Ccna2*, *E2f1*, and *Eef1a1*.

## 2. Materials and Methods

### 2.1. Materials

Female Swiss albino mice (7–9 weeks old, 27–40 g) were supplied by the Pasteur Institute in Ho Chi Minh City, Ho Chi Minh City, Vietnam. Animals were housed under a 12-h light/12-h dark cycle with free access to pelleted chow and clean water.

### 2.2. Methods

#### *2.2.1. Study design and induction of liver fibrosis*

Mice were randomly assigned to three groups and housed in three separate cages, with one cage per group (Figure 1A): a healthy group (H, n = 3) that received no intervention; a vehicle control group (VC, n = 6) that received intraperitoneal (i.p.) injections of 0.9% saline; and a TAA group (n = 6) that received i.p. injections of TAA (Sigma-Aldrich, USA) at 150 mg/kg body weight, prepared as a 30 mg/mL solution in 0.9% saline. Group allocation was not concealed, and investigators were not blinded to group assignment during the experiment or outcome assessment. The smaller healthy group was included to provide an untreated baseline reference, whereas the vehicle control and TAA groups were assigned more animals to support the primary comparison of TAA-associated effects against vehicle treatment. Injections were given three times per week for two consecutive weeks (six doses). Seventy-two hours after the last injection (day 15), mice were weighed, and the animals were euthanized by cervical dislocation. Blood was collected via heart puncture. Livers were excised,

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examined macroscopically, and weighed; the liver index was calculated as liver weight/body weight × 100 (%). The left lobe was fixed in 4% paraformaldehyde for histology, and the remaining tissue was frozen and stored at −80°C until RNA extraction. Liver index and serum aminotransferase activities were determined for all animals (H, n = 3; VC, n = 6; TAA, n = 6), whereas histological examination and RT-qPCR were performed on three randomly selected mice per group

#### 2.2.2. Serum aminotransferase assays

Blood (approximately 0.5 mL) was allowed to clot for 45 minutes at room temperature and was centrifuged at 3,500 rpm for 15 minutes at 4°C. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured with IFCC-modified kinetic UV kits (GPT (ALAT) and GOT (ASAT) IFCC mod. liquiUV (HUMAN, Germany)) by recording the decrease in NADH absorbance at 340 nm on a DTX 880 Multimode Detector (Beckman Coulter, USA), according to the manufacturer's instructions. Results are expressed in U/L.

#### 2.2.3. Histological examination

Fixed tissue was embedded in paraffin and sectioned at 4 µm at Cho Ray Hospital, Ho Chi Minh City, Vietnam. Sections were deparaffinized and stained with hematoxylin and eosin (H&E) (Abcam, USA) or with Picrosirius Red Stain Kit (Polysciences, USA) for collagen. Sections were examined by bright-field microscopy, and images were acquired with the Nikon Eclipse Ti2 (Nikon, Japan). One liver section per mouse was analyzed for each staining method. For collagen quantification, six non-overlapping fields per section were selected from different regions and captured with a 10× objective. Fields containing tissue-processing artifacts or a blood vessel whose lumen occupied more than 12% of the total image area were excluded. The total vascular lumen area across the selected fields was kept approximately comparable among groups.The Picrosirius red-positive area was segmented by color thresholding in ImageJ and expressed as a percentage of the total tissue area (excluding vascular lumina). Briefly, images were converted to RGB stacks, and the green channel was used for analysis. A threshold identifying Picrosirius red-positive areas was set on representative images and applied to all images, with minor adjustments for differences in staining intensity verified against the original images.

