1 **Title page**

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3 **Inhibiting TG2 sensitizes lung cancer to radiotherapy through interfering**

4 **TOPOII α -mediated DNA repair**

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6 Xiao Lei[#], Zhe Liu[#], Kun Cao[#], Yuanyuan Chen[#], Jianming Cai, Fu Gao*, Yanyong
7 Yang*

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9 [#]Authors contributed equally to this work.

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11 Department of Radiation Medicine, Faculty of Naval Medicine, Second Military
12 Medical University, 800, Xiangyin Road, 200433, Shanghai, P.R. China;

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14 *Corresponding author: Yanyong Yang, Fu Gao and Jianming Cai.

15 Address: Department of Radiation Medicine, Faculty of Naval Medicine, Second
16 Military Medical University; 800, Xiangyin Road, 200433, Shanghai, P.R. China. Fax:
17 +86-21-81871148. E-mail: yyyang2010@163.com, gaofusmmu@163.com,
18 cjm882003@163.com;

19

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Abstract

Radiotherapy is an indispensable strategy for lung cancer, however, treatment failure or reoccurrence is often found in patients due to the developing radioresistance. Novel approaches are required for radiosensitizing to improve the therapeutic efficacy. In present study, we found that transglutaminase 2 (TG2) confers radioresistance in non-small cell lung cancer (NSCLC) cells through regulating TOP2 α and promoting DNA repair. Our data showed that TG2 inhibitor or knockdown increased NSCLC radiosensitivity in vivo and in vitro. We found that TG2 translocated into nucleus and located to DSB sites, surprisingly, knockdown TG2 or glucosamine inhibited the phosphorylation of ATM, ATR and DNA-Pkcs. Through IP-MS assay and functional experiments, we identified that TOP2 α as a downstream factor of TG2. Moreover, we found that TGase domain account for the interaction with TOP2 α . Finally, we found that TG2 expression was correlated with poor survival in lung adenocarcinoma instead of squamous cell carcinoma. In conclusion, we demonstrated that inhibiting TG2 sensitize NSCLC to IR through interfere TOP2 α mediated DNA repair, suggesting TG2 as a potential radiosensitizing target in NSCLC.

Introduction

Radiotherapy is an indispensable strategy in treating lung cancer, of which 80% is non-small cell lung cancer (NSCLC) with poor outcomes (1, 2). Despite the advance in physical techniques, novel approaches in radiosensitizing from biological aspect are required to overcome the growing radioresistance during radiotherapy. The current research involving radiosensitization mainly falls in the following fields: DNA damage repair, poly (adenosine diphosphate–ribose) polymerase inhibitors, histone deacetylase inhibitors, tumor hypoxia and redox conditions, antiangiogenic drugs *etc* (3). However, most of these drugs are in research process or clinical trials, efficacy as well as normal tissue toxicity limits their application.

Transglutaminase 2 (TG2), a member of Transglutaminases family, exerts multiple physiological functions and is associated with cancer cell survival, metastatic behavior and chemoresistance (4-7). It has been proved that TG2 was related to multiple drug resistance including cisplatin, histone deacetylase inhibitor, EGFR-TKI *etc* (5, 8, 9). Recently, the prognostic value of elevated TG2 for patient survival has been illustrated in NSCLC and are attracting more and more attention (10, 11). These studies indicated that TG2 might be critical for radiation resistance in NSCLC. When we are preparing this manuscript, Sheng *et al.* reported that TG2 inhibitor KCC009 induces radiosensitization in lung adenocarcinoma cells(12). However, the detailed role of TG2 in NSCLC radioresistance and the underlying mechanism remains unclear.

Previous studies indicated that TG2 was related to DNA damage repair, which is aberrant active in cancer cells (13-15). Previous study had showed that ATM inhibitor KU55933 abrogated the constitutively activation of TG2 induced by genotoxic drug MNNG (13). ATM mediated NF- κ B activation increased the level of TG2. TG2 was also proved as a target of p53 and involved in DNA damage repair, and knockdown of p53 reduced the level of TG2 (14, 15). But the response of TG2 to ionizing radiation and the exact role of TG2 in DNA repair remains to be uncovered.

Here, we report that TG2 confers to radioresistance in NSCLC and enhanced

73 DNA repair capacity through directly interacting with DNA topoisomerase II α
74 (TOPOII α). We found that ionizing radiation (IR) resulted in a rapid nuclear
75 translocation of TG2 and knockdown TG2 significantly inhibited DNA repair. TG2
76 was found to bind and activate TOPOII α in nucleus to initial DNA damage repair
77 processes, such as phosphorylation of ATM, ATR and DNA-PKcs. Moreover, we used
78 a clinically used TG2 inhibitor, glucosamine, and found it significantly sensitized lung
79 cancer to IR in vivo and in vitro. Finally, we found TG2 was significantly correlated
80 with the survival in lung adenocarcinoma instead of squamous cell carcinoma patients,
81 which suggest possible prognostic value of TG2 in lung adenocarcinoma. These data
82 provide the possibility of clinical translation of TG2 inhibitor in the radiosensitization
83 of lung cancer.
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Results

Inhibition of TG2 sensitizes lung cancer cells to ionizing radiation

It has been proved that TG2 high expression was related to chemoresistance of multiple cancers (16-18). To determine whether TG2 participates in radioresistance in NSCLC, firstly we used a TG2 inhibitor, glucosamine, which was already used in clinics as an anti-inflammatory drug. We found that glucosamine effectively reduced cell viability in A549 cells, while showed little influence on normal lung BEAS-2B cells (Fig. 1A). Besides, glucosamine effectively inhibited TG2 level at the concentration of 5mM in A549 cells (Fig. 1B). Compared with normal lung BEAS-2B cells, TG2 expression was also found to be elevated in lung adenocarcinoma cell lines including A549, H1975 and H358 cells. (Fig. 1C, S1A). By using colony formation assay, we found that glucosamine or TG2 knockdown significantly sensitized A549, H1299, H460 cells to IR, while glucosamine showed no further sensitizing effects on TG2 knockdown cells (Fig. 1D-F). This data was also confirmed in CRISPR Cas9 mediated TG2 knockout cells (Fig. 1G). Alternatively, we used apoptosis assay to determine cellular damage in TG2 inhibited cells. It was found that glucosamine treatment resulted in more apoptotic cells in response to IR, while glucosamine showed no sensitizing effects on BEAS-2B cells (Fig. 1H, Fig. S1B, C).

Radiation induces TG2 nuclear translocation and initiates DNA damage response

To figure out how TG2 confers to radioresistance, we investigated its subcellular location and the relationship with DNA damage repair, the main effects of radiation response. By using Immunofluorescence staining and nuclear protein western blot assay, we found that radiation rapidly induced TG2 nuclear translocation, which could be inhibited by glucosamine (Fig. 2A, B, S2B). Based on distinct functions of TG2, we used calcium inhibitor perillyl alcohol (POH), TG2 activity inhibitor cystamine, and NF-kB inhibitor QNZ, our data showed that QNZ inhibited radiation-induced nuclear translocation of TG2 (Fig. S2A). Moreover, we found that glucosamine treatment significantly inhibited the phosphorylation of DNA-PKcs, ATM and ATR, which are critical for initiating DNA damage repair (Fig. 2C). Further, we used a

siRNA of TG2 to investigate its role in DDR, and found the same effects on DNA-PKcs, ATM and ATR inhibition. However, TG2 siRNA combined with glucosamine treatment didn't showed any additive effects (Fig. 2D). To investigate the influence of TG2 inhibitor on DNA damage, we examined γ H2AX foci and found that TG2 knockout significantly impaired DNA repair in response to IR (Fig. 2E, F). By using a comet assay, we confirmed that more DNA damage remains unrepaired in cells treated with glucosamine (Fig. 2G, H, I).

