

Selenomethionine suppresses head and neck squamous cell carcinoma progression through TopBP1/ATR and TCAB1 signaling

Bo Zhang^{1,2*}, Xiaodong Wei^{1,3*} and Jiwu Li⁴

¹Hubei Provincial Key Laboratory of Occurrence and Intervention of Rheumatic Diseases, Hubei Minzu University, ²Department of Stomatology, ³Department of Thoracic and Cardiovascular Surgery, Minda Hospital of Hubei Minzu University, Enshi, Hubei and ⁴Xiangya Stomatological Hospital and Xiangya School of Stomatology, Central South University, Changsha City, Hunan Province, China

*Bo Zhang and Xiaodong Wei are the co-first author

Summary. Objective. Head and neck squamous cell carcinoma (HNSCC) is a histological type of cancer originating from the head and neck. Selenium complexes have been considered as a potential treatment for HNSCC. Therefore, the present work focused on probing the mechanism of L-selenomethionine (SeMet) in HNSCC treatment.

Methods. MTT and colony formation assays were carried out to analyze the survival rate and proliferation of HNSCC cells, respectively. TUNEL staining was performed to examine apoptosis of HNSCC cells. Additionally, qRT-PCR and Western blotting assays were performed to measure mRNA and protein levels, separately.

Results. SeMet treatment significantly hindered the survival and promoted the apoptosis of HNSCC cells in a dose- and time-dependently. SeMet administration promoted expression of TopBP1, ATR, H2AX, p-ATR and γ -H2AX, and suppressed that of TCAB1. Importantly, SeMet treatment suppressed the proliferation and facilitated the apoptosis of HNSCC cells, which were partly reversed by down-regulation of TopBP1 or up-regulation of TCAB1. The activation of SeMet to TopBP1/ATR signaling was rescued by TCAB1 up-regulating, and the inhibition of SeMet to TCAB1 expression was rescued by TopBP1 silencing.

Conclusion. Our findings show that SeMet inhibits the proliferation of HNSCC cells and promotes their apoptosis by targeting TopBP1/ATR and TCAB1 signaling. SeMet is a potential method for HNSCC treatment.

Key words: Head and neck cancer, L-selenomethionine, TopBP1, ATR, TCAB1

Introduction

Head and neck squamous cell carcinoma (HNSCC) is a histological type of cancer that occurs in the head and neck. It ranks sixth place among cancers with regard to morbidity worldwide (Li et al., 2020). HNSCC accounts for over 90% of all head and neck cancer cases, because it usually develops from the squamous epithelium-covered pharynx, larynx and oral cavity (Wang et al., 2021). It has been reported that HNSCC affects more than 500,000 new individuals annually, and that the diagnosis of a majority of these cases is made at the advanced stage (Chan et al., 2019). Alcohol, snuff, cigarette smoke and human papillomavirus are the major risk factors for developing HNSCC. Currently, although excision of malignant tissues, chemotherapy and radiotherapy are available for HNSCC treatment, the five-year survival rate remains around 50% (Johnson et al., 2020; Jung et al., 2020; Liu et al., 2021).

Over the last decades, numerous studies have indicated that higher dosage of selenium may be an adjuvant method for treatment of HNSCC. Selenium compounds can induce apoptosis of cancer cells by disturbing the cell cycle while regulating the levels of protein associated with mitochondrial homeostasis, oxidative stress and other cellular events (Brozmanová et al., 2010; Fernandes and Gandin, 2015; Misra et al.,

Corresponding Author: Bo Zhang, Hubei Provincial Key Laboratory of Occurrence and Intervention of Rheumatic Diseases, Hubei Minzu University, No. 2, Wufengshan Road, Tuqiao Avenue, Enshi City 445000, Hubei Province, China. e-mail: zhboss1234@163.com www.hh.um.es. DOI: 10.14670/HH-18-665

Abbreviations. γ -H2AX, phosphorylated H2AX; ATR, ATM-and-Rad3-related kinase; CHK1, checkpoint kinase 1; DDR, DNA damage response; HNSCC, head and neck squamous cell carcinomas; SeMet, selenomethionine; TCAB1, telomerase cajal body protein 1; TopBP1, topoisomerase II β -binding protein 1



2015). Metabolites of selenium compounds promote the production of reactive oxygen species (ROS), which in turn exacerbate DNA strand breaking and oxidative modifications, finally resulting in cell apoptosis (Tapiero et al., 2003; Sanmartín et al., 2012). Typically, selenium compounds with anti-cancer properties include three main categories, namely inorganic selenium, organic selenium, and selenium nanoparticles selenium. Among them, organic selenium compounds can be better used and retained in the form of selenocysteine, selenomethionine (SeMet), and other amino acids (Tan and Mo, 2018). Michael et al. noted that the patients with HNSCC tolerated well the addition of SeMet to chemoradiotherapy (Mix et al., 2015). This work focused on investigating the regulatory mechanism of SeMet-induced apoptosis in HNSCC cells.

For the sake of maintaining genomic stability, eukaryotic cells have developed relevant signaling pathways to reply to DNA replication stress or DNA damage. Ataxia-telangiectasia mutated (ATM) together with ATM-and-Rad3-related kinase (ATR) have been identified as key mediators for DNA damage response (DDR) upon DNA damage (Maréchal and Zou, 2013). Both of them facilitate DNA repair and cell cycle arrest by targeting downstream factors like checkpoint kinase 1 (CHK1) (Weber and Ryan, 2015; Qiu et al., 2018). Moreover, topoisomerase II β -binding protein 1 (TopBP1) possesses an ATR-activating domain, which has an important effect on enhancing ATR kinase activity during DNA replication (Feng et al., 2020). A growing number of studies have demonstrated that TopBP1 and ATR promote cancer progression (Wilson et al., 2022; Zhao et al., 2022). Besides, telomerase cajal body protein 1 (TCAB1) has been identified as a regulator related to dyskerin, and exerts an important effect on the stability and assembly of telomerase by directly transporting telomerase to telomeres (Stern et al., 2012; Sun et al., 2014). TCAB1 also participates in regulating cell apoptosis and cell cycle arrest. It is lowly expressed within lung cancer cells (A549 as well as H1299), and decreasing TCAB1 facilitates the senescence and apoptosis of lung cancer cells (Niu et al., 2021). In Epstein-Barr virus-treated nasopharyngeal carcinoma cells, TCAB1 depletion enhances cell apoptosis and cell cycle arrest, which is accomplished by the down-regulation of ATR expression (Wang et al., 2017).