#### 2.2.4. RNA extraction and RT-qPCR

Frozen liver tissue was ground in liquid nitrogen, and total RNA was isolated with a silica-membrane spin-column kit, including on-column DNase I digestion (ISOLATE II RNA Mini Kit, Meridian Bioscience, UK). RNA was eluted in 60 µL of RNase-free water and quantified spectrophotometrically (Thermo Scientific NanoDrop™; Thermofisher Scientific, USA). Up to 1 µg of total RNA was reverse transcribed in a 20 µL reaction with the SensiFAST cDNA Synthesis Kit (Meridian Bioscience, UK). Quantitative PCR was performed with the SensiFAST SYBR Hi-ROX Kit (Meridian Bioscience, UK) on a Bio-Rad CFX96 system (Bio-Rad, USA) under the following conditions: 95°C for 10 min; 40 cycles of 95°C for 30 s, 59°C for 30 s, and 72°C for 40 s; and 72°C for 5 min, followed by melting-curve analysis (60–95°C). Primer sequences are listed in Table 1. Gene symbols follow mouse nomenclature: *Acta2* (α-SMA), *Col1a1* (collagen type I α1 chain), *Ccnd1* (cyclin D1), *Ccne1* (cyclin E1), *Ccna2* (cyclin A2), *E2f1* (E2F1), and *Eef1a1* (eEF1A1). qPCR was performed in three technical replicate wells for each target and reference gene per biological sample. Expression was normalized to *Gapdh* and calculated by the $2^{-\Delta\Delta Ct}$ method relative to the mean of the H group.

***Table 1. Primer sequences used for RT-qPCR***

| Gene (protein) | Forward primer (5′→3′) | Reverse primer (5′→3′) | Accession No. (RefSeq mRNA) |
|---|---|---|---|
| *Gapdh* (GAPDH) | AAGTTGTCATGGATGACC | TCACCATCTTCCAGGAGC | NM_008084 |
| *Acta2* (α-SMA) | GCATCCACGAAACCACCTA | CACGAGTAACAAATCAAAGC | NM_007392 |
| *Col1a1* (collagen I) | CAATGGCACGGCTGTGTGCG | AGCACTCGCCCTCCCGTCTT | NM_007742 |
| *Ccnd1* (cyclin D1) | CTGTGCTGCGAAGTGGAAACCAT | TTCATGGCCAGCGGGAAGACCTC | NM_007631 |
| *Ccne1* (cyclin E1) | AAGCCCTCTGACCATTGTGTCC | CTAAGCAGCCAACATCCAGGAC | NM_007633 |
| *Ccna2* (cyclin A2) | CTGCCTTCCACTTGGCTCTC | TTGTGGCGCTTTGAGGTAGG | NM_009828 |
| *E2f1* (E2F1) | GCTGCAACTGCTTTCGGAG | GATGTCTCCTGGCATGAGGTC | NM_007891 |
| *Eef1a1* (eEF1A1) | AATGGTGACAACATGCTGGA | ACGAGTTGGTGGCAGGATAC | NM_010106 |

### 2.3. Statistics

Data was recorded in Microsoft Excel 365 and analyzed with GraphPad Prism 9.3.0. All values are expressed as the mean ± standard error of the mean (SEM). Data normality within each group was assessed using the Shapiro–Wilk test, and homogeneity of variances among groups was assessed using the Brown–Forsythe test. Differences among the three groups were assessed by one-way ANOVA followed by Tukey's multiple comparisons test. A $p$-value < 0.05 was considered statistically significant.

### 2.4. Research Ethics

All animal experiments were approved by the Animal Ethics Committee of the Stem Cell Institute, University of Science, Vietnam National University Ho Chi Minh City, Vietnam (reference number: 230105/SCI-AEC).

## 3. Results and Discussion

### 3.1. TAA induces hepatomegaly and hepatocellular injury

All mice survived until the scheduled endpoint. Gross examination revealed smooth, glossy liver surfaces in the healthy (H) and vehicle control (VC) groups, whereas TAA-treated mice exhibited an irregular liver surface (Figure 1B). The liver index was significantly higher in the TAA group than in the VC group (6.93 ± 0.49% vs. 5.17 ± 0.40%; $p$ < 0.05) but did not differ significantly from that in the H group (5.50 ± 0.06%; Figure 1C). Serum ALT activity was elevated in the TAA group compared with both the VC group (51.60 ± 6.28 vs. 30.19 ± 2.40 U/L; $p$ < 0.05) and the H group (28.44 ± 6.72 U/L; $p$ < 0.05; Figure 1D). Similarly, serum AST activity was higher in the TAA group than in the VC group (75.85 ± 13.04 vs. 34.44 ± 2.24 U/L; $p$ < 0.05) and the H group (35.34 ± 5.06 U/L; $p$ < 0.05; Figure 1E). No statistically significant differences in liver index or serum ALT and AST activities were detected between the H and VC groups.

