DNA Microarray Analysis of HSC-3 Human Oral Squamous Cell Carcinoma Cells Following Knockdown of DDIT4

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Introduction

Oral squamous cell carcinomas (OSCCs) are the most common type of oral cancer. The 5-year survival rate and prognosis of patients with OSCC have a poor prognosis and a low 5-year survival rate. Longer survival is correlated with lower expression of DNA Damage Inducible Transcript 4 (DDIT4) from certain types of cancer tissue. The primary object of this study was to explore the associated role of DDIT4 in human oral cancers. HSC-3 human oral squamous cell carcinoma cells were used to examine the effect of DDIT4 knockdown by a DDIT4 siRNA and the silencing efficiency was evaluated by quantitative real-time PCR (qRT-PCR). DNA microarray analysis was carried out on DDIT4 siRNA transfected HSC-3 cells using the Human Exon 1.0 ST array. Ingenuity Pathway Analysis (IPA) and Gene Ontology (GO) analysis were used for bioinformatics analysis to identify potential molecular functions and related pathways of differentially expressed genes (DEGs). The microarray results showed that 182 DEGs were upregulated, and 424 DEGs were downregulated in DDIT4-deficient HSC-3 cells. Among them, 4 genes (RNASE7, IL-24, SCD and MMP13) were selected to confirm the microarray results using qRT-PCR. IPA showed 49 related pathways are involved in DDIT4-deficient HSC-3 cells. GO analysis revealed that the GO terms of upregulated DEGs were regulation of transcription, membrane and protein binding while the GO terms of downregulated DEGs were signal transduction, membrane and protein binding. In conclusion, DDIT4 may serve as a key regulator in carcinogenesis and could be a therapeutic target in patients with OSCC.

Keywords: DDIT4, DNA microarray, oral squamous cell carcinoma, IL-24, MMP13

Abstract

Oral squamous cell carcinomas (OSCCs) are the most common type of oral cancer and patients with OSCC have a poor prognosis and a low 5-year survival rate. Longer survival is correlated with lower expression of DNA Damage Inducible Transcript 4 (DDIT4) from certain types of cancer tissue. The primary object of this study was to explore the associated role of DDIT4 in human oral cancers. HSC-3 human oral squamous cell carcinoma cells were used to examine the effect of DDIT4 knockdown by a DDIT4 siRNA and the silencing efficiency was evaluated by quantitative real-time PCR (qRT-PCR). DNA microarray analysis was carried out on DDIT4 siRNA transfected HSC-3 cells using the Human Exon 1.0 ST array. Ingenuity Pathway Analysis (IPA) and Gene Ontology (GO) analysis were used for bioinformatics analysis to identify potential molecular functions and related pathways of differentially expressed genes (DEGs). The microarray results showed that 182 DEGs were upregulated, and 424 DEGs were downregulated in DDIT4-deficient HSC-3 cells. Among them, 4 genes (RNASE7, IL-24, SCD and MMP13) were selected to confirm the microarray results using qRT-PCR. IPA showed 49 related pathways are involved in DDIT4-deficient HSC-3 cells. GO analysis revealed that the GO terms of upregulated DEGs were regulation of transcription, membrane and protein binding while the GO terms of downregulated DEGs were signal transduction, membrane and protein binding. In conclusion, DDIT4 may serve as a key regulator in carcinogenesis and could be a therapeutic target in patients with OSCC.

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Introduction

Oral squamous cell carcinomas (OSCCs) are the most common type of oral cancer. The 5-year survival rate and prognosis of patients with OSCC have a poor prognosis and a low 5-year survival rate. Therefore, it is crucial to explore novel molecular mechanisms for a better understanding of oral carcinogenesis and progression and to identify new therapeutic targets.

DDIT4 (DNA Damage Inducible Transcript 4) overexpression correlates with microvessel density and overall survival rate in patients with OSCC (2), this could be a potential prognosis biomarker for OSCC. DDIT4 is local-
ized to human chromosome 10q24.33, and is induced by p53-dependent and p53-independent mechanisms (3). DDIT4 is induced in response to hypoxia and other cellular stresses that regulate cell growth and apoptosis through the inhibition of mTOR activity (4). Upstream signals from the phosphatidylinositol 3-kinase (PI3K)/AKT pathway are passed to the tuberous sclerosis complex (TSC) by DDIT4 (5). The upstream regulator Liver X receptor/retinoid X receptor (LXR/RXR) can inhibit the PI3K/AKT/mTOR signaling pathway and thus inhibit the migration and invasion of ovarian cancer cells (6). Upon oxidant stress, DDIT4 mediates p38 MAPK signaling that activates mTOR (7). Cholecystokinin/gastrin-mediated signaling plays an important role in regulating proliferation and angiogenesis. Cholecystokinin can inhibit oxidative stress-induced neurotoxicity (8), which could be associated with hypoxia responsive DDIT4.