TG2 interacts with TOP2 α and participates in DNA repair

To identify the specific target of TG2, we conducted an Immunoprecipitation–Mass Spectrometry (IP-MS) assay in A549 cells. Through bioinformatics analysis, we found that 134 proteins bind to TG2 in radiation group compared with normal group (Fig. 3A, Table S1). Among these, TOP2 α was found to be related to DNA damage, and TOP2 inhibitors were clinically used in cancer therapy, such as etoposide and doxorubicin. Then we used laser assay and found that after laser irradiation both TG2 and TOP2 α were recruited in DSB site (Fig. 3B, Fig. S3A). Then we used immunoprecipitation assay and proved that TG2 bind to TOP2 α after IR (Fig. 3C), as a consequence of its nuclear translocation. To confirm the direct binding of these two proteins, we transfected TG2 and TOP2 α into 293T cells. The interaction of TG2 and TOP2 α was further confirmed in co-immunoprecipitation experiments (Fig. 3D, E). Functionally, knockdown of TOP2 α resulted in more DNA damage after IR (Fig. 3F). Moreover, TOP2 α knockdown together with glucosamine didn't show additive effects on cellular DNA damage (Fig. 3F). TG2 knockdown also causes more γ H2AX accumulation in TG2 knockdown or TOP2 α knockdown cells after irradiation (Fig. S3C). Then we investigated the influence of TOP2 α on cellular radiosensitivity, and found that TOP2 α knockdown cells are more sensitive to radiation-induced cell death (Fig. S3B). However, no significant difference was found in TOP2 α knockdown cells compared with TOP2 α -TG2 double knockdown. To determine whether TOP2 α participate in DNA damage repair, we used a siRNA and found that TOP2 α siRNA inhibited radiation-induced phosphorylation of

DNA-PKcs as well as ATM (Fig. 3G). And similar results were observed in TOPOII α knockdown cells together with TG2 knockdown or glucosamine treatment (Fig. 3G). Then we transfected TOPOII α into TG2 knockdown cells, and found that TOPOII α overexpression rescued the inhibitory effects of TG2 knockdown on DDR pathway (Fig. 3G). However, TG2 overexpressing in TOPOII α knockdown cells showed no influence on DNA damage response (Fig. 3H).

TGase domain confers to the interaction with TOPOII α and radioresistance of NSCLC

TG2 is a multifunctional enzyme with different domains, structurally including four domains: an NH₂-terminal β -sandwich domain; a catalytic core domain containing a catalytic triad for the acyl-transfer reaction (Cys277, His335 and Asp358) for acyl-transfer reaction; a β -barrel1 domain, containing GDP/GTP-interacting residues, that is involved in receptor signaling and a β -barrel2 domain (19-21). To determine which domain was indispensable for the interaction with TOPOII α and confers to radioresistance, we generated constructs with different fragments, as well as constructs with mutation for dysfunction of distinct domain (Fig. 4A, B; Fig. S4A). We transfected these fragments or mutations into TG2 low expressing H1299 cells, and determined the cellular radiosensitivity based on survival fraction. Our data showed that TG2 full length expression increased radioresistance, which was only found in ABC domain expression of all fragments (Fig. 4C-D; Fig. S4B). However, in TG2 mutants transfected cells, only W241A mutant showed no increase on radioresistance (Fig. 4E, F; Fig. S4C). Then we performed immunoprecipitation assay in 293T cells transfected with both TG2 fragment or mutant and TOPOII α . It was found that none of TG2 fragment bind with TOPOII α efficiently (Fig. 4G), showing that the interaction of TG2 and TOPOII α might require the cooperation of multiple domains. Then we used TG2 expression vector with different functional mutations, and found that W241A mutant reduced the binding efficacy of TG2 and TOPOII α (Fig. 4H). Taken together, our data showed that TGase function might be critical for the role of TG2 in radioresistance.

TG2 inhibition sensitizes lung tumor to IR in vivo

To investigate the radiosensitizing effects of TG2 inhibition on NSCLC in vivo, we used an in situ lung cancer model generated by our group, together with local irradiation (Fig. S5A, B and C). It was found that if left untreated, all mice died within one month, while glucosamine treatment significantly increased survival of tumor bearing mice compared with single radiation group (Fig. 5A, B). The tumor size was reduced in glucosamine and radiation treated group (Fig. 5C). Through scanning the largest cross section of tumor, we found that the overall area was also significantly reduced in glucosamine combined with radiation group (Fig. 5D). HE staining of lung tissues observed that glucosamine treatment combined with radiation significantly reduced the size of lung cancer. And no invasion was observed in glucosamine treated group (Fig. 5D, E). Epithelial–mesenchymal transition (EMT) is an important process in the initiation of cancer metastasis. We performed IHC staining of tumor sample and found that glucosamine treatment reduced the level of Vimentin and α SMA, while elevated the expression of E-cadherin (Fig. 5F, G). These data indicated that glucosamine inhibited EMT process, which might accounts for the inhibition of cancer metastasis. TG2 is also found to participate in NF- κ B activation. We also found that glucosamine reduced Ki67 and p65 positive cells in tumor area (Fig. 5F, G).

High expression of TG2 predicts poor survival in lung adenocarcinoma instead of squamous cell carcinoma

It has been reported that TG2 was elevated in NSCLC patient tissues, and high expression of TG2 predicts poor outcome of disease free survival as well as overall survival (11). TG2 expression was also found to be related to survival of patients treated with EGFR-TKIs (22). However, we collected 80 pairs of tissues from NSCLC patients and also found that in some patients, TG2 mRNA expression was downregulated in lung cancer tissues compared with adjacent normal lung tissues (Fig. 6A). There is no evidence showing that TG2 expression was related to clinical features of age, gender and family cancer history (Table S2). When referred to cancer

pathological information, we found that TG2 was mainly highly expressed in glandular tubular adenocarcinoma (Figure 6A). However, TOP2A expression was not consistent with TG2, which indicates that maybe other mechanism is involved (Fig. 6B). TG2 protein expression was examined with IHC assay (Figure 6C, D). Then the association between TG2 expression and patient survival was examined with a Kaplan-Meier plotter tool (www.kmplot.com). Our data showed that high expression of TG2 was not associated with overall survival in all set of patients (Fig. S7, $P=0.0074$, dataset 216183; $P=0.075$, data set 211573; $P=0.19$, dataset 211003). Surprisingly, high TG2 expression was significantly associated with overall survival in lung adenocarcinoma lung cancer patients in three dataset (Fig. 6F to H, $P=0.0035$, dataset 216183; $P=4.7e-06$, data set 211573; $P=0.00056$, dataset 211003). While no significant difference was found in squamous cell carcinoma (Fig. S7, $P=0.61$, dataset 216183; $P=0.37$, data set 211573; $P=0.34$, dataset 211003). When compared with radiotherapy, no significant difference was found in patient with high TG2 or low TG2 expression (Fig. S8). With small sample size, we could still see the trends showing the possible association of TG2 and patients outcomes.

Discussion

In this study, we demonstrated that TG2 confers to radioresistance in NSCLC through promoting DNA repair. We found that TG2 inhibitor glucosamine significantly sensitize cancer cells and in vivo tumor to radiotherapy. For the first time, we observed significant nuclear translocation of TG2 in response to IR, and we found that TG2 knockdown abolished the activation of DDR signaling pathway. To identify the specific target of TG2, we used an IP-MS method and found that TG2 directly interact with TOP2 and promoted DNA repair, which might account for radioresistance. Finally, we identified that TGase catalytic function of TG2 was critical for DNA repair and radioresistance in NSCLC. Our data provide novel insight for the targeting TG2 in radiosensitization of lung cancer.

Targeting TG2 for radiosensitization in NSCLC

TG2 is ubiquitously expressed in many tissues and participate in multiple physiological functions, including wound healing, cancer metastasis, apoptosis as well as cell adhesion (23, 24). Recently, the increase level of TG2 was also shown was also related to chemoresistance in cancer, and targeting TG2 provide possibility of overcoming drug resistance (11, 25). In the present study, we found that TG2 inhibitor and siRNA significantly increased radiosensitivity of lung cancer cells. Then through an in vivo lung cancer model, we also proved that glucosamine sensitize lung cancer to IR. These findings indicate that TG2 contribute to resistance to drug or radiation induced cell death. However, it has been proved that TG2 play diverse roles in regulating cell death. On one hand, TG2 promote cell survival through activation of NF- κ B without degradation of I- κ B, and also induces cell resistance to chemotherapy (26). TG2 also promote chemoresistance through Akt activation, and it also inhibited cell apoptosis through downregulating Bax (27). On another hand, it was shown that TG2 promotes caspase dependent and independent apoptosis through Calpain/Bax Protein Signaling Pathway (28). Besides apoptosis and NF- κ B signaling pathway, it was found that the main mechanism of drug resistance was related to cathepsin D, nucleophosmin depletion through the crosslink activity (29, 30). However, targeting

TG2 for radiosensitization and its underlying mechanism was largely unknown.

