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Focal Adhesion Kinase Regulates Expression of Thioredoxin-interacting Protein (TXNIP) in Cancer Cells

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Abstract

Focal Adhesion Kinase (FAK) plays an important role in cancer cell survival. Previous microarray gene profiling study detected inverse regulation between expression of thioredoxin-interacting protein (TXNIP) and FAK, where down-regulation of FAK by siRNA in MCF-7 cells caused up-regulation of TXNIP mRNA level, and in contrast up-regulation of doxycyclin- induced FAK caused repression of TXNIP. In the present report, we show that overexpression of FAK in MCF-7 cells repressed TXNIP promoter activity. Treatment of MCF-7 cells with 1 α , 25-dihydroxyvitamin D₃ (1,25D) down-regulated endogenous FAK and up-regulated TXNIP protein level, and treatment with 5-FU decreased FAK protein expression and up-regulated TXNIP protein expression in 293 cells. Moreover, silencing of FAK with siRNA increased TXNIP protein expression, while overexpression of FAK inhibited TXNIP protein expression in 293 cells. In addition, treatment of DBTRG glioblastoma cells with FAK inhibitor Y15 increased TXNIP mRNA, decreased cancer cell viability and increased apoptosis. These results for the first time demonstrate FAK-regulated TXNIP expression which is important for apoptotic, survival and oxidative stress signaling pathways in cancer cells.

Keywords

Focal Adhesion Kinase; thioredoxin-interacting protein; breast cancer; brain cancer; apoptosis

Introduction

Focal Adhesion Kinase (FAK) is a 125 kDa protein which plays an important role in cell adhesion, motility, angiogenesis, and survival in cancer cells [1, 2]. It has three domains: N-terminal, Central Kinase and C-terminal domains. Many tumors overexpress FAK to increase survival [3], and inhibition of FAK with either anti-sense oligonucleotides [4], siRNA [5] or dominant-negative FAK-CD (FRNK) decreased cancer cell growth [6]. In a recent report, we performed microarray analysis of genes affected by FAK and detected that down-regulation of FAK with siRNA in MCF-7 breast cancer cells affected expression of many important genes and one of the genes was Thioredoxin-interacting protein [5], which mRNA level was inverse regulated by FAK: induced by down-regulation of FAK and repressed by overexpression of FAK.

Thioredoxin-interacting protein (TXNIP) or Vitamin D3 up-regulated protein 1 (VDUP-1) is a 46 kDa protein was first identified in an HL-60 leukemia cell line treated with 1 α ,25-

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dihydroxyvitamin D₃ (1,25(OH)₂D₃) [7]. TXNIP was induced by Vitamin D₃, but not induced by another monocyte or macrophage differentiation inducer: phorbol 12-myristate 13-acetate (PMA) [7]. TXNIP bound catalytic active center of thioredoxin (TRX) [8], which protected cells against oxidative stress. TXNIP was found to be a negative regulator of thioredoxin activity and inducer of the intracellular level of reactive oxygen species (ROS) [9]. TXNIP is a tumor and metastasis suppressor in many tumor types and its expression is reduced in tumors and can be up-regulated TGF-beta 1 [10]. Overexpression of TXNIP inhibited tumor growth and caused cell cycle arrest and apoptosis. TXNIP was also shown to play a role as a transcriptional repressor of cyclin A 2 [10]. Recently, TXNIP-deficient cells up-regulated WNT pathway, reduced p21 and regulated hematopoietic stem cell proliferation and cell quiescence [11]. Future studies on the therapeutic and diagnostic potential of TXNIP will be important for the cancer research field.

This report is focused on regulation of TXNIP expression by FAK. We demonstrate that FAK down-regulates TXNIP mRNA, promoter activity and protein level in cancer cells that is important for cancer cell survival and intracellular signaling. We show that inhibition of FAK with FAK inhibitor Y15 up-regulated TXNIP. This inverse regulation of TXNIP by FAK down-regulation and overexpression is important for cancer research field.