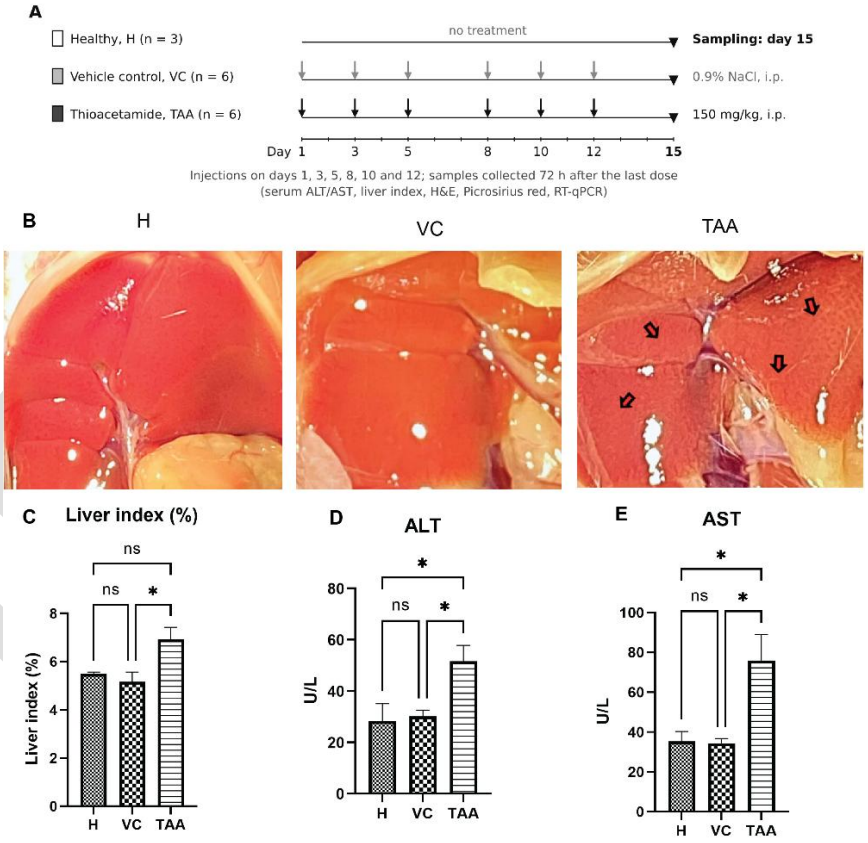



**Figure 1.** Experimental design and assessment of thioacetamide-induced liver injury in mice. (A) Experimental design. (B) Representative gross appearance of the liver; arrows indicate the rough, granular surface in the TAA group. (C) Liver index (liver weight/body weight × 100). (D, E) Serum ALT and AST activities. H, healthy group; VC, vehicle control group; TAA, thioacetamide group. Sample sizes were n = 3, 6, and 6 mice for the H, VC, and TAA groups, respectively. Bars show the mean ± SEM. ns, not significant; *$p$ < 0.05 (one-way ANOVA).

### 3.2. TAA treatment induces hepatic collagen deposition and upregulates fibrogenic markers

H&E-stained liver sections from the H and VC groups showed preserved hepatic architecture, with regularly arranged hepatocyte cords. In contrast, sections from TAA-treated mice exhibited prominent hepatocellular vacuolation and disruption of the normal hepatocyte arrangement (Figure 2A). Picrosirius red staining revealed limited collagen deposition around vascular structures in the H and VC groups, whereas the TAA group showed increased collagen deposition extending into the surrounding parenchyma (Figure 2B). The collagen-positive area was significantly greater in the TAA group (5.53 ± 0.45%) than in the VC group (1.57 ± 0.18%; $p < 0.001$) and the H group (1.55 ± 0.09%; $p < 0.01$), corresponding to approximately 3.5 times that in the VC group (Figure 2C).

Consistent with these histological findings, relative hepatic *Acta2* mRNA levels were significantly higher in the TAA group (3.32 ± 0.97) than in the VC (0.90 ± 0.32) and H (1.04 ± 0.20) groups (both $p < 0.05$; Figure 2D). Relative *Col1a1* mRNA levels were also elevated in the TAA group (10.97 ± 3.57) compared with the VC (1.04 ± 0.54) and H (1.00 ± 0.59) groups (both $p < 0.01$; Figure 2E). The mean expression levels of *Acta2* and *Col1a1* in the TAA group were approximately 3.7 and 10.6 times those in the VC group, respectively. Together, these findings indicate that the two-week TAA regimen induced liver injury accompanied by collagen accumulation and increased fibrogenic marker expression.