Radiation induces TG2 translocation to nuclear and participate in DDR

On normal status, TG2 is predominantly located in cytoplasm, even there is some located in the nucleus, the mitochondria, on the plasma membrane, or in the extracellular cell surface (31, 32). Extracellular TG2 is mainly involved in wound healing and scarring, tissue fibrosis and cancer metastasis (33). And under some stimuli, TG2 was shown to translocate to nucleus. In hepatocellular carcinoma cells, acyclic retinoid induced significant nuclear translocation of TG2 and promoted cell death (34). To our knowledge, it is the first time that we observed nuclear translocation of TG2 in response to ionizing radiation, although the significance of TG2 nuclear localization had also been illustrated in other studies. To determine the reason of TG2 nuclear translocation, we used calcium blocker, NF- κ B inhibitor, TG2 inhibitor etc, and found that NF- κ B might play a critical role in this process.

Then by using TG2 inhibitor and siRNA, we found that TG2 inhibition impaired the activation of DNA repair pathway, including both NHEJ and HR, which is key mechanism for radiation response (35, 36). We found that the phosphorylation of ATM, DNA-PKcs, ATR were all suppressed, while more unrepaired DNA damage was observed. These data indicate that TG2 confers to DNA repair, the elevation of which promote radioresistance. It has been proved that TG2 is related to p53 and ATM function, but our data showed an earlier role of TG2 in DNA damage response, as TG2 translocate into nucleus at several minutes after irradiation. Through a laser assay, we observed that TG2 as well as TOP2 α were recruited to the DSB site, which suggest TG2 as a direct regulator of DNA repair. However, the exact role of TG2 in DNA repair was to be uncovered.

Interaction of TG2 and TOP2 α in DDR response

The significance of TG2 in many pathological processes has drawn much attention, and it has been proved that TG2 regulating many key proteins, including p65, p53 (37-39). To study its role in DNA repair, we performed an IP-MS analysis

and found several potential interacting proteins. Among these, we identified TOP2A as a target of TG2 and proved their interaction through IP assay. Surprisingly, we found that TOP2A knockdown inhibited activation of DNA repair pathway, and TG2 and TOP2A double knockdown didn't produce an additive effect. And for many years, multiple TOP2A inhibitors, such as etoposide, doxorubicin, have been used clinically to treating cancer, as well as in radiosensitization (40-42). Thus, inhibition of TG2- TOP2A signaling might account for the radiosensitizing effects of TG2 inhibitors. Then based on the distinct function and domain of TG2, we constructed several plasmid expressing different domain or different mutation of TG2. and we found that the TGase function of TG2 was indispensable for its interaction with TOP2A and accounts for radioresistance. Our data provide novel mechanism of TG2 in radioresistance. The TGase domain was also required for radioresistance.

Taken together, our findings suggest TG2 as a potential radiosensitizing target for lung cancer radiosensitization in vivo and in vitro. We also provide novel mechanism for the role of TG2 in radioresistance: after irradiation, TG2 translocated into nucleus, bind to DSB site, and initiate DNA damage repair through interacting with TOP2A. TG2 was correlated with outcomes of lung adenocarcinoma, and glucosamine provide possibility of translation in clinical applications.

Materials and methods

Reagents and plasmids

Glucosamine was purchased from Sigma. The following antibodies were used: anti-TG2 (Abcam, US; 1:1000), anti-Flag (Abcam, US; 1:1000), anti- γ -H2AX (Abcam, US; 1:1000), anti-Rad51 (Abcam, US; 1:1000), anti-P-ATM, anti-ATM (Abcam, US; 1:1000), anti-pT2069-DNA-PKcs, anti-DNA-PKcs (Abcam, US; 1:1000), anti-P-ATR, anti-ATR (Abcam, US; 1:1000), anti-TOPOII α (Abcam, US; 1:1000), anti-TBP, anti-GAPDH (sampler kit from Cell signaling technology, US; 1:1000), horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse IgG (all purchased from Cell Signaling Technology). Plasmids encoding different TGM2 fragments plasmids and different mutants (C277S, W241A, R580A, Y516F), cloned in pLenO-GTP were constructed by Biolink biotechnology (Shanghai) Co., Ltd.

Animals and glucosamine treatments

The whole protocols were approved by the Ethics Committee of Second Military Medical University, China. Female C57BL/6 mice, 8 weeks old, obtained from the Experimental Animal Center of Chinese Academy of Sciences, Shanghai, China, were used for the animal experiment. Mice were fed in daily-changed individual cages, at 25 $\pm$ 1 $^{\circ}$ C with food and water provided for free access. All of the animals were implanted with mouse Lewis lung cancer (LLC) cells (25 μ l, 2 $\times$ 10⁵ cells) in the fixed location which was 5mm distance from the lower end of the xiphoid process and parallel to it in right lung, the needle inserted depths was 5mm. The treated mice were randomly divided into four groups: group 1, non-irradiated +saline control; group 2, irradiation + saline; group 3, irradiation + glucosamine. Either glucosamine (150 mg/kg/d) was delivered to the corresponding groups by intraperitoneal injection 3 days before expose to whole lung irradiation.

Cell culture and glucosamine treatments

Mouse lewis lung cancer (LLC), human NSCLC (A549, H460, H1299, H1975, H358) and human bronchial epithelial cell line BEAS-2B were purchased from the ATCC (USA). YFP-53BP1-HT1080 was provided by Division of Molecular Radiation

Biology, Department of Radiation Oncology, University of Texas Southwestern Medical Center. Mouse Lewis lung cancer (LLC), human NSCLC (A549, H460, H1299, H1975, H358) and YFP-53BP1-HT1080 was maintained in DMEM with 10% fetal bovine serum at 37°C in a 5% CO₂ humidified chamber. Human bronchial epithelial cell line BEAS-2B was maintained in RPMI 1640 medium (10% fetal bovine serum) at 37°C in a 5% CO₂ humidified chamber as well. A549, H460, H1299, H1975, H358 and BEAS-2B cell was pre-treated with glucosamine at 1 hour before irradiation and further cultured for another 24 hours then switched to normal medium.

SiRNA and transfections

Small interfering RNA (siRNA) oligonucleotide duplexes were designed against TG2 (sense, 5'-AAGGGCGAACCACCTGAACAA-3' and antisense, 5'-TTGTTTCAGGTGGTTCGCCCTT-3') and TOPO II α siRNA (purchased from Thermo Fisher, Catalog # AM16708). Plasmids and miRNA were transfected with Lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions. At different time after transfection, cells were subjected to further experiment.

Irradiation

The ⁶⁰Co γ -rays in Radiation Center (Faculty of Naval Medicine, Second Military Medical University, Shanghai, China) were applied for the irradiation exposure. After anesthetization with 10% chloralhydrate (350mg/kg), the mice were treated whole lung irradiation. All radiated animals received a single dose of 15Gy with a dose rate of 1Gy/min and were monitored up to 2 weeks post-irradiation. Cells were treated with 2, 4, 8Gy of γ -rays irradiation at a dose rate of 1Gy/min.

Laser micro-IR

A 365-nm pulsed nitrogen laser (Spectra-Physics) was directly coupled to the epifluorescence path of the microscope (Axiovert 200M; Carl Zeiss) as described (43). Find the cell which placed on the cover slip two days before by using a Plan-Apochromat 63X/NA 1.40 oil immersion objective (Carl Zeiss, Inc). Then laser was used to generate DSBs in a defined area of the nucleus. At different time point, cells were fixed and subjected to immunofluorescence staining.

Cell viability assay (CCK-8 assay) and clonogenic survival

A549 and BEAS-2B cells in good condition were seeded in 96-well plates in triplicate and after adherence treated with glucosamine. After 24h treatment, cell viability was measured by using a CCK-8 assay (Beyotime, Shanghai, China) according to the manufacture's protocol.

Clonogenic survival was used to assess the potential of cell proliferation. Cells were calculated and seeded in the 6-wells plates, and then the pretreated cells were irradiated with 0, 2, 4, 8Gy. After incubated for 10 days, the plated were fixed with paraformaldehyde and stain with 1% methylene blue. The survival fractions were analyzed using a more target and one-hit model: $f=1-(1-\exp(-b \cdot x))^c$, where x is the dose in Gary and f is the survival fraction at dose x. b and c are the parameters of the survival curve.