Materials and Methods

Cell Lines

MCF-7 cells and 293 cells were obtained from ATCC and cultivated according to the manufacturer's protocol. The stable MCF-7 with doxycyclin-inducible FAK, MCF-7-FAK and control MCF-7 vector cells; and also MCF-7 siRNA#1 and #2 were generated and described [5]. DBTRG glioblastoma cells were obtained from Dr. Brian Eliceiri (University of California, San Diego) [12] and were maintained in DMEM medium supplemented with 10% fetal bovine serum. 1µg/mL streptomycin, 1µg/mL L-Glutamine, 1µg/mL sodium pyruvate, 1µg/mL nonessential amino acids, and 500 µL of Insulin 10mg/mL.

Plasmids

FAK-pcDNA3 plasmid was described before [13]. The human 1.5 kb TXNIP-pGL3 promoter construct was obtained from Dr. Don Ayer (Huntsman Cancer Institute, University of Utah, Salt Lake City, UT). FAK siRNA#1 and FAKsiRNA#2 hyg-pRNAT4.1 and control siRNA plasmids were described [5]. The human TXNIP-pcDNA3 plasmid was obtained from Dr. Parth Patwari (Brigham and Women's Hospital, Cambridge, Massachusetts).

Antibodies and Reagents

Monoclonal anti-FAK antibody, caspase-8 and anti-PARP antibodies were obtained from *Millipore, Inc.* Polyclonal anti-phospho-Tyrosine 397-FAK antibody was obtained from *Enzo Life Sciences* (#ADI-KAP-TK131). Monoclonal anti-TXNIP (VDUP-1) antibody was ordered from *MBL International Corporation*. Monoclonal anti-β-actin antibody was obtained from *Sigma*. FAK inhibitor Y15 (1,2,4,5-benzenetetraamine tetrahydrochloride) was obtained from *Sigma*. 1α,25-dihydroxyvitamin D(3) (1,25(OH)₂D(3) and 5-fluorouracil were obtained from *Sigma*.

Western Blotting

Cells were plated on 6 well or 100 mm³ plates, and after treatment with different agents or after transfection with plasmids Western blotting was performed by the standard procedure [13].

Cell Viability

Cells were plated in a 96-well plate and after 24 hours cells were treated with for 24 hours. The 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium compound from Promega 96 well Viability kit according to the manufacture's protocol.

Real-time PCR

Real-time PCR with forward and reverse primers and fluorescent probe 5'FAM and 3'TAMPA was performed, as described in [14]. The TXNIP probe was 6'FAM-TTCGAGCA GCGCACGACACCC-TAMRA, and primers: forward: 5-TGGT GGATGTCAATA CCCCT-3'; and reverse primer: 5'ATTGGC AAGGTAAGTGTGGC-3'. GAPDG was used as endogenous control. RQ was calculated for each gene tested.

Dual-luciferase Assay

For dual luciferase assay, 2×10^5 293 cells were plated on 6-well plates, cultured overnight and co-transfected with TXNIP promoter luciferase plasmid, FAK plasmid (1 μ g/well) and pPRL-TK plasmid containing the herpes simplex virus thymidine kinase promoter encoding Renilla luciferase (0.1 μ g/well) using Lipofectamine (*Invitrogen*) transfection agent according to the manufacture's protocol. The pRL-TK control encoding Renilla luciferase vector was used for normalization of luciferase activity (*Promega*). For all experiments, cells were cultured for 48 hours after transfection and lysed with the 1x Passive Lysis Buffer (*Promega*). Lysates were analyzed using a Dual-Luciferase Reporter Assay System kit (*Promega*). All experiments were performed at least three times.

RNA Isolation

The untreated and treated for 24 hours with 10 microM of Y15 DBTRG cells were used for isolation of RNA. Total RNA was prepared from the frozen cells using the RNeasy midi kits (*Qiagen, Inc.*) following manufacturer's instructions. Before labeling, RNA samples were quantitated using a ND-1000 spectrophotometer (*NanoDrop*).

Apoptosis

Apoptosis was analyzed by staining with Hoechst or by FACS, flow cytometry analysis, which was performed by the standard protocol at Roswell Park Flow Cytometry Core Facility. The percentage of G1, G2, S phase-arrested and/or apoptotic cells was calculated.

Statistical Analyses

Student's t test was performed to determine significance. The difference between treated and untreated samples with $P < 0.05$ was considered significant.