This work aimed to preliminary explore the regulation of SeMet on TopBP1/ATR and TCAB1 signaling in HNSCC cell lines, and probe the regulatory mechanism by which SeMet regulates the proliferation and apoptosis of HNSCC cells. Our findings will offer new evidence for the application of SeMet in treating HNSCC.

Materials and methods

Cell culture and treatment

HNSCC cells, including HSC-3, Cal-27, CNE1 and

ACC2, were provided by the American Type Culture Collection (ATCC; Manassas, VA). Cell culture medium used in the present work consisted of Dulbecco's modified Eagle's medium (DMEM; Gibco, USA), 10% fetal bovine serum (FBS) and 1% antibiotics (Sigma Aldrich, St. Louis, MO, USA). L-selenomethionine was purchased from Sigma Aldrich, and was dissolved within PBS solution to prepare the 10 mM stock solution. Here, SeMet at 0, 20, 40, 60, 80, and 100 μ M was added to treat HNSCC cells. Cells were inoculated within an incubator at 37°C and 5% CO₂ conditions. For cell transfection, HNSCC cells were transfected with three different sequences of specific TopBP1 siRNA (si-TopBP1), negative control siRNA (si-NC), TCAB1 overexpression plasmid (pcDNA-TCAB1) or empty plasmid (vector) by adopting Lipofectamine 2000 (Invitrogen). The sequences of siRNAs included: si-NC: 5'-AGUACUGCUUACGAUACGGTT-3'; si-TopBP1-1: 5'-GGAUUAUUCUUUGCGGUUUTT-3'; si-TopBP1-2: 5'-GCAGAA CUGUUGCGGAUUATT-3'; si-TopBP1-3: 5'-GCUCUGUAAUAGUCGACUATT-3'. The above si-NC, si-TopBP1, pcDNA-TCAB1 and empty plasmid were purchased from GenePharma (Shanghai, China).

3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2 tetrazolium bromide assay

For analyzing how SeMet affects HNSCC cell proliferation, this work inoculated cells (1x10⁴/well) in the 96-well plates. After 48h treatment using SeMet at 0, 20, 40, 60, 80, or 100 μ M, or 0, 24, 48 or 72h treatment using 100 μ M SeMet, MTT assay was conducted to measure HNSCC cell survival rate (Marconi and Gallorini, 2019). After 24h of the attachment period, SeMet at varying concentrations was added for cell treatment, while PBS solution was introduced to treat cells in Control group. Later, 5 mg/ml MTT solution (10 μ l; Solarbio, Beijing, China) was introduced to maintain HNSCC cells at 37°C for a 3h period. Then, the cells were incubated with MTT lysis buffer supplemented with 10% SDS together with 0.01 N HCl, for 12h to release formazan. Finally, the optical density of cells was measured by a Spectrophotometer (DU-640; Beckman Coulter, Inc. Fullerton, CA) at 570 nm. Data acquired without cells were recognized to be the background. Cell viability was measured relative to the control group.

RT-qPCR assay

TopBP1, ATR, TCAB1 and H2A.X variant histone (H2AX) gene levels were determined by RT-qPCR assay. Firstly, total RNA was extracted from HSC-3, Cal-27, CNE1 and ACC2 cells with Trizol reagent (Takara, Dalian, China). Then, cDNA was synthesized with the PrimeScript RT reagent kit (Takara). Afterwards, relative mRNA expression levels of TopBP1, ATR, TCAB1 and H2AX were examined by RT-qPCR assay by utilizing the SYBR Green Master Mix (Takara). The above steps were accomplished

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following specific protocols, with *GAPDH* being the endogenous reference. In this work, $2^{-\Delta\Delta Ct}$ approach was employed for calculating those aforementioned gene levels. Sequences of all primers utilized are shown below: TopBP1: 5'-TGAGTGTGCCAAGAGATGGAA-3' (F) and 5'-TGCTTCTGGTCTAG GTTCTGT-3' (R); ATR: 5'-AACATTCGTGGCATTGACTG-3' (F) and 5'-AAGCAA GGTGATCTCATCCG-3' (R); H2AX: 5'-TTGATTGCCGGGC TTAGAGG-3' (F) and 5'-CTGCCGCAGGTATAGAATC-3' (R); TCAB1: 5'-TCAA GAAGGTGGTGAA GCA-3' (F) and 5'-GTCAAAGGTGGAGGAGTGG-3' (R); *GADPH*: 5'-GGAGCGA GATCCCTCCAAAAT-3' (F) and 5'-GGCTGTTGTCATACTTCTCATGG-3' (R).

Western blotting assay

TopBP1, ATR, TCAB1, H2AX, p-ATR and phosphorylated H2AX (γ -H2AX) protein levels were examined by Western blotting assay. Firstly, total cellular protein was isolated by RIPA buffer (Solarbio), then cell lysates (25 μ g) were separated on the 12% SDS-PAGE gel, followed by transfer of proteins onto the polyvinylidene difluoride (PVDF) membranes. Afterwards, 5% skimmed milk was added to block membranes at ambient temperature for 60 min, followed by overnight incubation of primary antibodies at 4°C, including anti- β -actin (1:2000, ab8226), anti-TCAB1 (1:1000, ab224444, Abcam), anti-TopBP1 (1:500, #14342), anti-ATR (1:500, #2790), anti-H2AX (1:500, #7631), anti-p-ATR (1:500, #2853) and anti- γ -H2AX (1:500, #5438, Cell Signaling Technology) antibodies. Thereafter, peroxidase-conjugated goat anti-mouse or anti-rabbit secondary antibody was introduced to incubate membranes at ambient temperature for another 1h period. Finally, membranes were developed using the enhanced chemiluminescence (ECL) system, and the protein bands were visualized with Image J software. β -actin served as the loading reference (Marconi et al., 2022).

Colony formation assay

HSC-3, Cal-27, CNE1 and ACC2 cells (600/well) were subject to inoculation in the 6-well plates. After 100 μ M SeMet treatment, cells were transfected with si-TopBP1 or TCAB1 overexpression plasmid, followed by culture in an incubator for about 7 days until large colonies were formed. After that, formaldehyde was introduced to fix the cells by incubation for 15 min, then 0.5% crystal violet was added to stain cells for 10 min. The number of colonies containing more than 50 cells was then calculated by using Image J software (Dong et al., 2018; Xu et al., 2020). To be specific, the number of colonies was counted according to the following process: images of colonies were opened by Image J software, the type of 8 bit was selected, the clicking threshold in the directory of the adjust and red color was used to mark colonies, then clicking watershed in the directory of the binary was used to select the particles to

be analyzed finally. Meanwhile, the colony number was also counted under an optical microscope.