Apoptosis assay

After 24h post-irradiation, cell apoptosis were measured by double-staining with Annexin V-fluorescein isothiocyanate (Annexin V-FITC) and Propidium Iodide (PI) by Apoptosis Detection Kit (Invitrogen, Carlsbad, California, USA) and analyzed by flow cytometry (Beckman Cytoflex) according to the manufacturer's instructions.

Comet Assay

The DNA double-strand breaks of A549 cells were determined by ameliorating the aforementioned neutral comet assay. This part uses two-layer-agarose style, 1% normal melting agarose (NMA) as the base layer on the slide, 0.65% low melting point agarose (LMA) as the upper layer, suitable for smoothing and agarose adhesion, and The clarity of micrograms. Firstly, we prepared the slides by immersing the clean slides in a molten 1% NMA and wiping it immediately. All slides are pre-painted in advance to make sure they are thoroughly dry the next use (previous day). Next, the concentration of the single cell suspension prepared in ice-free Ca^{2+} and Mg^{2+} -containing PBS was adjusted to 2×10^4 cells / ml, and 0.4 ml of the solution was then immersed in 1.2 ml of LMA, 40°C water bath. Thirdly, 1.2ml of the cell

suspension was mixed and rapidly pipetted onto the surface of the precoated slide. Fourth, once the solid solution, neutral solution (58.44 g NaCl, 5.584 g Na₂EDTA and 0.61 g Tris) was dissolved in 500 ml of double distilled water, pH 8.2-8.5, Triton X-100 final concentration was 1% before use) Stay in the darkness. The slides were then gently soaked in TBE rinse buffer (0.744 g Na₂EDTA, 10.902 g Tris and 5.564 g boric acid in 500 ml double distilled water PH8.2-8.5) and then incubated at 4 °C in the dark for 25 min at 25 V and 7 mA in fresh TBE. Fifth, the gel washed with ddH₂O (double distilled water) was stained with PI (10µg/ml) for 20 minutes and then rinsed gently with ddH₂O. Finally, all gels were examined by a fluorescence microscope (Olympus BX60) under a 10X objective. A total of 100 comet images in each slide were analyzed using special analysis software named CASP 1.2.3b2 (CASPlab, Wroclaw, Poland), which contained several features of DNA content, tail length, olive tail and tail.

Western blotting and immunoprecipitation

Total proteins were obtained from cell lines using ProtecJETTM Mammalian Cell Lysis Reagent (Fermentas, Vilnius, Baltic, Lithuania) according to the manufacturer's instruction. For nuclear and cytoplasmic protein, we using ProteinExt[®] Mammalian Nuclear and Cytoplasmic Protein Extraction Kit. Briefly, cell pellets were resuspended(4×10^7 cells/ml) in PBS by low-speed centrifugation (1000× g, 3min, 4°C) , the pellets were lysed by 500 µl CPEB I and incubated in ice for 10mins, then added 55µl CPEB II and vortexed for 5s and incubated on ice for 1 min. After high-speed centrifugation (16000×g, 4°C) for 15min, the supernatant were collected as the cytoplasmic protein. Resuspended the pellets with 500 µl CPEB I, vortexed for 5s. Carefully discarded the supernatant, resuspended the pellets with 200 µl NPEB, incubated on ice for 30 mins and high-speed centrifugation(16000× g, 4°C) for 10mins. Collected the supernatant which was the nuclear protein. Then the samples were analyzed by western blotting with chemiluminescent detection as described elsewhere. For immunoprecipitation (IP), Cells were lysed in IP buffer (#9803,CST) and incubated overnight with pulled antibody-protein A beads(#9863,CST). The

beads were washed with IP buffer and resuspended in 3X SDS Sample Buffer: (#7722,CST) for WB.

Histopathology and immunohistochemistry

On 7 days after local lung irradiation, lung and tumor tissues were isolated, fixed and subjected to sectioning. Tissues were stained with H&E and antibodies for Ki67 (1:200; Cell Signaling Tech.), p65 (1:200; Cell Signaling Tech.), E-cadherin (1:200; Cell Signaling Tech.), Vimentin (1:200; Cell Signaling Tech.) and α -SMA (1:200; Cell Signaling Tech.). Five fields per section at $\times 200$ magnifications were randomly selected per mouse, and two blinded pathologists independently examined 30 fields per group using Nikon DS-Fi1-U2 microscope (Nikon, Tokyo, Japan).

Immunofluorescence analysis

We used an immunofluorescence assay to detect γ H2AX foci (DNA double strand break marker), the subcellular location of 53BP1, TG2 and TOP2 α . Briefly, A549 cells were seeded on 22X22mm² cover glasses in 6-well plates at the concentration of 2×10^5 per well. After different treatment, cells were fixed in 4% paraformaldehyde for 10min and permeabilized in 0.5% Triton X-100 for 10min. After blocked in serum, cells were stained with primary antibody (1:200) and then with the secondary antibody (1:1000). Cellular images were obtained using an Olympus BX60 fluorescent microscope (Olympus America Inc., Center Valley, PA, USA) equipped with a Retiga 2000R digital camera (Q Imaging Inc., Surrey, BC, Canada). Image Pro Plus (Media Cybernetics, Silver Springs, MD) were used to count the γ H2AX foci per cell according to our previous studies, and at least 100 cells per group were counted.

Patients samples and realtime PCR

Paired NSCLC and adjacent normal tissues resected surgically used for qRT-PCR were collected from 80 patients during operation at Shanghai Pulmonary Hospital (Shanghai, China). All the experiments were conducted with the informed consent of the patients and were approved by the ethics committee of Shanghai Pulmonary Hospital. Tissue array was performed by Zhuhao Tech., Shanghai. Total RNA was extracted from normal lung tissues and lung cancer tissues with TRIzol reagent, as

described by the manufacturer (Invitrogen). The isolated RNA concentration was measured by Genequant pro (Biochrom Ltd, Cambridge England). The RNA (1 µg) was applied to generate cDNA by means of PrimeScript™RT Master Mix (Takara, RR036A). The real-time quantitative PCR was performed by using SYBR Green Master Mix (Takara, RR420A) in StepOnePlus 96 Real-Time PCR System (Applied Biosystems). The average threshold cycle (Ct) of quadruplicate reactions was determined, and amplification was analysed by the $\Delta\Delta C_t$ method. Gene expression was normalized to that of GAPDH. Real-time quantitative PCR with reverse transcription data were representative of at least three independent experiments. Primer sequences used to amplify human TG2 and TOPOII α and GAPDH were as follows: TG2 forward: CCTGATCGTTGGGCTGAAG, TG2 reverse: TCGGCCAGTTTGTTCAGGTG; TOPOII α forward: CCCACATCAAAGGCTTGCTG, TOPOII α reverse: GATGTGCTGGTGCCCAAACC. GAPDH forward: AGCCACATCGCTCAGACAC, GAPDH reverse: GCCCAATACGACCAAATCC.

Kaplan Meier Survival Analysis

We used an online tool Kaplan Meier (KM) plotter to analyze overall survival in lung cancer patients as described previously (44). Briefly, KM plot was obtained from the KM Plotter web-based (kmplot.com/analysis) curator, which includes relapse-free and overall survival data on 54,675 genes from 2437 lung cancer patients. In our analysis, patients were differed with TG2 expression, in combination with histology subtype, and radiotherapy. Populations were separated by median TG2 expression and plots were generated accordingly. From the KM database, three set of microarray Affymetrix probe 216183, 211573, 211003 were used in our analysis.

Statistical analysis

Data were expressed at the means $\pm$ standard error of mean (SEM). Between group differences were tested using a one-way ANOVA. Two-group comparisons were performed using independent-samples Student's t-test. $P < 0.05$ was considered significant. All experiments were performed at least 3 independent times.

467

Author contributions

Y.Yang. X.Lei. and Z.Liu.: study concept and design, carried out experiments, preparation of manuscript, obtain funding. K.Cao, Y.Chen: carried out experiments, data analysis, figures preparation. H.Qin, H.Qu, L.Liu. and Z.Liao: carried out experiments. J.Cai F.Gao Y.Yang: study design, obtained funding.