Results

Doxycyclin-inducible FAK Repressed TXNIP and Silenced FAK by siRNA Induced TXNIP mRNA Level

We performed microarray profiling and analyzed genes that will be inversely regulated by down- and up-regulation of FAK. Microarray analysis of stable MCF-7 cells, transfected with FAKsiRNA#1 and #2 and control siRNA and MCF-7 with doxycyclin-inducible FAK (Dox+) and (Dox-) MCF-7, described previously [5] identified the top affected gene: TXNIP, which expression was 3.25 fold induced by FAKsiRNA compared with Control siRNA cells, and which expression was 0.36-fold repressed by FAK overexpression in

MCF-7 cells (Fig. 1). Thus, TXNIP is inversely regulated by FAK overexpression and by FAK silencing with siRNA in MCF-7 cells.

Overexpression of FAK Reduces TXNIP Promoter Activity

To test if FAK represses TXNIP mRNA through inhibiting TXNIP promoter, we co-transfected MCF-7 cells with human TXNIP promoter either with HA-FAK-pcDNA3 or with control pcDNA3 plasmid and performed a dual-luciferase assay to measure activity of TXNIP promoter. Overexpression of FAK significantly decreased TXNIP promoter activity (Fig. 2) which was consistent with microarray data on repressed TXNIP mRNA level by FAK.

Vitamin D Or 5-Fluorouracil Decreased Endogenous FAK and Induced TXNIP Protein Levels

To test the effect of Vitamin D ((1,25(OH)₂)D₃ or 1,25D on FAK and TXNIP protein expression, we treated MCF-7 cells with Vitamin D and performed Western blotting with FAK and TXNIP antibodies (Fig. 3, left panel). Vitamin D decreased FAK expression and increased TXNIP expression, which supports data on inverse regulation between FAK and TXNIP. We also performed a treatment of 293 cells with 5-fluorouracil and analyzed FAK and TXNIP protein expression by Western blotting (Fig. 3, right panel). 5-fluorouracil decreased FAK and increased TXNIP protein levels, which supports data in MCF-7 cells.

FAK siRNA Induced TXNIP, While Overexpression of FAK Decreased TXNIP Protein Expression

We transfected 293 cells with control pCMV4 vector, Control siRNA and two FAK siRNA#1 and siRNA#2 and performed Western blotting with FAK and TXNIP antibodies. FAKsiRNA decreased expression of FAK but increased TXNIP expression (Fig. 4, left panel). We also treated 293 cells with control and FAKsiRNA with 5-fluorouracil and performed Western blotting with FAK and TXNIP antibodies. FAKsiRNA decreased FAK and increased TXNIP expression. In addition, 5-FU treatment and FAKsiRNA decreased FAK and induced TXNIP expression even more than FAK siRNA alone (Fig. 4, right panel). Thus, these data show that FAK siRNA induced TXNIP protein expression, and in combination with 5-FU this effect was more pronounced.

To test the effect of overexpressed FAK on TXNIP protein expression, we transfected 293 cells with TXNIP plasmid alone or with TXNIP and FAK plasmids, and then performed Western blotting with FAK and TXNIP antibodies. Overexpression of FAK decreased TXNIP protein expression (Fig. 5). The data are consistent with microarray data in doxycycline-inducible MCF-7-FAK cells, where overexpression of FAK repressed TXNIP level.

Inhibition of FAK with FAK Inhibitor Y15 Down-Regulated FAK and Induced TXNIP mRNA Level

To test if pharmacologic inhibition of FAK with FAK autophosphorylation inhibitor, which decreased FAK auto-phosphorylation and expression in glioblastoma cells [12], we treated DBTRG glioblastoma cells with FAK inhibitor Y15 and performed Real-time PCR with TXNIP to test regulation of TXNIP mRNA by FAK (Fig. 6A). FAK inhibitor Y15 inhibitor decreased Y397-FAK and FAK, activated caspase-8 in DBTRG cells (Fig. 6A, left panel), and it dramatically increased TXNIP mRNA in DBTRG glioblastoma cells (Fig. 6A, right panel). Y15 treatment decreased DBTRG cell viability (Fig. 6B), and increased apoptosis (Fig. 6C, left panel). The apoptotic Hoechst stained Y15-treated fragmented nuclei are shown in Fig. 6C, right panel, white arrows). Thus, FAK inhibitor decreased FAK

expression, induced TXNIP expression and decreased cancer cell viability, activated caspase-8 and induced apoptosis.