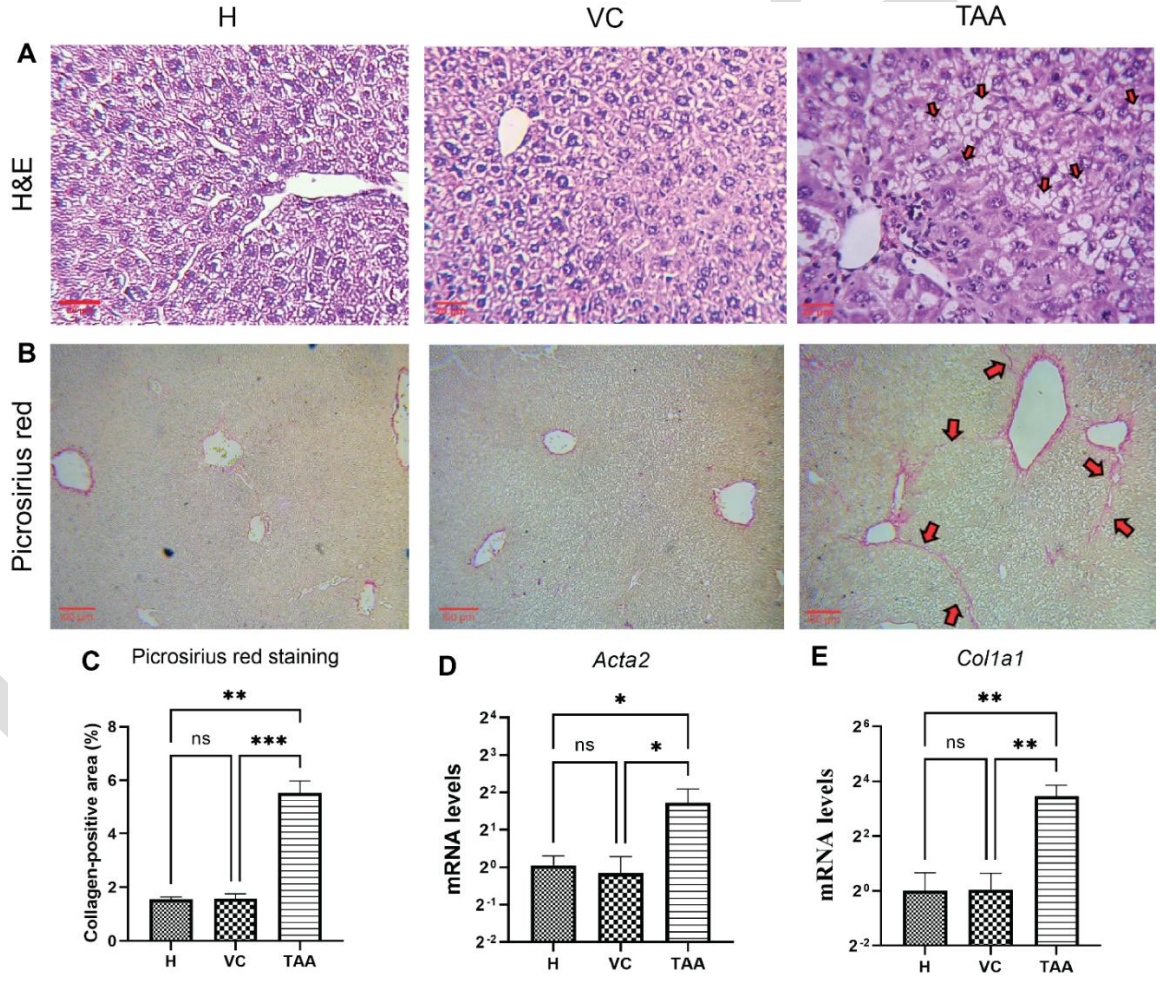



**Figure 2.** Histological alterations and fibrogenic responses in thioacetamide-treated mouse livers

(A) Representative hematoxylin and eosin (H&E)-stained liver sections from healthy (H), vehicle control (VC), and thioacetamide-treated (TAA) mice. Arrows indicate hepatocellular vacuolation in the TAA group. (B) Representative Picrosirius red-stained sections showing collagen deposition (red); arrows highlight collagen accumulation in TAA-treated livers. (C) Quantification of the collagen-positive area as a percentage of the total tissue area. (D, E) Relative hepatic mRNA levels of *Acta2* and *Col1a1*, respectively, measured by RT-qPCR. Scale bars: 20 μm (A) and 100 μm (B). n = 3 mice per group. Bars show the mean ± SEM. ns, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (one-way ANOVA).