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611 **Figures and Figure legends**

Figure1. Inhibition of TG2 sensitizes lung cancer cells to ionizing radiation

(A) A549 and BEAS-2B cell lines were analyzed for their cell viability after treated with different concentration of glucosamine. (B) Expression of TG2 in A549 cells with 0, 1, 5mM glucosamine pretreated. (C) Expression of TG2 in A549 and BEAS-2B cells. (D-F) A549, H1299, H460 and their TG2 knock down cell lines were analyzed for their colony forming ability against IR with/without glucosamine (5mM) pretreated. (G) A549 WT and CRISPR KO cells were analyzed for their colony forming ability against IR. (H, I) Flow cytometric analysis of A549 cell line against 8Gy irradiation with/without glucosamine (5mM) pretreated. *P < 0.05, **P < 0.01 versus radiation group.

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624 **Figure2. Radiation induces TG2 nuclear translocation and initiates DNA damage**

625 **response.** (A) Immunofluorescence of TG2 and 53BP1 in A549 cells exposed to IR with/without

626 glucosamine (5mM) pretreated. (B) Immunoblot of endogenous TG2 in the cytoplasmic and

627 nuclear fractions of A549 cells exposed to 8Gy irradiation with/without glucosamine (5mM)

628 pretreated. (C) A549 cells were exposed to IR with/without glucosamine (5mM) pretreated and

629 harvested at the indicated time points. Whole cell lysates were analyzed with indicated antibodies.

(D) A549 and A549 TG2 knock down cells were exposed to IR with/without glucosamine (5mM) pretreatments and harvested at 0, 0.5h. Then whole cell lysates were analyzed with indicated antibodies. (E) A549 and A549 TG2 KO cells exposed to IR were immunofluorescent stained against γ -H2AX (green) and DAPI (blue). (F) The average numbers of γ -H2AX foci per cell among A549 and A549 TG2 KO cells exposed to 2Gy irradiation. (G) Representative comet assay showing the tail moment of A549 cells exposed to IR with/without glucosamine (5mM). $n = 3$ independent experiments. Quantification in H and J; data represent mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$ versus radiation group.

Figure3. TG2 interacts with TOP2IIα and participate in DNA repair

(A) Venn diagram about bioinformatics analysis in Immunoprecipitation–Mass Spectrometry (IP-MS) assay in A549 cells. (B) Representative immunofluorescence of TG2 and 53BP1 at DNA damage sites, in HT1080 cells following laser microirradiation. (n = 3 independent experiments). (C) A549 cells exposed to IR were harvested at 0, 0.5h. Whole cell lysates were subjected to IP

with anti-TG2 antibody and immunoblotted with TOPOII α antibody. (D) 293T cells transfected with FLAG-tagged TG2 and TOPOII α constructs were exposed to IR (8Gy) and harvested for 30mins. Whole cell lysates were subjected to co-IP with anti- TOPOII α antibody and western blotted against anti-TG2 and anti- TOPOII α antibodies. (E) 293T cells transfected with FLAG-tagged TG2 and TOPOII α constructs were exposed to IR (8Gy) and harvested for 30mins. Whole cell lysates were subjected to co-IP with anti- FLAG antibody and western blotted against anti-TOPOII α and anti-TG2 antibodies. (F) Representative comet assay showing the tail moment of A549 cells transfected with TOPOII α siRNA were exposed to IR with/without glucosamine (5mM) or TG2siRNA. (n = 3 independent experiments). (G) A549 cells transfected with TG2 construct/ TG2 siRNA/ TG2 siRNA and TOPOII α construct were treated with IR (8Gy) and harvested at the indicated time points. Whole cell lysates were analyzed with indicated antibodies. (H) A549 cells transfected with TOPOII α siRNA/ TOPOII α siRNA and TG2 construct were treated with IR (8Gy) and harvested at the indicated time points. Whole cell lysates were analyzed with indicated antibodies.

Figure 4 TGase function confers the interaction with TOP2 α and radioresistance of NSCLC. (A) Schematic structure of TG2 full length, and fragments including AB, ABC, AB+C, B+C, CD. (B) Plasmids encoding wild-type TGM2, W241A, C277S,

664 R580A, Y516F cloned in pLenO-GTP were constructed by Biolink biotechnology(Shanghai
 665 Co.,Ltd. (C-D) Survival assay of wild type TG2 and ABC fragment in response to different doses
 666 of radiation. (E-F) Analyzing TG2 mutants transfected cells for their colony forming ability
 667 against IR (WT and W241A). (I) immunoprecipitation assay in 293T cells transfected with both
 668 TG2 fragment and TOPOII α . (J) immunoprecipitation assay in 293T cells transfected with both
 669 TG2 mutant and TOPOII α .

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671

Figure 5. TG2 inhibition sensitizes lung cancer to IR in vivo. (A) Tumors formed in female C57BL/6 mice by injection of LLC cells. The recipient mice were treated with whole lung irradiation (15Gy) with/without glucosamine (150 mg/kg/d) for 3 days before IR. (B) Survival rate of tumor-bearing mice treated with whole lung irradiation (15Gy) with/without glucosamine (150 mg/kg/d) for 3 days before IR. (C) The mean diameter of each tumor in different groups. (D) the

images of each group scanning the largest cross section of tumor in different days. (E) HE staining of lung tissues with tumor in each group. (F)E-cadherin, SMA, Vimentin, Ki67 and p65 staining of lung tissues with tumor after IR with/without glucosamine treated.(G) Quantification of protein expression levels of E-cadherin, SMA, Vimentin, Ki67 and p65 staining of lung tissues with tumor after IR with/without glucosamine treated. Values are given as mean± SEM (n=10), *P<0.05 and **P<0.01 versus single radiation group.

Figure6. High expression of TG2 predicts poor survival in lung adenocarcinoma instead of squamous cell carcinoma.

(A) TG2 mRNA expression in lung cancer tissues compared with adjacent normal lung tissues. (B)

690 TOPOII α level in lung cancer tissues compared with adjacent normal lung tissues. (C) scanning of
691 tissue array of the samples we collected from NSCLC patients. (D) representative images of TG2
692 negative and positive staining of lung cancer samples. (E-G) Kaplan-Meier survival curve
693 showing significant association between TG2 IHC staining and overall survival of patients from
694 lung adenocarcinoma from three set of microarray (P=0.0035, dataset 216183; P=4.7e-06, data set
695 211573; P=0.00056, dataset 211003).
696

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698

699 **Figure 7 Proposed model illustrating the mechanism how TG2 confers to**
700 **radioresistance.** In response to radiation, TG2 translocate into nucleus and is
701 recruited to DSB sites. The recruitment of TG2 initiate phosphorylation of DNA-PKcs
702 and ATM through interacting with TOPOIIα, which promoted DNA repair. After
703 glucosamine treatment or TG2 knockdown, TG2-TOPOIIα mediated DNA repair was
704 abrogated, and the cancer cells were radiosensitized.

705

Figure S1

Figure S1 (S1A) Expression of TG2 in lung cancer cells including A549, H1975, H1299, H460, LLC and H358 cells, as well as normal BEAS-2B cells. (S1B, C) Representative images and column chart of flow cytometric analysis of BEAS-2B cell line against 8Gy irradiation with/without glucosamine (5mM) pretreated.

Figure S2

Figure S2 (S2A) Immunofluorescence of TG2 and DAPI in A549 cells exposed to IR with POH, cystamine or QNZ pretreatment. (S2B) Immunofluorescence of TG2 and 53BP1 in A549 cells exposed to IR with/without glucosamine (5mM) pretreated in delicate time point.

Figure S3

Figure S3 (S3A) Representative immunofluorescence of TOPOIIα and 53BP1 at DNA damage sites, in HT1080 cells following laser microirradiation. (n = 3 independent experiments).

(S3B) A549 WT and TG2 KD cells transfected with TOPOIIα siRNA were analyzed for their colony forming ability against IR.

(S3C) Expression of γ-H2AX in WT, TG2 knockdown or TOPOIIα knockdown A549 cell lines

722 after IR.