Discussion

This report demonstrates for the first time direct regulation of TXNIP mRNA and protein levels by FAK. Inhibition of FAK with FAKsiRNA increased TXNIP mRNA expression, while overexpression of FAK decreased TXNIP mRNA and protein level in MCF-7 and 293 cells. In addition, overexpression of FAK decreased TXNIP promoter activity, which can explain down-regulation of TXNIP mRNA by FAK. Pharmacologic inhibition of FAK with Y15 inhibitor increased TXNIP mRNA, decreased cancer viability, and increased apoptosis. This study links FAK to TXNIP-one of the major players in oxidation, apoptosis, metabolism processes in cancer cells.

FAK is overexpressed in many tumors, while TXNIP is down-regulated in tumors. The present data can explain this inverse regulation. It will be interesting to study the correlation between FAK and TXNIP expression. FAK can inhibit TXNIP to increase tumor survival and metastasis. Both proteins were involved in metastasis and angiogenesis. TXNIP^{-/-} mice developed proliferated more efficiently than control mice.

Oxidative stress induced FAK phosphorylation and increased cell migration in an intestinal epithelium by a Src and PI-3-dependent mechanism [15]. Reduced expression of FAK decreased hydrogen peroxide-induced cell migration [15]. This effect can be explained by increased TXNIP expression, which is repressor of Trx and metastasis.

The data on decreased FAK and increased TXNIP by 1 α ,25-dihydroxyvitamin D3 are consistent with data on inhibition of cell proliferation by vitamin D3 in breast cancer cells [16].

We show in this report that FAK decreased TXNIP promoter activity. This study reveals a novel function of FAK in the nucleus. FAK and TXNIP can both shuttle between cytoplasm and nucleus [13]. FAK has been shown to regulate cyclin D promoter [17]. The future study will define the regulation of TXNIP promoter by FAK in detail. The correlative population-based study of expression of FAK and TXNIP by immunohistochemistry analysis in tumor samples is important to confirm the inverse regulation of TXNIP by FAK in different types of tumors.

It is known that TXNIP promoter is regulated by Mondo A factor (negative regulator of glucose uptake), which is involved in glucose metabolism pathway [18]. This can explain the mechanism of negative regulation of glucose uptake and glycolysis by TXNIP. Murine embryonic fibroblasts of TXNIP^{-/-} mice expressed increased glucose uptake and lactate production, switching metabolism toward aerobic glycolysis [19]. Thus, the link between FAK-regulated TXNIP promoter activity and cellular glucose and metabolism in different types of tumors is important to study in the future.

We showed that FAK inhibitor Y15 decreased FAK and increased TXNIP and decreased cancer cell viability and induced apoptosis. This, FAK inhibitor targeting FAK and increasing TXNIP expression can be used for developing anti-cancer therapies. It will be interesting to study mechanisms of other FAK inhibitors on FAK and TXNIP expression.

In conclusion, the present study demonstrated novel signaling of FAK-regulated TXNIP promoter activity, TXNIP mRNA and protein expression that can be used for novel FAK-related therapeutics.