TUNEL staining

HSC-3, Cal-27, CNE1 and ACC2 cells were treated with 100 μ M SeMet, followed by transfection with si-TopBP1 or TCAB1 overexpression plasmid. After 48h of cell transfection, TUNEL staining assay was performed to analyze apoptotic cancer cells. In brief, 4% paraformaldehyde was added to fix cells, and later 0.1% Triton X-100 was introduced for maintenance at 4°C for a 5 min period. After that, cells in each well were maintained using TUNEL reaction mixture (50 μ l, Solarbio) for staining apoptotic cells, while nuclei were stained with DAPI solution (Solarbio) for 5 min in dark.

Statistical analysis

Statistical analysis was completed with GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA). In the present study, two independent groups were compared by Student's t-test, while several groups were compared by one-way ANOVA plus Tukey's post hoc test. Results were represented by mean \pm SD. $P < 0.05$ stood for significant difference. Each assay was carried out three times.

Results

SeMet suppressed proliferation of HNSCC cells and facilitated their apoptosis

Firstly, the influences of SeMet on the proliferation and apoptosis of HNSCC cells (ACC2, CNE1, Cal-27 and HSC-3) were detected. As revealed by the results of MTT assay, SeMet inhibited HNSCC cell growth dose-dependently (Fig. 1A) or time-dependently (Fig. 1C). In the meantime, SeMet facilitated apoptosis of HNSCC cells dose-dependently (Fig. 1B) or time-dependently (Fig. 1D). These findings indicate that SeMet suppress HNSCC cell proliferation and facilitate their apoptosis.

SeMet activated TopBP1/ATR signaling and inhibited TCAB1 in HNSCC cells

Thereafter, the gene and protein expression of TopBP1, ATR, p-ATR, TCAB1, H2AX, and phosphorylated H2AX (γ -H2AX) in HNSCC cells treated with 0, 80 or 100 μ M SeMet was detected. Our data proved that SeMet significantly increased expression of TopBP1 gene in HSC-3, Cal-27, CNE1 and ACC2 cells dose-dependently (Fig. 1E). ATR gene expression also was up-regulated after SeMet treatment in HSC-3, Cal-27, CNE1 and ACC2 cells dose-dependently (Fig. 1F). Oppositely, SeMet treatment significantly decreased expression of TCAB1 gene in HSC-3, Cal-27, CNE1 and ACC2 cells dose-dependently (Fig. 1G). Besides, the effect of SeMet on H2AX gene expression was also

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detected. H2AX is one of the substrates of ATM/ATR (Zhou et al., 2003). SeMet treatment facilitated H2AX gene expression in HSC-3, Cal-27, CNE1 and ACC2 cells dose-dependently (Fig. 1H). Moreover, Western blotting assay was used to detect the protein levels of those aforementioned factors. According to our results, SeMet treatment dramatically increased TopBP1 protein levels in the above HNSCC cells dose-dependently (Fig. 2A,C). Additionally, ATR protein and p-ATR expression also increased by SeMet induction in these HNSCC cells dose-dependently (Fig. 2A,D,G). Oppositely, SeMet treatment significantly decreased TCAB1 protein expression in the above HNSCC cells dose-dependently (Fig. 2A,E). Furthermore, SeMet treatment enhanced expression of H2AX and γ -H2AX in the aforementioned HNSCC cells dose-dependently (Fig. 2A,B,F). Overall, our findings indicate that SeMet activate TopBP1/ATR and inhibit TCAB1 signaling in HNSCC cells. Based on the above results, SeMet at a dose of 100 μ M was used

in the following studies.

SeMet regulated the proliferation and apoptosis of HNSCC cells through TopBP1/ATR and TCAB1 signaling

To probe the regulatory mechanism of SeMet in HNSCC cell proliferation and apoptosis, TopBP1 expression in SeMet-treated HNSCC cells was silenced by transfection of TopBP1 specific siRNA. Three different sequences of siRNA were designed. As shown in Figure 3A,B, si-TopBP1-1 exhibited the best interference effect on TopBP1 expression. Therefore, si-TopBP1-1 or si-NC was subsequently transfected into SeMet-treated HNSCC cells. As a result, SeMet remarkably suppressed proliferation of HNSCC cells, which was rescued by TopBP1 silencing (Fig. 4A). Meanwhile, SeMet apparently facilitated HNSCC cell apoptosis, which was reversed by TopBP1 silencing