Figure S4

723

724 **Figure S4** (S4A) Overlap of TG2 loss-function mutants and different fragment. (S4B) Survival
725 of cells transfected with different TGM2 fragments plasmids (AB,AB+C, B+C,CD) were
726 performed after irradiation. (S4C) Survival of cells transfected with different TG2 mutants (C277S,

727 R580A, Y516F).

728

Figure S5

729

730 **Figure S5** (S5A) The establishment of the in situ lung cancer model. (S5B)

731 representative images of in situ lung cancer model and HE staining of each tumor.

732 (S5C). time schedule of tumor implantation, radiotherapy and drug delivery.

733

Figure S6 IHC staining of TG2 in two sets of lung cancer samples (total 160 pairs).

Figure S7

Figure S7 Kaplan-Meier survival curve showing little associations between TG2 IHC staining and overall survival of patients from lung squamous cell carcinoma and all patients from three sets of microarray.

Figure S8

Figure S8 Kaplan-Meier survival curve showing no significant association between TG2 IHC staining and overall survival of patients from lung cancer patients with and without radiotherapy.

747 **Table S2 TG2 expression and clinical parameters**

Parameters	TG2 expression		Total (n=80)	P value
	Low TG2	High TG2		
Age (yr)				
<60	20	21	41	1
>60	20	19	39	
Sex				
Male	29	25	54	0.4739
Female	11	15	26	
Invasion				
Yes	8	9	17	1
No	32	31	63	
Histology				
Adenocarcinoma	21	28	47	0.1685
No-Ade.	19	12	31	
Adjuvant chemotherapy				
Yes	12	20	32	0.1101
No	28	20	48	
Smoking history				
Yes	18	11	29	0.1629
No	22	29	51	

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750 Note: see Table S1 in attached excel file.

134 elements included exclusively in "IR":

381 common elements in "List 1", "List 2" and "List 3":

tr|B4E3A4|B4E3A4_HUMAN cDNA FLJ57283, highly similar to Actin, cytoplasmic 2 OS=Homo sapiens PE=2 SV=1
tr|B4E335|B4E335_HUMAN cDNA FLJ52842, highly similar to Actin, cytoplasmic 1 OS=Homo sapiens PE=2 SV=1
sp|P35579|MYH9_HUMAN Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4
tr|A0A024R1N1|A0A024R1N1_HUMAN Myosin, heavy polypeptide 9, non-muscle, isoform CRA_a OS=Homo sapiens
tr|W8QEH3|W8QEH3_HUMAN Lamin A/C OS=Homo sapiens GN=LMNA PE=3 SV=1
tr|Q5I6Y5|Q5I6Y5_HUMAN Lamin A/C transcript variant 1 OS=Homo sapiens GN=LMNA PE=2 SV=1
tr|Q0VAS5|Q0VAS5_HUMAN Histone H4 OS=Homo sapiens GN=HIST1H4H PE=2 SV=1
tr|E9PKE3|E9PKE3_HUMAN Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1
tr|B3KTV0|B3KTV0_HUMAN cDNA FLJ38781 fis, clone LIVER2000216, highly similar to HEAT SHOCK COGNATE 71
tr|Q53HF2|Q53HF2_HUMAN Heat shock 70kDa protein 8 isoform 2 variant (Fragment) OS=Homo sapiens PE=1 SV=1
tr|Q9BSV4|Q9BSV4_HUMAN SFPQ protein (Fragment) OS=Homo sapiens GN=SFPQ PE=2 SV=2
tr|Q86VG2|Q86VG2_HUMAN Splicing factor proline/glutamine-rich (Polypyrimidine tract binding protein associated)
tr|Q0D2M2|Q0D2M2_HUMAN HIST1H2BC protein OS=Homo sapiens GN=HIST1H2BC PE=2 SV=1
tr|Q8J014|Q8J014_HUMAN Ribosomal protein S2 OS=Homo sapiens GN=rps2 PE=2 SV=1
tr|Q3KQT6|Q3KQT6_HUMAN Ribosomal protein S2 OS=Homo sapiens GN=RPS2 PE=2 SV=1
sp|P07814|SYEP_HUMAN Bifunctional glutamate/proline--tRNA ligase OS=Homo sapiens GN=EPRS PE=1 SV=5
tr|A8JZY9|A8JZY9_HUMAN Tubulin alpha chain OS=Homo sapiens PE=2 SV=1
sp|P68363|TUBA1B_HUMAN Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1
tr|B7Z1V7|B7Z1V7_HUMAN cDNA FLJ51811, highly similar to Stress-70 protein, mitochondrial OS=Homo sapiens
tr|Q6MZX8|Q6MZX8_HUMAN Putative uncharacterized protein DKFZp686K06110 OS=Homo sapiens GN=DKFZp6
tr|B7Z597|B7Z597_HUMAN cDNA FLJ54373, highly similar to 60 kDa heat shock protein, mitochondrial OS=Homo sapiens
tr|B7Z4F6|B7Z4F6_HUMAN cDNA FLJ54912, highly similar to 60 kDa heat shock protein, mitochondrial OS=Homo sapiens
tr|A2J422|A2J422_HUMAN Anti-HER3 scFv (Fragment) OS=Homo sapiens PE=2 SV=1
tr|A2J423|A2J423_HUMAN Anti-Mpl scFv (Fragment) OS=Homo sapiens PE=2 SV=1
tr|Q65ZC9|Q65ZC9_HUMAN Single-chain Fv (Fragment) OS=Homo sapiens GN=scFv PE=2 SV=1
tr|E5RH77|E5RH77_HUMAN 40S ribosomal protein S14 OS=Homo sapiens GN=RPS14 PE=1 SV=1
tr|E9PPU1|E9PPU1_HUMAN 40S ribosomal protein S3 OS=Homo sapiens GN=RPS3 PE=1 SV=1
tr|Q65ZQ3|Q65ZQ3_HUMAN FBRNP OS=Homo sapiens GN=D10S102 PE=2 SV=1
tr|D2KTB2|D2KTB2_HUMAN Helicase-like protein (Fragment) OS=Homo sapiens GN=Si-11 PE=2 SV=1
tr|D2KTB3|D2KTB3_HUMAN Helicase-like protein (Fragment) OS=Homo sapiens GN=Si-11-6 PE=2 SV=1
tr|B4DYS5|B4DYS5_HUMAN cDNA FLJ50297, weakly similar to ATP-dependent DNA helicase MER3 (EC 3.6.1.-) OS=Homo sapiens
tr|B4DGT2|B4DGT2_HUMAN cDNA FLJ50147, weakly similar to ATP-dependent DNA helicase MER3 (EC 3.6.1.-) OS=Homo sapiens
sp|A2PYH4|HFM1_HUMAN Probable ATP-dependent DNA helicase HFM1 OS=Homo sapiens GN=HFM1 PE=1 SV=1
tr|B7ZM00|B7ZM00_HUMAN HFM1 protein (Fragment) OS=Homo sapiens GN=HFM1 PE=2 SV=1
tr|B4E132|B4E132_HUMAN cDNA FLJ53122, highly similar to ATP-dependent RNA helicase DDX3Y (EC 3.6.1.-) OS=Homo sapiens
tr|A0A024R9A4|A0A024R9A4_HUMAN DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked, isoform CRA_a OS=Homo sapiens
sp|O15523|DDX3Y_HUMAN ATP-dependent RNA helicase DDX3Y OS=Homo sapiens GN=DDX3Y PE=1 SV=2
tr|Q5S4N1|Q5S4N1_HUMAN Putative uncharacterized protein (Fragment) OS=Homo sapiens PE=2 SV=1
sp|O14746|TERT_HUMAN Telomerase reverse transcriptase OS=Homo sapiens GN=TERT PE=1 SV=1
tr|Q9UBR6|Q9UBR6_HUMAN Telomerase reverse transcriptase (Fragment) OS=Homo sapiens GN=TERT PE=4 SV=1
tr|O94807|O94807_HUMAN Telomerase transcriptase (Fragment) OS=Homo sapiens GN=hTERT PE=4 SV=1
sp|O60307|MAST3_HUMAN Microtubule-associated serine/threonine-protein kinase 3 OS=Homo sapiens GN=MAST3 PE=1 SV=1
sp|O75400|PRPF40A_HUMAN Pre-mRNA-processing factor 40 homolog A OS=Homo sapiens GN=PRPF40A PE=1 SV=1
tr|B4DPY2|B4DPY2_HUMAN cDNA FLJ59286, highly similar to Pre-mRNA-processing factor 40 homolog A (Fragment)
sp|O75643|U520_HUMAN U5 small nuclear ribonucleoprotein 200 kDa helicase OS=Homo sapiens GN=SNRNP200 PE=1 SV=1