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References

1. Cance WG, Golubovskaya VM. Focal adhesion kinase versus p53: Apoptosis or survival? *Sci Signal*. 2008; 1:pe22. [PubMed: 18493017]
2. Hanks SK, Polte TR. Signaling through focal adhesion kinase. *Bioessays*. 1997; 19:137–145. [PubMed: 9046243]
3. Cance WG, Harris JE, Iacocca MV, Roche E, Yang X, Chang J, Simkins S, Xu L. Immunohistochemical analyses of focal adhesion kinase expression in benign and malignant human breast and colon tissues: Correlation with preinvasive and invasive phenotypes. *Clin Cancer Res*. 2000; 6:2417–2423. [PubMed: 10873094]
4. Smith CS, Golubovskaya VM, Peck E, Xu LH, Monia BP, Yang X, Cance WG. Effect of focal adhesion kinase (FAK) downregulation with FAK antisense oligonucleotides and 5-fluorouracil on the viability of melanoma cell lines. *Melanoma Res*. 2005; 15:357–362. [PubMed: 16179862]
5. Golubovskaya VM, Zheng M, Zhang L, Li JL, Cance WG. The direct effect of focal adhesion kinase (FAK), dominant-negative FAK, FAK-CD and FAK siRNA on gene expression and human MCF-7 breast cancer cell tumorigenesis. *BMC Cancer*. 2009; 9:280–288. [PubMed: 19671193]
6. Xu LH, Yang XH, Bradham CA, Brenner DA, Baldwin AS, Craven RJ, Cance WG. The focal adhesion kinase suppresses transformation-associated, anchorage-Independent apoptosis in human breast cancer cells. *J Biol Chem*. 2000; 275:30597–30604. [PubMed: 10899173]
7. Chen KS, DeLuca HF. Isolation and characterization of a novel cDNA from HL-60 cells treated with 1,25-dihydroxyvitamin D-3. *Biochim Biophys Acta*. 1994; 1219:26–32. [PubMed: 8086474]
8. Junn E, Han SH, Im JY, Yang Y, Cho EW, Um HD, Kim DK, Lee KW, Han PL, Rhee SG, Choi I. Vitamin D3 up-regulated protein 1 mediates oxidative stress *via* suppressing the thioredoxin function. *J Immunol*. 2000; 164:6287–6295. [PubMed: 10843682]
9. Lee KN, Kang HS, Jeon JH, Kim EM, Yoon SR, Song H, Lyu CY, Piao ZH, Kim SU, Han YH, Song SS, Lee YH, Song KS, Kim YM, Yu DY, Choi I. VDUP1 is required for the development of natural killer cells. *Immunity*. 2005; 22:195–208. [PubMed: 15723808]
10. Han SH, Jeon JH, Ju HR, Jung U, Kim KY, Yoo HS, Lee YH, Song KS, Hwang HM, Na YS, Yang Y, Lee KN, Choi I. VDUP1 upregulated by TGF-beta1 and 1,25-dihydroxyvitamin D3 inhibits tumor cell growth by blocking cell-cycle progression. *Oncogene*. 2003; 22:4035–4046. [PubMed: 12821938]
11. Jeong M, Piao ZH, Kim MS, Lee SH, Yun S, Sun HN, Yoon SR, Chung JW, Kim TD, Jeon JH, Lee J, Kim HN, Choi JY, Choi I. Thioredoxin-interacting protein regulates hematopoietic stem cell quiescence and mobilization under stress conditions. *J Immunol*. 2009; 183:2495–2505. [PubMed: 19625652]
12. Golubovskaya V, Huang G, Ho B, Yemma M, Morrison CD, Lee J, Eliceiri B, Cance WG. Pharmacological blockade of FAK autophosphorylation decreases human glioblastoma tumor growth and synergizes with temozolomide. *Mol Cancer Ther*. 2012 Epub ahead of print.
13. Golubovskaya VM, Finch R, Cance WG. Direct Interaction of the N-terminal Domain of Focal Adhesion Kinase with the N-terminal Transactivation Domain of p53. *J Biol Chem*. 2005; 280:25008–25021. [PubMed: 15855171]
14. Golubovskaya VM, Conway-Dorsey K, Edmiston SN, Tse CK, Lark AA, Livasy CA, Moore D, Millikan RC, Cance WG. FAK overexpression and p53 mutations are highly correlated in human breast cancer. *Int J Cancer*. 2009; 125:1735–1738. [PubMed: 19521985]
15. Basuroy S, Dunagan M, Sheth P, Seth A, Rao RK. Hydrogen peroxide activates focal adhesion kinase and c-Src by a phosphatidylinositol 3 kinase-dependent mechanism and promotes cell migration in Caco-2 cell monolayers. *Am J Physiol Gastrointest Liver Physiol*. 2010; 299:G186–195. [PubMed: 20378826]