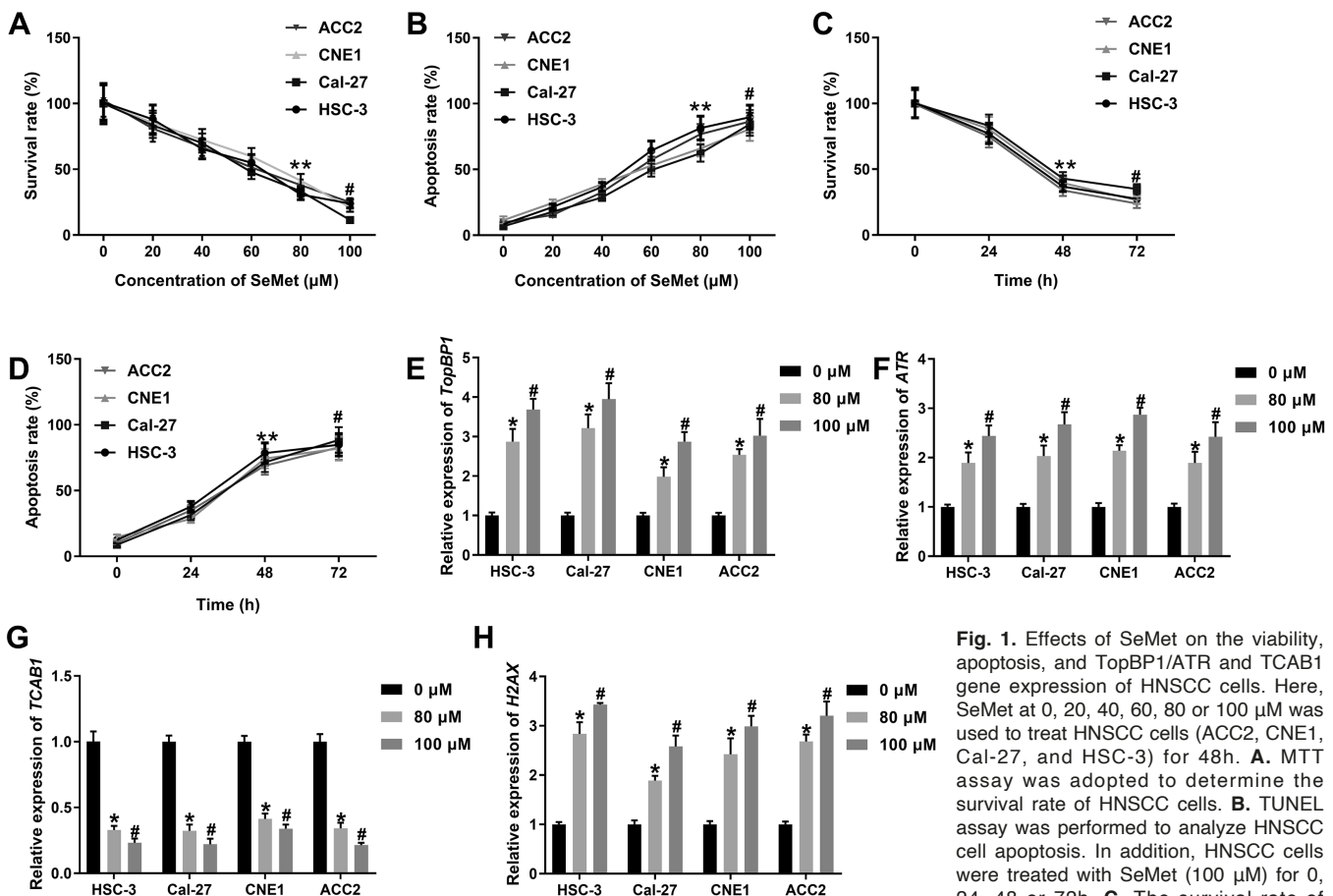


Fig. 1. Effects of SeMet on the viability, apoptosis, and TopBP1/ATR and TCAB1 gene expression of HNSCC cells. Here, SeMet at 0, 20, 40, 60, 80 or 100 μ M was used to treat HNSCC cells (ACC2, CNE1, Cal-27, and HSC-3) for 48h. **A.** MTT assay was adopted to determine the survival rate of HNSCC cells. **B.** TUNEL assay was performed to analyze HNSCC cell apoptosis. In addition, HNSCC cells were treated with SeMet (100 μ M) for 0, 24, 48 or 72h. **C.** The survival rate of HNSCC cells was determined by MTT assay. **D.** HNSCC cell apoptosis was measured with TUNEL assay. Besides, HNSCC cells were treated with SeMet (0, 8 or 100 μ M) for 48 h. **E.** The expression of TopBP1 gene in HSC-3, Cal-27, CNE1 and ACC2 cells was then identified by RT-qPCR assay. **F.** The expression of ATR gene in HSC-3, Cal-27, CNE1 and ACC2 cells was determined by RT-qPCR assay. **G.** The expression of TCAB1 gene in HSC-3, Cal-27, CNE1 and ACC2 cells was determined through RT-qPCR assay. **H.** The expression of H2AX gene in HSC-3, Cal-27, CNE1 and ACC2 cells was examined with RT-qPCR assay. * P <0.05 vs. 0 μ M group, and # P <0.05 vs. 80 μ M group.

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(Fig. 4B). Additionally, the inhibition of SeMet to TCAB1 protein expression also was rescued by TopBP1 silencing (Fig. 4C). In summary, the inhibition of SeMet to proliferation of HNSCC cells and the promotion on their apoptosis are partly reversed by TopBP1 silencing.

To analyze the impact of TCAB1 on regulating proliferation and apoptosis of HNSCC cells by SeMet, TCAB1 expression was over-expressed within SeMet-treated HNSCC cells. According to Figure 3C,D, the transfection of TCAB1 overexpression plasmid significantly up-regulated TCAB1 expression in HSC-3 cells. TCAB1 overexpression plasmid or empty plasmid was transfected into SeMet-treated HNSCC cell lines. According to colony formation analysis, TCAB1 overexpression rescued the inhibition of SeMet to

HNSCC cell proliferation (Fig. 5A). SeMet treatment markedly facilitated HNSCC cell apoptosis, which was rescued by TCAB1 overexpression (Fig. 5B). Furthermore, the expression levels of TopBP1, ATR and p-ATR in HNSCC cells transfected with TCAB1 overexpression plasmid were detected. Therefore, the promotion of SeMet to TopBP1 expression in HSC-3, Cal-27, CNE1 and ACC2 cells was rescued by TCAB1 overexpression (Figs. 5C, 6A,B). Meanwhile, the promotion of SeMet to ATR and p-ATR expression in HSC-3, Cal-27, CNE1 and ACC2 cells was also rescued by TCAB1 overexpression (Figs. 5C, 6C-F). In conclusion, the inhibition of SeMet to HNSCC cell proliferation and its promotion to cell apoptosis are partly reversed by TCAB1 overexpression.