To get a deeper insight into the role of DDIT4 in OSCCs, DNA microarray analysis was performed after knockdown of DDIT4 in HSC-3 cells, and a total of 606 differentially expressed genes (DEGs) were identified. The potential functions of DDIT4 and its related molecular pathways were explored using Ingenuity Pathway Analysis (IPA) and Gene Ontology (GO) analysis.

**Materials and Methods**

**Cell culture**

The human oral squamous carcinoma cell line, HSC-3, was purchased from the ATCC (Manassas, VA, USA). HSC-3 cells were cultured in RPMI-1640 medium (Gibco, Tokyo, Japan) containing 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 U/ml). Cells were incubated in 5% CO2 at 37°C.

**Short interference RNA (siRNA)**

siRNAs against DDIT4 were synthesized by Qiagen (Hilden, Germany). HSC-3 cells were seeded at 5 × 10^4 cells per 35-mm dish in preparation for transfection. After 16 h, a scramble siRNA or a DDIT4 siRNA was transfected into cells using Lipofectamine RNAiMAX reagent (Invitrogen, Carlsbad, CA, USA). Cell lysates were collected after 48 h transfection, then subjected to qRT-PCR to determine the transfection and silencing efficiency.

**Quantitative real-time PCR (qRT-PCR)**

Gene expression levels were evaluated by qRT-PCR using TaqMan Gene Expression Assays (Thermo Fisher Scientific, Waltham, MA, USA). Briefly, total RNA was extracted with QIAZOL (Qiagen KK, Tokyo, Japan) and was reverse-transcribed using a High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA). Each cDNA was subjected to qRT-PCR with the TaqMan Probe Hs01111686_g1 for DDIT4, Hs00922963_s1 for RNASE7, Hs01114274_m1 for IL-24, Hs01682761_m1 for SCD, Hs00942584_m1 for MMP13 and Hs09999903_m1 for ACTB (Applied Biosystems, Framingham, MA, USA). All reactions were done in triplicate.

**DNA microarray analysis**

A NanoDrop (Thermo Fisher Scientific) and a Bioanalyzer (Agilent, CA, USA) were used to determine RNA integrity. Microarray hybridization was performed according to the manufacturer’s standard protocols (Affymetrix, exonExprChip). One sample from each group (treated with a scramble siRNA or a DDIT4 siRNA) was subjected to the analysis. After scanning, the data were analyzed using Agilent GeneSpring GX software. Biological function descriptions of DEGs were conducted using Ingenuity Pathway Analysis (Qiagen) and Gene Ontology analysis.

**Statistical analysis**

SPSS 22.0 was used for statistical analysis. The statistical significance of observed differences was determined by unpaired t-tests. Data are expressed as means ± standard error of the mean (SEM). A p<0.05 is considered to indicate a statistically significant difference.

**Results**

**Knockdown of DDIT4 in HSC-3 cells**

HSC-3 cells were transfected with a scramble siRNA or a DDIT4 siRNA. The expression of DDIT4 was evaluated by qRT-PCR after 48 h. The results showed that the efficiency of the siRNA against DDIT4 was 96% (Fig. 1).

**Gene expression profile of DDIT4 Knockdown in HSC-3 cells**

To identify genes that are differentially expressed in HSC-3 cells following knockdown of DDIT4, we conducted DNA microarray analysis between HSC-3 cells trans-
fected with a scramble siRNA or a DDIT4 siRNA and a differential gene expression profile was established. That analysis identified 606 DEGs (≥ 2.0-fold change) that were differentially regulated between the DDIT4 knockdown and the control group, and among those, 182 DEGs were significantly upregulated and 424 DEGs were significantly downregulated. The 20 most significantly upregulated DEGs are listed in Table 1, and the 20 most significantly downregulated DEGs are listed in Table 2. The DEGs were uploaded to Ingenuity Pathway Analysis software (Qiagen), which identified 5 primary pathways related to: (a) p38 MAPK signaling; (b) PI3K/AKT signaling; (c) Cholecystokinin/Gastrin-mediated signaling; (d) LXR/RXR activation; and (e) IL-8 signaling. The top 20 related pathways are listed in Table 3.

**Validation of DNA microarray results**

Four of those DEGs were selected to validate the DNA microarray results using qRT-PCR. Consistent with the microarray results, mRNA expression levels of RNASE7 and MDA-7/IL-24 were significantly upregulated (fold-change: 16.0 and 7.2, respectively) while mRNA levels of SCD and MMP13 were downregulated (fold-change: 0.1 and 0.2, respectively) after DDIT4 knockdown (Fig. 2).