tr|F8W079|F8W079_HUMAN ATP synthase subunit beta, mitochondrial (Fragment) OS=Homo sapiens GN=ATP5F1
tr|I6L957|I6L957_HUMAN HNRNPA2B1 protein OS=Homo sapiens GN=HNRNPA2B1 PE=2 SV=1
tr|A0A087WUI2|A0A087WUI2_HUMAN Heterogeneous nuclear ribonucleoproteins A2/B1 OS=Homo sapiens GN=HNRNPA2B1
sp|P38919|IF4A3_HUMAN Eukaryotic initiation factor 4A-III OS=Homo sapiens GN=EIF4A3 PE=1 SV=4
tr|A0A024R8W0|A0A024R8W0_HUMAN DEAD (Asp-Glu-Ala-Asp) box polypeptide 48, isoform CRA_a OS=Homo sapiens GN=TOP2B
sp|Q02880|TOP2B_HUMAN DNA topoisomerase 2-beta OS=Homo sapiens GN=TOP2B PE=1 SV=3
sp|P11388|TOP2A_HUMAN DNA topoisomerase 2-alpha OS=Homo sapiens GN=TOP2A PE=1 SV=3
tr|B4DKD0|B4DKD0_HUMAN DNA topoisomerase 2 (Fragment) OS=Homo sapiens PE=2 SV=1
tr|E9PCY5|E9PCY5_HUMAN DNA topoisomerase 2 (Fragment) OS=Homo sapiens GN=TOP2B PE=1 SV=1
tr|Q71UH4|Q71UH4_HUMAN DNA topoisomerase 2 (Fragment) OS=Homo sapiens GN=TOP2B PE=3 SV=1
sp|Q12788|TBL3_HUMAN Transducin beta-like protein 3 OS=Homo sapiens GN=TBL3 PE=1 SV=2
tr|A0A087WYP7|A0A087WYP7_HUMAN Transducin beta-like protein 3 OS=Homo sapiens GN=TBL3 PE=1 SV=1
tr|A0JLS5|A0JLS5_HUMAN TBL3 protein (Fragment) OS=Homo sapiens GN=TBL3 PE=2 SV=1
tr|J3KNP2|J3KNP2_HUMAN Transducin beta-like protein 3 (Fragment) OS=Homo sapiens GN=TBL3 PE=1 SV=1
sp|Q68DL7|CR063_HUMAN Uncharacterized protein C18orf63 OS=Homo sapiens GN=C18orf63 PE=2 SV=2
sp|Q6ZMW3|EMAL6_HUMAN Echinoderm microtubule-associated protein-like 6 OS=Homo sapiens GN=EML6 PE=2 SV=1
sp|Q9UPN7|PP6R1_HUMAN Serine/threonine-protein phosphatase 6 regulatory subunit 1 OS=Homo sapiens GN=PPP6R1
sp|Q9UPR3|SMG5_HUMAN Protein SMG5 OS=Homo sapiens GN=SMG5 PE=1 SV=3
tr|Q96SX4|Q96SX4_HUMAN cDNA FLJ14580 fis, clone NT2RM4001204 OS=Homo sapiens PE=2 SV=1
tr|A0A024R4Q8|A0A024R4Q8_HUMAN Ribosomal protein S5, isoform CRA_a OS=Homo sapiens GN=RPS5 PE=3 SV=1
tr|M0R0F0|M0R0F0_HUMAN 40S ribosomal protein S5 (Fragment) OS=Homo sapiens GN=RPS5 PE=1 SV=1
tr|M0R0R2|M0R0R2_HUMAN 40S ribosomal protein S5 OS=Homo sapiens GN=RPS5 PE=1 SV=1
sp|P46782|RS5_HUMAN 40S ribosomal protein S5 OS=Homo sapiens GN=RPS5 PE=1 SV=4
tr|Q53G25|Q53G25_HUMAN Ribosomal protein S5 variant (Fragment) OS=Homo sapiens PE=2 SV=1
tr|M0QZN2|M0QZN2_HUMAN 40S ribosomal protein S5 OS=Homo sapiens GN=RPS5 PE=1 SV=1
tr|A0A024R5K8|A0A024R5K8_HUMAN Serpin peptidase inhibitor, clade H (Heat shock protein 47), member 1, (C) OS=Homo sapiens GN=SERPINH1
tr|E9PKH2|E9PKH2_HUMAN Serpin H1 OS=Homo sapiens GN=SERPINH1 PE=1 SV=1
sp|P50454|SERPH_HUMAN Serpin H1 OS=Homo sapiens GN=SERPINH1 PE=1 SV=2
tr|A8K259|A8K259_HUMAN cDNA FLJ78501, highly similar to Homo sapiens serpin peptidase inhibitor, clade H (Heat shock protein 47), member 1, (C)
tr|B4DN87|B4DN87_HUMAN cDNA FLJ52569, highly similar to Collagen-binding protein 2 OS=Homo sapiens PE=2 SV=1
tr|A0A087WXY2|A0A087WXY2_HUMAN Plakophilin-2 (Fragment) OS=Homo sapiens GN=PKP2 PE=1 SV=1
tr|A0A0G2JNU3|A0A0G2JNU3_HUMAN Transcription factor TFIIIB component B'' homolog OS=Homo sapiens GN=TFIIIB
tr|B1WB49|B1WB49_HUMAN BDP1 protein (Fragment) OS=Homo sapiens GN=BDP1 PE=2 SV=1
sp|A6H8Y1|BDP1_HUMAN Transcription factor TFIIIB component B'' homolog OS=Homo sapiens GN=BDP1 PE=1 SV=1
tr|A0A1B0GUM4|A0A1B0GUM4_HUMAN Cyclin-dependent kinase-like 5 OS=Homo sapiens GN=CDKL5 PE=1 SV=1
tr|A0A1B0GTX4|A0A1B0GTX4_HUMAN Cyclin-dependent kinase-like 5 (Fragment) OS=Homo sapiens GN=CDKL5 PE=2 SV=1
tr|A0A096LNR9|A0A096LNR9_HUMAN Cyclin-dependent kinase-like 5 (Fragment) OS=Homo sapiens GN=CDKL5 PE=2 SV=1
tr|A0A096LPI4|A0A096LPI4_HUMAN Cyclin-dependent kinase-like 5 (Fragment) OS=Homo sapiens GN=CDKL5 PE=2 SV=1
tr|A0A096LPG3|A0A096LPG3_HUMAN Cyclin-dependent kinase-like 5 (Fragment) OS=Homo sapiens GN=CDKL5 PE=2 SV=1
tr|A0A096LP32|A0A096LP32_HUMAN Cyclin-dependent kinase-like 5 (Fragment) OS=Homo sapiens GN=CDKL5 PE=2 SV=1
tr|A7E2A5|A7E2A5_HUMAN ARAP2 protein (Fragment) OS=Homo sapiens GN=ARAP2 PE=2 SV=1
sp|Q8WZ64|ARAP2_HUMAN Arf-GAP with Rho-GAP domain, ANK repeat and PH domain-containing protein 2 OS=Homo sapiens GN=ARAP2
tr|Q2M2Z8|Q2M2Z8_HUMAN ARAP2 protein (Fragment) OS=Homo sapiens GN=ARAP2 PE=2 SV=1
tr|A9XA89|A9XA89_HUMAN Alpha-helix coiled-coil rod homologue (Fragment) OS=Homo sapiens GN=HCR PE=4 SV=1
tr|A9XA72|A9XA72_HUMAN Alpha-helix coiled-coil rod homologue (Fragment) OS=Homo sapiens GN=HCR PE=4 SV=1
tr|A9XA71|A9XA71_HUMAN Alpha-helix coiled-coil rod homologue (Fragment) OS=Homo sapiens GN=HCR PE=4 SV=1
tr|A9XA75|A9XA75_HUMAN Alpha-helix coiled-coil rod homologue (Fragment) OS=Homo sapiens GN=HCR PE=4 SV=1