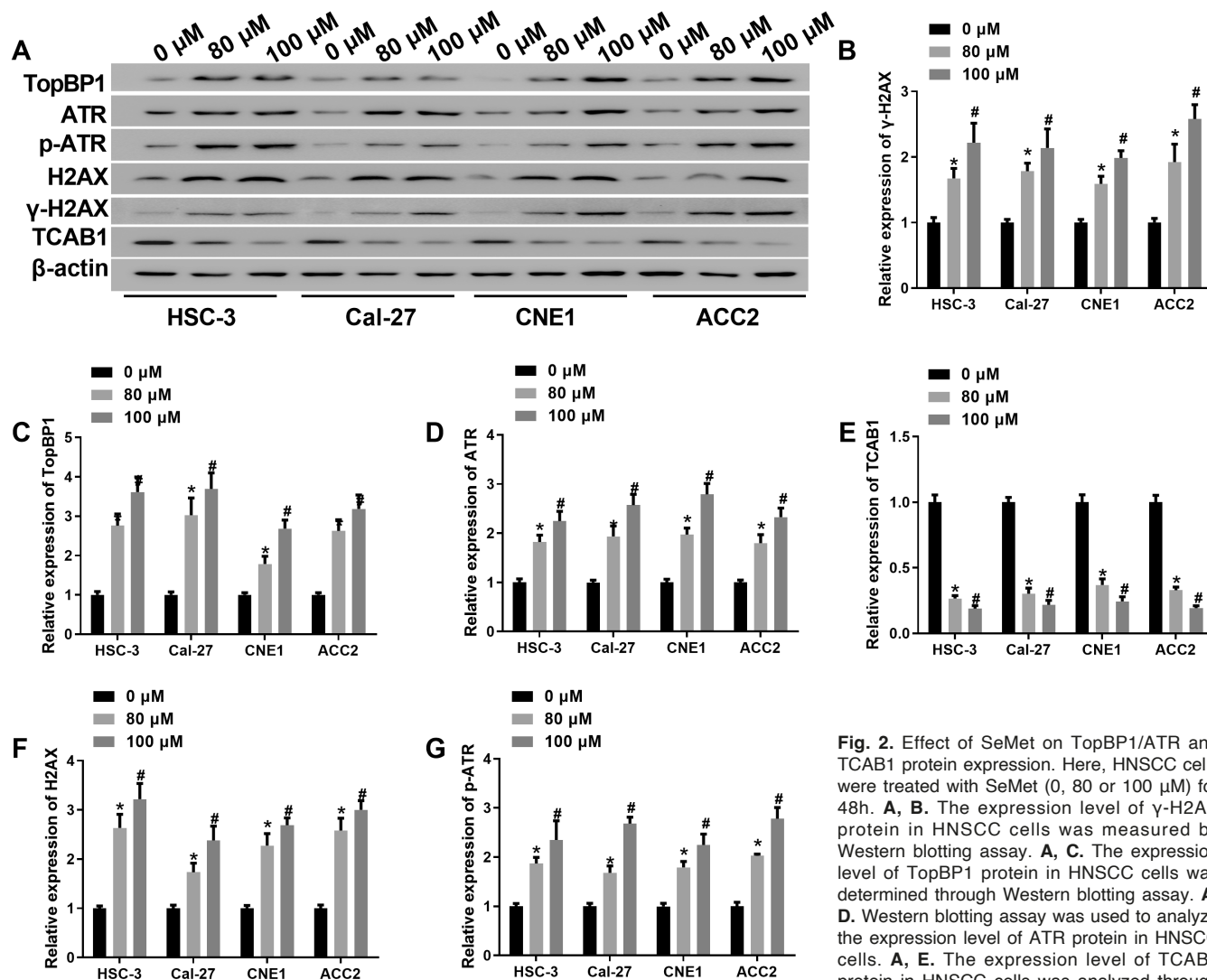


Fig. 2. Effect of SeMet on TopBP1/ATR and TCAB1 protein expression. Here, HNSCC cells were treated with SeMet (0, 80 or 100 μ M) for 48h. **A, B.** The expression level of γ -H2AX protein in HNSCC cells was measured by Western blotting assay. **A, C.** The expression level of TopBP1 protein in HNSCC cells was determined through Western blotting assay. **A, D.** Western blotting assay was used to analyze the expression level of ATR protein in HNSCC cells. **A, E.** The expression level of TCAB1 protein in HNSCC cells was analyzed through

Western blotting assay. **A, F.** The expression level of H2AX protein in HNSCC cells was measured by Western blotting assay. **A, G.** The expression level of p-ATR protein in HNSCC cells was examined by Western blotting assay. * $P < 0.05$ vs. 0 μ M group, and # $P < 0.05$ vs. 80 μ M group.

Discussion

Although SeMet may cause some damage to the human body, some researchers have identified its anti-cancer activity. For instance, Burke et al. authenticated that SeMet application during or after UVB exposure was effective in ameliorating UV-induced photodamage and skin cancer (Burke et al., 2014). Korbut et al. certified that SeMet at a certain dosage suppressed the growth of colorectal cancer cells and facilitated their apoptosis by inactivating Wnt/ β -catenin and GSK-3 β signaling (Korbut et al., 2018). Additionally, more and more studies have confirmed that selenium complexes are potential candidates for the treatment of head and neck cancer. Selenium nanoparticles at a dosage of 20-55 μ g/ml effectively induce apoptosis of HNSCC cells, but had little influence on the growth of human dermal fibroblasts (Hassan and Webster, 2016). In this study, the impacts of SeMet on the growth and apoptosis of HNSCC cells were probed. To be specific, this study used the salivary adenoid cystic carcinoma cell line ACC2, nasopharyngeal carcinoma cell line CNE1, and oral cancer lines Cal-27 and HSC-3. Our findings show that SeMet treatment significantly inhibit the survival and proliferation of HNSCC cells and facilitate their apoptosis dose- and time-dependently.

Furthermore, this work preliminarily investigated the roles of SeMet in TopBP1/ATR signaling, and demonstrated that SeMet significantly promoted expression of TopBP1, ATR and H2AX mRNAs in the above-mentioned four HNSCC cell lines. The cell cycle has a critical role in cell apoptosis, growth and survival, and it is regulated by numerous regulatory proteins like the p53 family proteins. In the course of cancer treatment, exposure to ionizing radiation will interrupt G1/S transition and cause cell cycle arrest in the S-phase. Meanwhile, ATM/ATR signaling is activated to regulate the cell cycle checkpoints during ionizing radiation (Saldivar et al., 2017; Huang and Zhou, 2020). According to the studies by Lai et al., selenium-bearing ruthenium complex triggered DNA damage in prostate cancer cells time-dependently, accompanied by increased expression of p-ATR, p-ATM and γ -H2AX (Lai et al., 2019). H2AX is a downstream target of ATR, and its phosphorylation is one of the hallmarks of DNA damage. Furthermore, TopBP1 is an activator of ATR (Saldivar et al., 2017; Thada and Cortez, 2019). In the present study, SeMet was proved to activate TopBP1/ATR signaling and increase H2AX phosphorylation in HNSCC cells dose-dependently. Importantly, the inhibition of SeMet to HNSCC cell proliferation was reversed by TopBP1 silencing, and its