### 3.3. Cell cycle-related genes respond selectively to TAA

Hepatic mRNA expression of selected cell cycle regulators and the translation elongation factor gene *Eef1a1* was assessed following two weeks of TAA exposure (Figure 3A–E). Relative *Ccnd1* mRNA levels were significantly higher in the TAA group (6.70 ± 0.75) than in the VC (0.62 ± 0.19) and H (1.07 ± 0.25) groups (both $p < 0.001$), corresponding to approximately 10.9 times the level in the VC group (Figure 3A). No statistically significant differences in *Ccne1* mRNA levels were detected among the groups (Figure 3B). Relative *Ccna2* mRNA levels were elevated in the TAA group (19.25 ± 7.16) compared with the VC (1.10 ± 0.05) and H (1.02 ± 0.14) groups (both $p < 0.05$), corresponding to 17.5 times the level in the VC group (Figure 3C).

In contrast, relative *E2f1* mRNA levels did not differ significantly among the H (0.98 ± 0.03), VC (0.89 ± 0.37), and TAA (1.04 ± 0.16) groups (Figure 3D). Relative *Eef1a1* mRNA levels were significantly higher in the TAA group (3.14 ± 0.33) than in the VC group (0.63 ± 0.26; $p < 0.01$) and the H group (1.11 ± 0.33; $p < 0.05$), corresponding to approximately 5.0 times the level in the VC group (Figure 3E). No statistically significant differences between the H and VC groups were detected for any of the five genes. Together, these results show that two-week TAA exposure was associated with increased hepatic *Ccnd1*, *Ccna2*, and *Eef1a1* mRNA levels, without statistically significant changes in *Ccne1* or *E2f1* (Figure 3F).