tr|B3KR57|B3KR57_HUMAN Calcium-transporting ATPase OS=Homo sapiens PE=2 SV=1
 tr|A0A0A0MSP0|A0A0A0MSP0_HUMAN Calcium-transporting ATPase OS=Homo sapiens GN=ATP2C2 PE=1 SV=1
 tr|B7ZA13|B7ZA13_HUMAN Calcium-transporting ATPase OS=Homo sapiens PE=2 SV=1
 sp|O75185|AT2C2_HUMAN Calcium-transporting ATPase type 2C member 2 OS=Homo sapiens GN=ATP2C2 PE=1
 tr|B4DQQ8|B4DQQ8_HUMAN cDNA FLJ60806, highly similar to RalBP1-associated Eps domain-containing proteir
 tr|Q59H22|Q59H22_HUMAN RalBP1 associated Eps domain containing protein 2 variant (Fragment) OS=Homo sa
 sp|Q8NFH8|REPS2_HUMAN RalBP1-associated Eps domain-containing protein 2 OS=Homo sapiens GN=REPS2 PE
 tr|B4DSF8|B4DSF8_HUMAN cDNA FLJ57278 OS=Homo sapiens PE=2 SV=1
 tr|B3KX96|B3KX96_HUMAN cDNA FLJ45003 fis, clone BRAWH3011623, highly similar to Heterogeneous nuclear r
 tr|F5H676|F5H676_HUMAN T-complex protein 1 subunit alpha (Fragment) OS=Homo sapiens GN=TCP1 PE=1 SV=
 tr|F5H726|F5H726_HUMAN T-complex protein 1 subunit alpha (Fragment) OS=Homo sapiens GN=TCP1 PE=1 SV=
 tr|F5H136|F5H136_HUMAN T-complex protein 1 subunit alpha (Fragment) OS=Homo sapiens GN=TCP1 PE=1 SV=
 tr|E7ERJ7|E7ERJ7_HUMAN Polyadenylate-binding protein OS=Homo sapiens GN=PABPC1 PE=1 SV=1
 tr|A0A024R9E2|A0A024R9E2_HUMAN Poly(A) binding protein, cytoplasmic 1, isoform CRA_c OS=Homo sapiens (C
 tr|B4DQX0|B4DQX0_HUMAN Polyadenylate-binding protein OS=Homo sapiens PE=2 SV=1
 tr|F1T0J8|F1T0J8_HUMAN REST corepressor 2 OS=Homo sapiens GN=RCOR2 PE=2 SV=1
 sp|Q9P2K3|RCOR3_HUMAN REST corepressor 3 OS=Homo sapiens GN=RCOR3 PE=1 SV=2
 sp|Q8IZ40|RCOR2_HUMAN REST corepressor 2 OS=Homo sapiens GN=RCOR2 PE=1 SV=2
 sp|Q9UKL0|RCOR1_HUMAN REST corepressor 1 OS=Homo sapiens GN=RCOR1 PE=1 SV=2
 tr|E9PQE5|E9PQE5_HUMAN REST corepressor 3 (Fragment) OS=Homo sapiens GN=RCOR3 PE=1 SV=1
 tr|B4DV59|B4DV59_HUMAN REST corepressor 3 OS=Homo sapiens GN=RCOR3 PE=1 SV=1
 tr|E9PLA9|E9PLA9_HUMAN Caprin-1 (Fragment) OS=Homo sapiens GN=CAPRIN1 PE=1 SV=1
 tr|H6QX63|H6QX63_HUMAN Hepatocellular carcinoma related protein 2 OS=Homo sapiens PE=2 SV=1
 sp|Q6PK04|CC137_HUMAN Coiled-coil domain-containing protein 137 OS=Homo sapiens GN=CCDC137 PE=1 SV=
 tr|I3L0U5|I3L0U5_HUMAN Coiled-coil domain-containing protein 137 (Fragment) OS=Homo sapiens GN=CCDC13
 tr|H7BYF9|H7BYF9_HUMAN XK-related protein (Fragment) OS=Homo sapiens GN=XKR6 PE=1 SV=1
 sp|Q5GH73|XKR6_HUMAN XK-related protein 6 OS=Homo sapiens GN=XKR6 PE=2 SV=1
 tr|A4D1R5|A4D1R5_HUMAN Similar to Ubiquinol-cytochrome C reductase iron-sulfur subunit, mitochondrial (Rie
 tr|A4D1R6|A4D1R6_HUMAN Similar to 60S ribosomal protein L17 (L23) (Amino acid starvation-induced protein) (I
 tr|A0A0A0MRF8|A0A0A0MRF8_HUMAN RPL17-C18orf32 readthrough OS=Homo sapiens GN=RPL17-C18orf32 PE
 tr|J3QLC8|J3QLC8_HUMAN 60S ribosomal protein L17 OS=Homo sapiens GN=RPL17 PE=3 SV=1
 tr|M0QY43|M0QY43_HUMAN Myosin-14 (Fragment) OS=Homo sapiens GN=MYH14 PE=1 SV=8
 tr|B3KWH4|B3KWH4_HUMAN cDNA FLJ43092 fis, clone COLON2002520, highly similar to Myosin-14 (Fragment)
 sp|Q7Z406|MYH14_HUMAN Myosin-14 OS=Homo sapiens GN=MYH14 PE=1 SV=2
 tr|Q5NV79|Q5NV79_HUMAN V5-4 protein (Fragment) OS=Homo sapiens GN=V5-4 PE=4 SV=1
 sp|A0A075B6I1|LV460_HUMAN Immunoglobulin lambda variable 4-60 OS=Homo sapiens GN=IGLV4-60 PE=3 SV=
 sp|Q15149|PLEC_HUMAN Plectin OS=Homo sapiens GN=PLEC PE=1 SV=3
 tr|Q96J91|Q96J91_HUMAN Aortic aneurysm antigenic protein clone 4 (Fragment) OS=Homo sapiens PE=2 SV=1
 tr|Q9NYI7|Q9NYI7_HUMAN Pyruvate kinase M2 (Fragment) OS=Homo sapiens PE=4 SV=1
 tr|Q9UKK4|Q9UKK4_HUMAN Pyruvate kinase M2 (Fragment) OS=Homo sapiens PE=4 SV=1
 tr|Q9UN47|Q9UN47_HUMAN Frameshifted pyruvate kinase M2 (Fragment) OS=Homo sapiens PE=4 SV=1

ens GN=MYH9 PE=3 SV=1

kDa PROTEIN OS=Homo sapiens PE=2 SV=1
v=1

ted) OS=Homo sapiens GN=SFPQ PE=2 SV=1

PE=2 SV=1
i86K06110 PE=2 SV=1
s sapiens PE=2 SV=1
s sapiens PE=2 SV=1

s=Homo sapiens PE=2 SV=1
S=Homo sapiens PE=2 SV=1
2

=Homo sapiens PE=2 SV=1
lomo sapiens GN=DDX3Y PE=3 SV=1

=1

AST3 PE=1 SV=2
=2
ent) OS=Homo sapiens PE=2 SV=1
0 PE=1 SV=2

3 PE=1 SV=1

=HNRNPA2B1 PE=1 SV=1

apiens GN=DDX48 PE=3 SV=1

=2 SV=2

=PPP6R1 PE=1 SV=5

iV=1

ollagen binding protein 1), isoform CRA_a OS=Homo sapiens GN=SERPINH1 PE=3 SV=1

eat shock protein 47), member 1, (collagen binding protein 1) (SERPINH1), mRNA OS=Homo sapiens PE=2 SV=1
? SV=1

=BDP1 PE=1 SV=1

SV=3

1

PE=1 SV=1

PE=1 SV=1

=1 SV=6

PE=1 SV=6

E=1 SV=1

=Homo sapiens GN=ARAP2 PE=1 SV=3

SV=1

SV=1

SV=1

SV=1

. SV=2

1 2 OS=Homo sapiens PE=2 SV=1

sapiens PE=2 SV=1

=1 SV=2

ribonucleoproteins C OS=Homo sapiens PE=2 SV=1

:1

:1

:1

3N=PABPC1 PE=4 SV=1

:1

7 PE=1 SV=1

iske iron-sulfur protein) (RISP) OS=Homo sapiens GN=LOC202789 PE=4 SV=1

(ASI) OS=Homo sapiens GN=LOC402695 PE=3 SV=1

:3 SV=1

OS=Homo sapiens PE=2 SV=1

:1