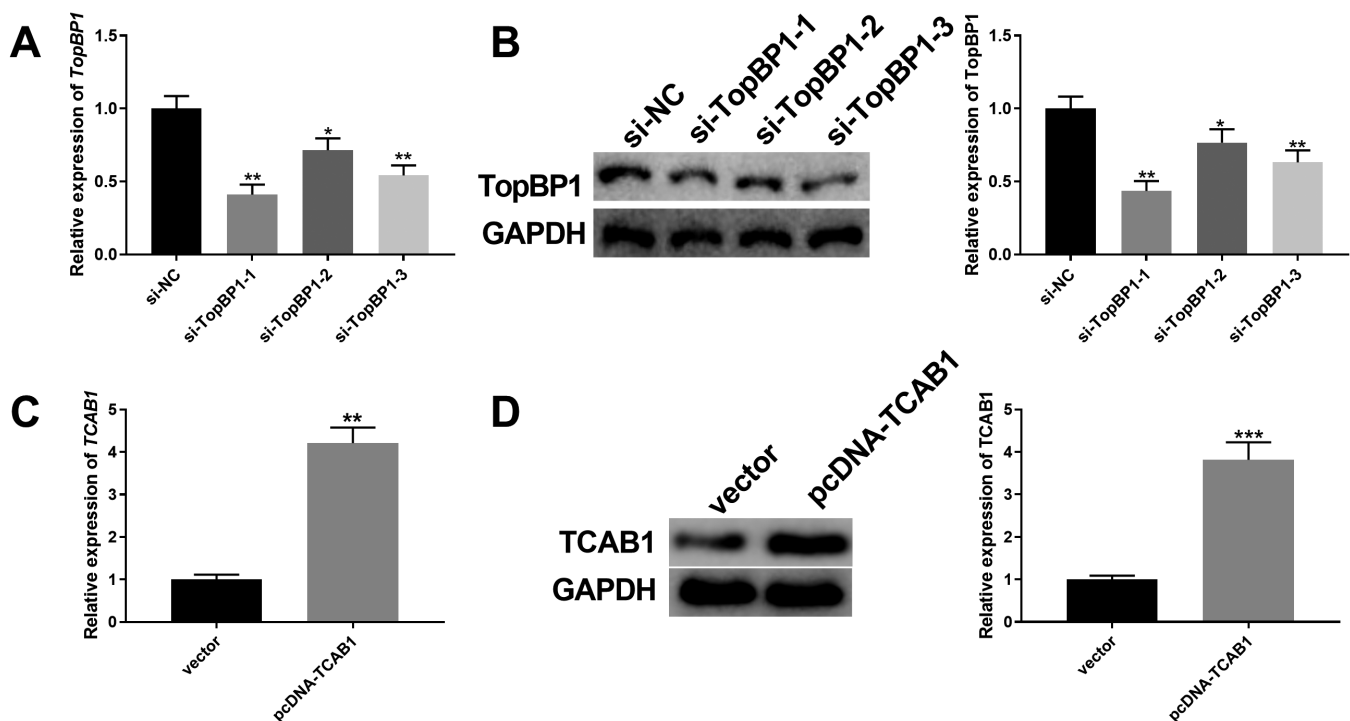


Fig. 3. Verification of transfection efficiency. **A, B.** Three different sequences of TopBP1 siRNA or negative control siRNA were transfected into HSC-3 cells. At 48 h post-transfection, the gene or protein expression of TopBP1 was measured by RT-qPCR or Western blotting assay, respectively. **C, D.** TCAB1 overexpression plasmid or empty plasmid was transfected into HSC-3 cells. At 48h post-transfection, the gene or protein expression of TCAB1 was determined through RT-qPCR or Western blotting assay, respectively. ** $P < 0.01$; *** $P < 0.001$.