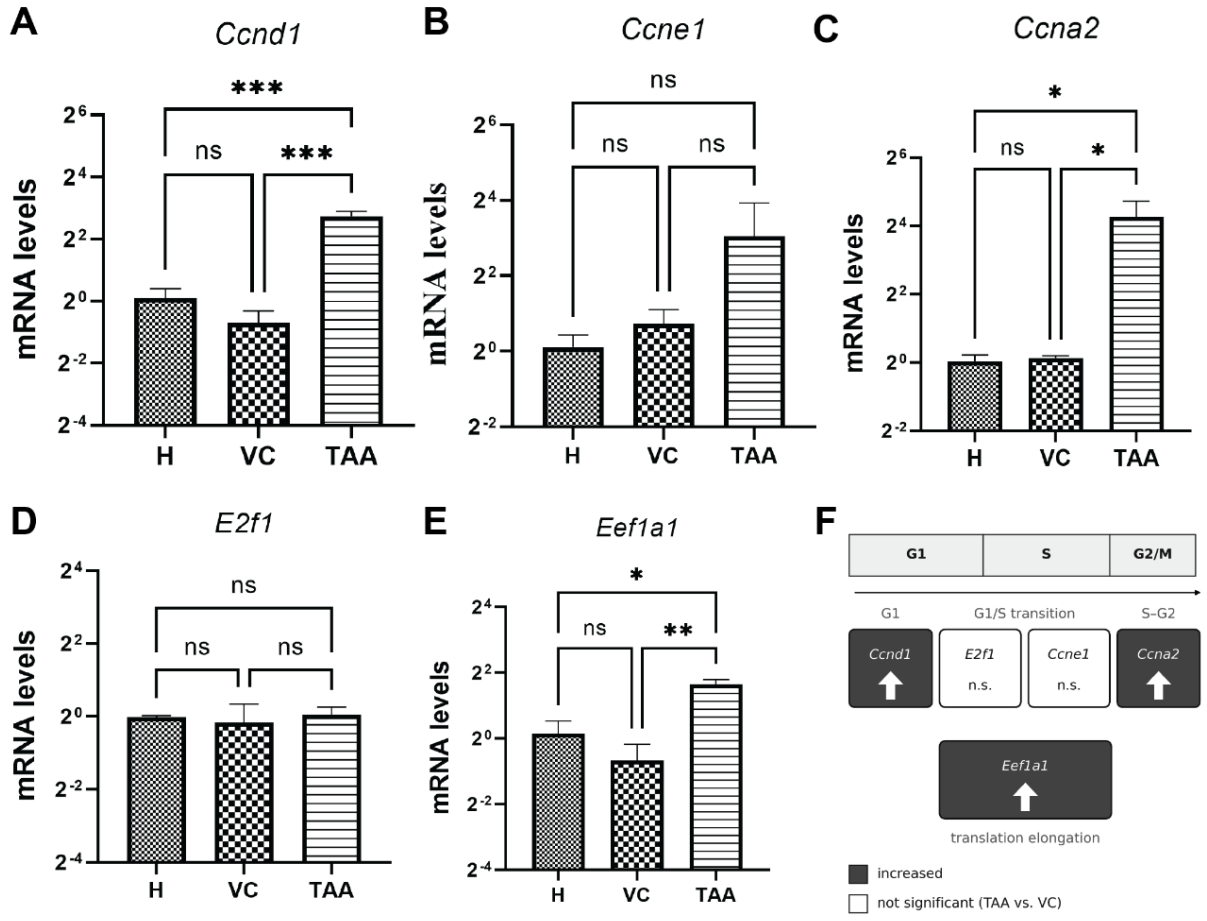



**Figure 3.** Hepatic expression of cell cycle regulatory genes and Eef1a1 in thioacetamide-treated mice

*(A–E) Relative mRNA levels of Ccnd1, Ccne1, Ccna2, E2f1, and Eef1a1 determined by RT-qPCR (normalized to Gapdh). (F) Schematic summary of the observed expression changes in relation to cell cycle regulation and translation elongation. Dark boxes with upward arrows indicate significantly increased expression in the TAA group compared with the VC group; white boxes indicate no statistically significant difference. The schematic summarizes transcriptional changes and does not represent direct measurements of cell cycle progression; n.s., not significant. n = 3 mice per group. Bars show the mean ± SEM. ns, not significant; *p < 0.05; **p < 0.01; ***p < 0.001 (one-way ANOVA).*

### 3.4. Discussion

Six intraperitoneal administrations of TAA at 150 mg/kg over two weeks produced liver injury accompanied by collagen accumulation and increased fibrogenic gene expression in female Swiss albino mice. The collagen-positive area was approximately 3.5 times that in vehicle controls, while hepatic *Acta2* and *Col1a1* mRNA levels were approximately 3.7 and 10.6 times the corresponding control levels, respectively (Figure 2C–E). These findings support a fibrogenic response involving myofibroblast-associated gene expression and extracellular matrix deposition [2]. Similar fibrogenic features have been reported in other TAA models, including studies in rats [19]. However, the severity of TAA-induced injury and fibrosis varies with dose, exposure duration, administration schedule, and animal characteristics [14, 15]. The present results demonstrate that collagen accumulation and fibrogenic transcriptional responses were detectable after two weeks of exposure, although the absence of formal histological staging limits classification of fibrosis severity.

Serum ALT and AST activities were approximately 1.7 and 2.2 times those in vehicle controls, respectively (Figure 1D, E). Together with hepatocellular vacuolation, these elevations indicate that liver injury remained evident at the sampling endpoint, 72 h after the final TAA administration. This interval was selected to reduce the influence of immediate post-injection toxic responses and evaluate cell cycle-related gene expression in the context of liver injury following repeated TAA exposure over two weeks. Nevertheless, it should not be interpreted as an obvious distinction between acute toxicity and subsequent tissue responses. The single-endpoint design prevents determination of the temporal profiles of aminotransferase activities and gene expression. Differences in exposure regimens and sampling intervals should therefore be considered when comparing these findings with acute or longer-term TAA models.

The principal molecular finding was the non-uniform response of the selected cell cycle regulators. Hepatic *Ccnd1* and *Ccna2* mRNA levels were approximately 10.9 and 17.5 times those in vehicle controls, respectively (Figure 3A, C). Cyclin D1 and cyclin A2 participate in G1 and S-phase progression [6], and their regulation is relevant to hepatocyte proliferation during liver regeneration [3, 4]. Their increased transcript abundance is consistent with an injury-associated transcriptional response involving cell cycle regulators. Previous investigations of these regulators in TAA-treated rats provide a relevant experimental context [16]. However, the relationship between the transcriptional changes observed here and actual cell cycle progression remains unresolved because cyclin protein abundance, CDK activity, and DNA synthesis were not measured.