16. Jensen SS, Madsen MW, Lukas J, Binderup L, Bartek J. Inhibitory effects of 1 α ,25-dihydroxyvitamin D(3) on the G(1)-S phase-controlling machinery. *Mol Endocrinol*. 2001; 15:1370–1380. [PubMed: 11463860]
17. Zhao J, Pestell R, Guan JL. Transcriptional activation of cyclin D1 promoter by FAK contributes to cell cycle progression. *Mol Biol Cell*. 2001; 12:4066–4077. [PubMed: 11739801]
18. Elgort MG, O'Shea JM, Jiang Y, Ayer DE. Transcriptional and translational downregulation of thioredoxin interacting protein is required for metabolic reprogramming during G(1). *Genes Cancer*. 2010; 1:893–907. [PubMed: 21779470]
19. Hui TY, Sheth SS, Diffley JM, Potter DW, Lusk AJ, Attie AD, Davis RA. Mice lacking thioredoxin-interacting protein provide evidence linking cellular redox state to appropriate response to nutritional signals. *J Biol Chem*. 2004; 279:24387–24393. [PubMed: 15047687]

Fig. (1). Overexpression of FAK decreased TXNIP and down-regulation of FAK with siRNA increased TXNIP mRNA level

Microarray analysis was performed on MCF-7 cells with doxycyclin-inducible FAK and in MCF-7 stable clones with FAK siRNA and Control siRNA, as described in Materials and Methods. Overexpression of FAK decreased 0.36-fold expression of TXNIP, while silencing of FAK with siRNA induced TXNIP 3.25-fold.

Fig. (2). Overexpression of FAK decreased TXNIP promoter activity

The dual-luciferase assay was performed in MCF-7 cells co-transfected with TXNIP with HA-tagged FAK-pcDNA3 or with control pcDNA3 plasmid and with control Renilla plasmid that was used for normalization, as described in Materials and Methods. * $p < 0.05$, TXNIP promoter luciferase activity in HA-FAK-transfected cells is significantly less than in versus control pcDNA3 transfected cells.

Fig. (3). Vitamin D treatment and 5-fluorouracil treatment decreased FAK and increased TXNIP protein levels in MCF-7 and 293 cells

MCF-7 cells were treated with 1α,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) or Vitamin D (labeled 1.25 D) at 0.1 μM (left panel), and 293 cells were treated with 5-fluorouracil at 0.5 mM dose for 24 hours and Western blotting was performed with FAK, TXNIP and beta-Actin. Structures of 5-FU and D₃ are shown below Western blots. The image is a composite from the same blot.

Fig. (4). Down-regulation of FAK with FAKsiRNA increased TXNIP protein level, which was more increased with 5-fluorouracil

Left panel: 293 cells were transfected with FAKsiRNA#1 and #2 and Western blotting was performed with FAK, TXNIP and beta-Actin antibodies. FAKsiRNA increased TXNIP protein level. Right panel: Control, FAKsiRNA-transfected cells were treated with 5-fluorouracil (5-FU) and compared with the same cells without 5-FU treatment. Western blotting showed that FAK siRNA increased TXNIP protein level, which was more increased with 5-FU.

Fig. (5). Overexpression of FAK decreased TXNIP protein level

293 cells were co- transfected with TXNIP-pcDNA3 plasmid either with control pcDNA3 plasmid or with HA-tagged FAK plasmid. Western blotting demonstrated that overexpression of FAK decreased TXNIP protein level in 293 cells.

Fig. (6). A. FAK inhibitor Y15 down-regulating FAK increased TXNIP mRNA level. Left panel DBTRG glioblastoma cells were treated with 10 microM Y15 inhibitor for 24 hours and Western blotting was performed to test the effect on Y397-FAK and FAK. Western blotting with caspase-8 antibody demonstrates a decrease of uncleaved caspase-8 in Y15-treated cells. Y15 decreased Y397 and FAK. Right panel: Real-time analysis demonstrated increased TXNIP in Y15-treated DBTRG cells. The structure of Y15 is shown below. **B.** Y15 decreased DBTRG viability. MTT assay on DBTRG cells treated with Y15 inhibitor at 10 microM for 24 demonstrated significant decreased cell viability. * $p < 0.05$ Y15 versus Untreated cells. **C. Left panel:** Y15 increased apoptosis in DBTRG cells. Untreated and Y15-treated cells as above were analyzed by FACS analysis. Y15-treated cells had increased % of apoptosis compared with untreated cells. **Right panel:** Hoechst staining demonstrated apoptosis fragmented nuclei in DBTRG cells, treated with Y15.