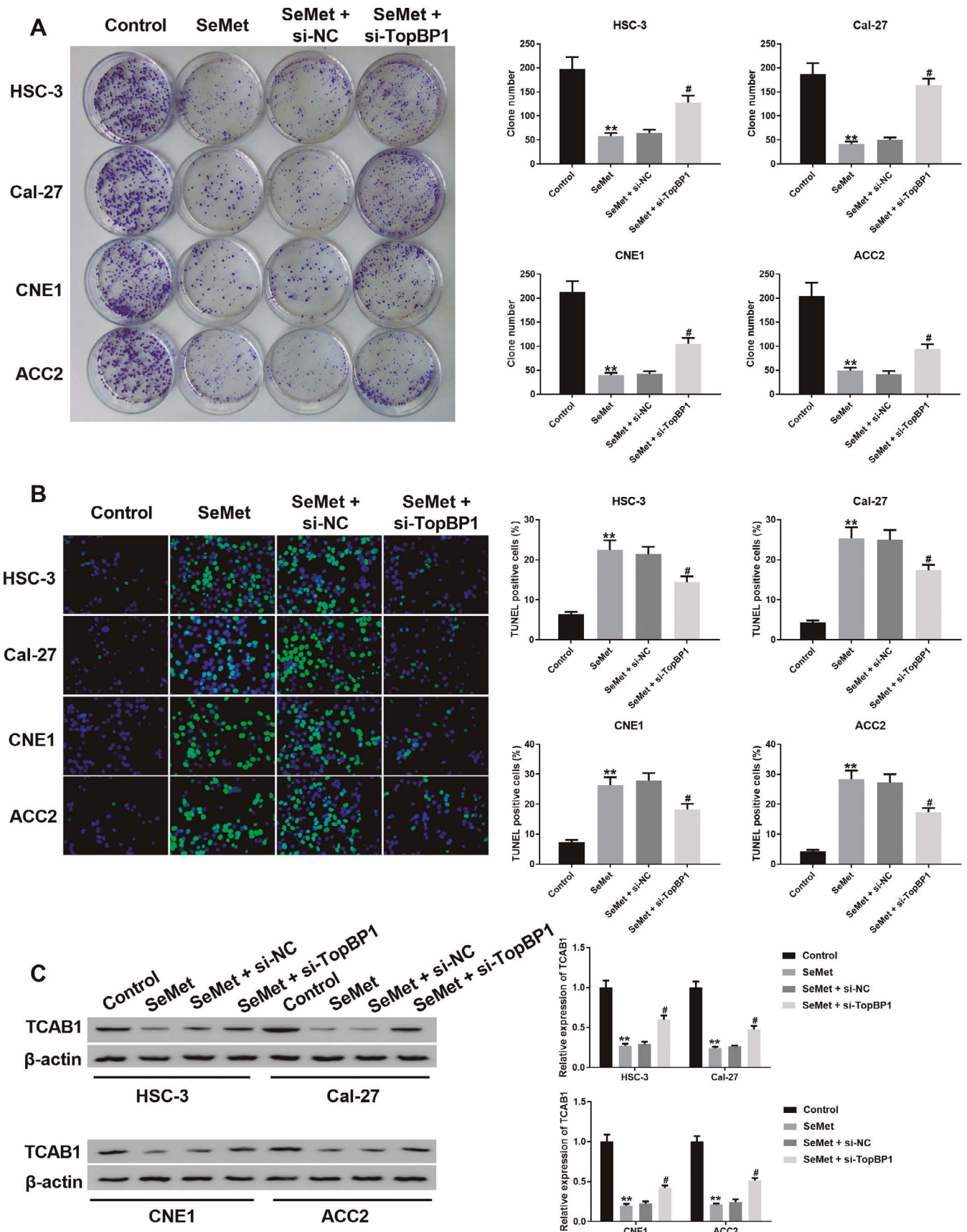


Fig. 4. SeMet affected the proliferation and apoptosis of HNSCC cells through TopBP1/ATR signaling. TopBP1 siRNA or negative control siRNA was transfected into the SeMet-treated HSC-3, Cal-27, CNE1 and ACC2 cells. **A.** Colony formation assay was performed to analyze cancer cell proliferation. **B.** TUNEL assay was carried out to determine HNSCC cell apoptosis. **C.** The expression of TCAB1 protein in HSC-3, Cal-27, CNE1 and ACC2 was measured by Western blotting assay. ** $P < 0.01$ vs. Control group, and # $P < 0.05$ vs. SeMet group.

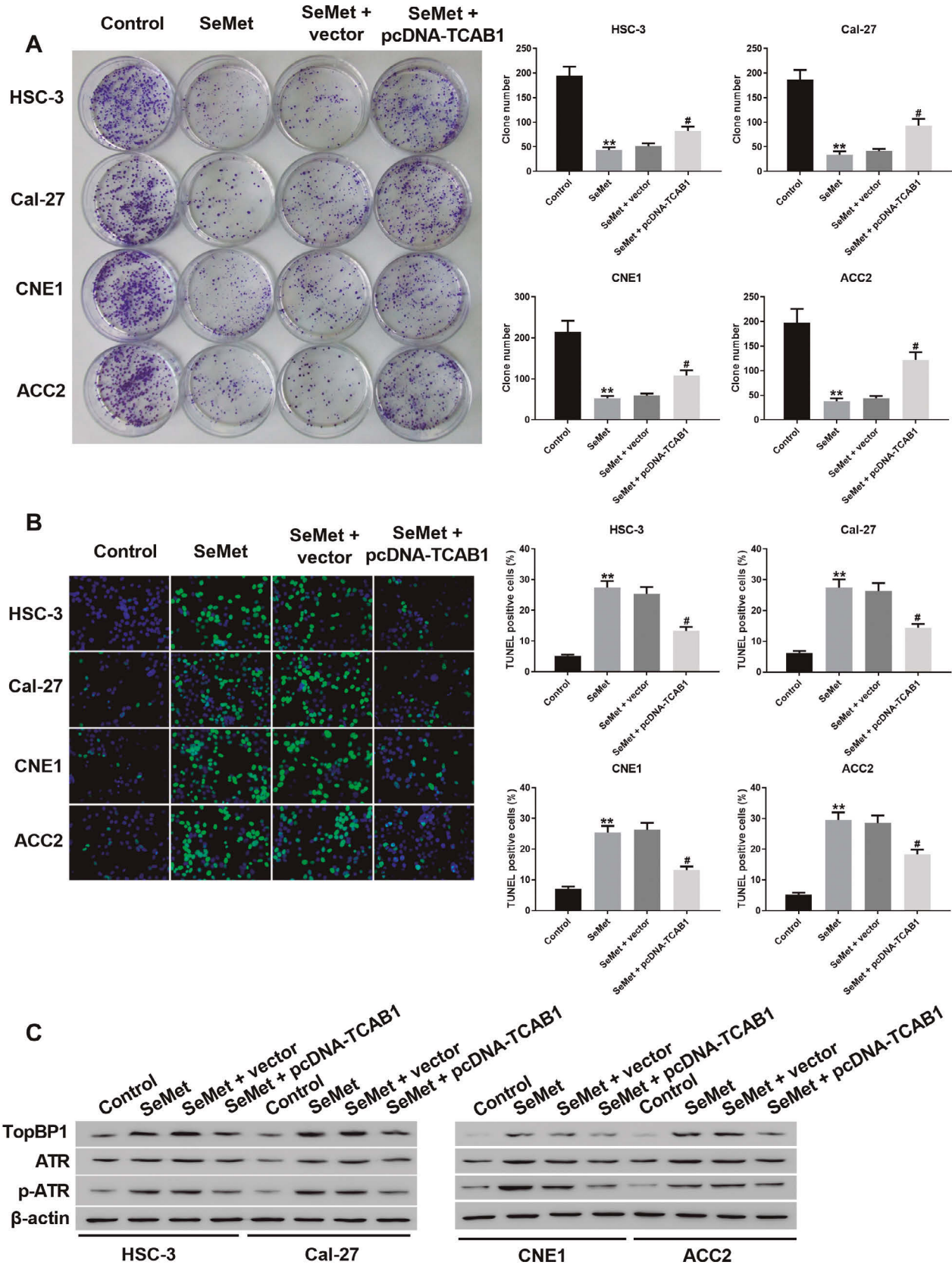


Fig. 5. SeMet affected the proliferation and apoptosis of HNSCC cells by targeting TCAB1. The overexpression plasmid of TCAB1 and empty plasmid were transfected into the SeMet-treated HNSCC cells. **A.** Colony formation assay was performed to analyze cancer cell proliferation. **B.** TUNEL assay was carried out to determine HNSCC cell apoptosis. **C.** The expression of TopBP1, ATR and p-ATR in HSC-3, Cal-27, CNE1 and ACC2 was measured by Western blotting assay. ** $P < 0.01$ vs. Control group, and # $P < 0.05$ vs. SeMet group.

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promotion to cancer cell apoptosis was also rescued by TopBP1 silencing. Besides, it was also found that SeMet suppressed expression of TCAB1 in HNSCC cells, which was also rescued by TopBP1 silencing.