Whole-liver expression reflects contributions from multiple hepatic cell populations. The increases in *Ccnd1* and *Ccna2* could arise from altered expression within hepatocytes, activated HSCs, or other non-parenchymal cells, as well as changes in cellular composition following injury. These possibilities are relevant to the interconnected regenerative, inflammatory, and fibrogenic responses of injured liver [2-4, 20]. Although *Acta2* and *Col1a1* expressions increased concurrently, this does not localize the cell cycle-associated transcriptional changes to HSCs. The relative contributions of the different hepatic cell populations cannot be distinguished from the present bulk-tissue measurements.

No statistically significant differences in *E2f1* or *Ccne1* mRNA levels were detected among the groups (Figure 3B, D). This contrasts with the increases in *Ccnd1* and *Ccna2 but* does not exclude functional involvement of E2F1 or cyclin E1 in the response to TAA. Global and HSC-specific deletion studies have demonstrated roles for cyclin E1 in experimental liver fibrosis [5, 9], while E2F1 has been implicated in cholestatic fibrogenesis [10]. The functional involvement demonstrated in these models may not be accompanied by increased transcript abundance at the endpoint examined here. E2F1 activity is also regulated through protein interactions and post-translational mechanisms [7], while cyclin E function is subject to regulation beyond transcript abundance [8]. Thus, the expression pattern observed here does not establish whether the activities of these regulators were altered.

Temporal and cellular heterogeneity offer additional possible explanations for these findings. Transient changes outside the sampled interval or responses restricted to a small cell population may be obscured in whole-liver measurements. Our previous observation of dynamic *E2f1* regulation during primary HSC activation supports the relevance of sampling time when interpreting its expression [13]. Small sample sizes and inter-animal variability further limit the detection of modest differences. Consequently, the lack of statistically significant differences in *E2f1* and *Ccne1* mRNA levels at this endpoint does not exclude transient or cell type-specific responses to TAA.

Hepatic Eef1a1 mRNA levels were approximately 5.0 times those in vehicle controls (Figure 3E). eEF1A1 functions in translation elongation and participates in several non-canonical cellular processes [11]. Its increased transcript abundance may therefore accompany changes in biosynthetic or stress-associated responses to liver injury, although translation rates and eEF1A1 protein abundance were not assessed. In HCC cells, eEF1A1 has been reported to promote G1 progression through a STAT1–cyclin D1 pathway [12]. The concurrent increases in Eef1a1 and Ccnd1 observed here are compatible with a coordinated response to injury but do not establish a regulatory relationship or implicate the same pathway in non-malignant liver tissue.

Taken together, the main finding is the concurrent increase in hepatic *Ccnd1*, *Ccna2*, and *Eef1a1* mRNA levels associated with collagen accumulation and increased fibrogenic marker expression after two weeks of TAA exposure. The absence of statistically significant differences in *E2f1* and *Ccne1* highlights the non-uniform transcriptional response of the selected regulators at this endpoint. By examining these genes together, the study extends the characterization of short-term TAA injury beyond conventional biochemical and histological measures and identifies an expression pattern involving both cell cycle regulators and a translation elongation factor. This pattern provides a basis for investigating how regenerative and fibrogenic responses are accompanied by changes in cell cycle regulation and translation-associated gene expression. The study is limited by small and unequal sample sizes, a single sampling endpoint, and whole-liver mRNA measurements, which leave the cellular sources and functional significance of these changes unresolved. In addition, outcome assessment was not blinded, and because each group was housed in a single cage, cage effects could not be separated from treatment effects. Importantly, increased mRNA expression does not necessarily reflect increased protein abundance or activity and cannot, by itself, establish that cells are actively proliferating. In the absence of protein-level validation and proliferation assessments, these findings should be interpreted as transcriptional changes in cell cycle-related genes rather than direct evidence of increased cell proliferation. Time-course studies incorporating Western blotting, Ki-67 immunostaining or EdU incorporation, and cell-resolved analyses would help clarify this transcriptional response's contribution to liver injury and fibrogenesis. In addition, gene expression was normalized to a single reference gene, Gapdh. Although Gapdh Ct values showed low variability and did not differ significantly among groups, its stability was not validated against additional candidate reference genes as recommended by the MIQE guidelines; normalization to multiple validated reference genes would strengthen future analyses.

## 4. Conclusion

Intraperitoneal administration of TAA at 150 mg/kg three times per week for two weeks induced liver injury accompanied by collagen accumulation and increased hepatic *Acta2* and *Col1a1* mRNA levels in Swiss albino mice. These changes were associated with increased expression of *Ccnd1*, *Ccna2*, and Eef1a1, whereas *E2f1* and *Ccne1* mRNA levels showed no statistically significant differences. The findings identify a selective hepatic transcriptional response involving cell cycle regulators and a translation elongation factor during short-term TAA-induced injury and fibrogenesis, providing a basis for further investigation of their cellular origins and functional contributions.

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### Declaration of Competing Interest

The authors declare no competing interests.

### Declaration of Generative AI and AI-Assisted Technologies

The authors used ChatGPT and Google Gemini to assist in improving the English language and readability during the research process and in writing this manuscript.

### Author Contributions

Truong Hai Nhung, Dang Minh Thanh: Conceptualization and study design; Dang Minh Thanh, Le Quoc Trung: performing experiments, data analysis, interpretation of results, preparing figures; Dang Minh Thanh, Le Quoc Trung: drafting the manuscript; Truong Hai Nhung: editing and critical review of the manuscript, and final approval of the manuscript.

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In press

# Biểu hiện mRNA của một số gen điều hòa chu kỳ tế bào và Eef1a1 trong mô hình tổn thương và xơ hóa gan ngắn hạn do thioacetamide ở chuột

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**TÓM TẮT**

Xơ hóa gan là đáp ứng với tổn thương gan kéo dài; tuy nhiên, đặc điểm biểu hiện tại gan của các yếu tố điều hòa chu kỳ tế bào trong giai đoạn sớm của quá trình xơ hóa do thioacetamide (TAA) vẫn chưa được làm rõ đầy đủ. Nghiên cứu này đánh giá tổn thương gan, sự lắng đọng collagen, cũng như mức độ biểu hiện của một số gen điều hòa chu kỳ tế bào chọn lọc và gen mã hóa yếu tố kéo dài dịch mã *Eef1a1* sau 2 tuần tiêm TAA. Chuột cái chủng Swiss albino được chia thành các nhóm: nhóm khỏe mạnh không can thiệp ($n$ = 3), nhóm chứng dung môi ($n$ = 6), và nhóm TAA ($n$ = 6). TAA (150 mg/kg) hoặc dung môi (nước muối sinh lý 0,9%) được tiêm ổ bụng chuột 3 lần mỗi tuần trong 2 tuần; các mẫu được thu thập tại thời điểm 72 giờ sau mũi tiêm cuối cùng. Chỉ số hoạt độ men gan ALT, AST huyết thanh, mô bệnh học gan và mức độ biểu hiện gen tại gan được đánh giá. So với nhóm chứng dung môi, chuột được xử lý TAA có chỉ số gan tăng, hoạt độ aminotransferase huyết thanh tăng, thoái hóa tế bào gan và tăng lắng đọng collagen. Diện tích dương tính với collagen tăng 3,5 lần, kèm theo sự gia tăng mức mRNA của *Acta2* (3,7 lần) và *Col1a1* (10,6 lần). Biểu hiện của *Ccnd1*, *Ccna2* và *Eef1a1* tại gan tăng lần lượt là 10,9 lần, 17,5 lần và 5,0 lần ($p$ < 0,05), trong khi biểu hiện của *E2f1* và *Ccne1* không cho thấy sự khác biệt có ý nghĩa thống kê. Các phát hiện này chứng minh rằng phác đồ TAA trong 2 tuần gây tổn thương gan và kích hoạt các đáp ứng sinh xơ, đi kèm với sự gia tăng có chọn lọc trong biểu hiện của *Ccnd1*, *Ccna2* và *Eef1a1*. Kết quả này là cơ sở quan trọng để tiếp tục làm rõ loại tế bào biểu hiện cũng như vai trò chức năng của các gen trên trong quá trình xơ hóa gan.

**Từ khóa/keywords**: chu kỳ tế bào; cyclin; E2F1; eEF1A1; thioacetamide; xơ hóa gan