TCAB1 is an essential and structural component factor for the Cajal body, and is also a key regulator for the active telomerase synthesis within human cancer cells (Venteicher and Artandi, 2009). Up-regulation of TCAB1 facilitates γ H2AX clearance from ionizing radiation, thus increasing DDR efficiency (Henriksson et al., 2014). Besides, TCAB1 has been extensively identified as an oncogene due to the mechanism of telomerase trafficking to telomeres. Yuan et al. reported that the down-regulation of TCAB1 induced cell cycle arrest of lung cancer cells (Yuan et al., 2014). Moreover, TCAB1 was considered to be a possible therapeutic target for head and neck cancer due to its effect on inhibiting TCAB1 depletion in HNSCC tumor formation (Sun et al., 2014). In the current study, our results

showed that SeMet treatment remarkably inhibited TCAB1 gene and protein expression in HNSCC cells dose- and time-dependently. SeMet-induced activation of TopBP1/ATR signaling was reversed by TCAB1 overexpression. TCAB1 overexpression reversed the inhibition of SeMet to HNSCC cell proliferation, and its promotion to cell apoptosis.

To sum up, SeMet treatment inhibit HNSCC cell proliferation and facilitate their apoptosis by activating TopBP1/ATR signaling and decreasing TCAB1 signaling. Our study provides new evidence that SeMet can serve as a potential therapeutic method for HNSCC. Nevertheless, there are some limitations in our current study, such as the lack of animal experiments, the unclear molecular mechanism by which SeMet regulates TopBP1/ATR and TCAB1 signaling, and the unknown molecular mechanisms of TopBP1 in regulating TCAB1 and of TCAB1 in regulating TopBP1/ATR signaling. Our future studies will focus on exploring the above limitations.

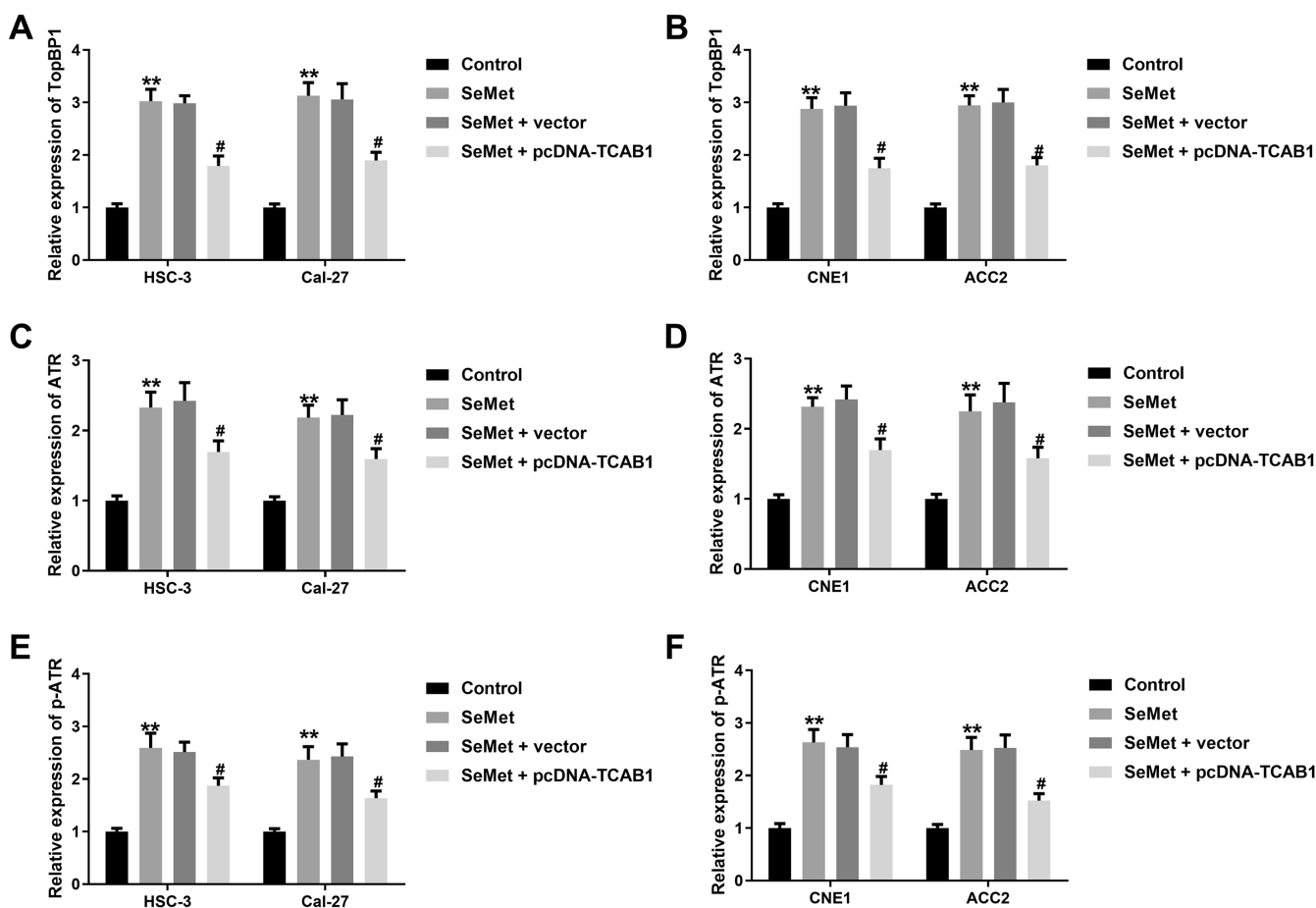


Fig. 6. Analysis of protein expression. The overexpression plasmid of TCAB1 and empty plasmid were transfected into the SeMet-treated HNSCC cells. **A-F.** The relative expression of TopBP1, ATR and p-ATR in HSC-3, Cal-27, CNE1 and ACC2 cells was analyzed. ** $P < 0.01$ vs. Control group, and # $P < 0.05$ vs. SeMet group.

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Author contributions. Bo Zhang: design, conduction, data analysis and manuscript preparation. Xiaodong Wei: sample collection and data analysis. Jiwu Li: data analysis and manuscript preparation. All authors provided final approval for the submission.

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Data availability. The datasets generated during and/or analyzed during the current study are not publicly available due to privacy or ethical restrictions but are available from the corresponding author on reasonable request.

Declarations. Conflict of interest

The authors declare that they have no conflict of interest to disclose.

Ethical approval. Not applicable.

Consent to participate. Not applicable.

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