**GO analysis**

The DEGs were annotated by GO enrichment analysis and were composed of GO biological process, GO cellular component and GO molecular function. The most significant GO terms for the upregulated DEGs after DDIT4 knockdown were regulation of transcription, membrane and protein binding (Fig. 3A-C) while the most significant GO terms for the downregulated DEGs were signal transduction, membrane and protein binding (Fig. 3D-F).

**Discussion**

Despite advances in treatment and diagnosis, OSCCs are the most common type of oral cancer and patients with OSCCs have a poor prognosis and a low 5-year survival rate. Thus, the need for novel mechanisms and strategies for therapy is critical.

In this study, DNA microarray analysis was performed to explore the different gene expression profiles in DDIT4 knockdown HSC-3 cells. In response to transfection with the DDIT4 siRNA, 606 DEGs were found to be involved in multiple bio-function pathways related to inflammation, cell growth and apoptosis.

DDIT4 is regulated in response to several cellular stresses, including DNA damage (3) and hypoxia (9). DDIT4 regulates cell growth, proliferation, survival and apoptosis via the inhibition of mTORC1 activity, which is consistent with our IPA analysis that DDIT4 knockdown affected the PI3K/AKT signaling pathway.

In the present study, we demonstrated other promising areas in which DDIT4 might be involved. Upon knockdown of DDIT4, the 5 most upregulated DEGs were RNASE7 (Ribonuclease A Family Member 7), ADAMTS1 (ADAM Metallopeptidase with Thrombospondin Type1 Motif 1), MDA-7/IL-24 (Melanoma Differentiation Associated Gene-7/Interleukin 24), PTGS2 (Prostaglandin-Endoperoxide Synthase 2) and DEFB103 (Defensin Beta 103). RNASE7 and DEFB103 are gene-encoded antimicrobial peptides (AMPs), which are synthesized by keratinocytes (10) to control microbial proliferation (11). AMPs can modulate cytokine production and cell proliferation in addition to their antimicrobial activities. IL-24 also inhibits tumor angiogenesis (12), which can be speculated from our study showing that HIF1α targets the expression of DDIT4 and is a mediator in preventing angiogenesis. The downregulation of DDIT4 promotes the expression of IL-24 and hence inhibits tumor growth. ADAMTS1 belongs to a group of extracellular proteases, which may play important roles in inflammatory and immune responses (13) as well as in the development of cancer. MDA-7/IL-24
Table 1. The top 20 upregulated DEGs in HSC-3 cells after knockdown of DDIT4.

| Gene Symbol | Gene description                                      | Fold change |
|-------------|-------------------------------------------------------|-------------|
| RNASE7      | ribonuclease, RNase A family, 7                       | 16.0        |
| ADAMTS1     | ADAM metallopeptidase with thrombospondin type 1 motif, 1 | 7.4         |
| IL24        | interleukin 24                                        | 7.2         |
| PTGS2       | prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) | 6.5         |
| DEFB103A/B  | defensin, beta 103A/B                                 | 6.0         |
| ARL14       | ADP-ribosylation factor-like 14                       | 5.8         |
| SLC7A11     | solute carrier family 7, (cationic amino acid transporter, y+ system) member 11 | 4.7         |
| SERPINE2    | serpin peptidase inhibitor, clade B (ovalbumin), member 2 | 4.2         |
| NCF2        | neutrophil cytosolic factor 2 (65 kDa, chronic granulomatous disease, autosomal 2) | 3.8         |
| AOX1        | aldehyde oxidase 1                                    | 3.7         |
| ALOXE3      | arachidonate lipoygenase 3                            | 3.6         |
| ASNS        | asparagine synthetase                                 | 3.4         |
| HIST1H2BK   | histone cluster 1, H2b                                  | 3.4         |
| DSC2        | desmocollin 2                                         | 3.3         |
| FLNC        | filamin C, gamma (actin binding protein 280)          | 3.3         |
| LCP1        | lymphocyte cytosolic protein 1 (L-plastin)             | 3.3         |
| TM4SF19     | transmembrane 4 L six family member 19                | 3.2         |
| CD163L1     | CD163 molecule-like 1                                 | 3.2         |
| OCLN        | occludin                                              | 3.1         |
| IMPAD1      | inositol monophosphatase domain containing 1           | 3.1         |

Table 2. The top 20 downregulated DEGs in HSC-3 cells after knockdown of DDIT4.

| Gene Symbol | Gene description                                      | Fold change |
|-------------|-------------------------------------------------------|-------------|
| SCD         | stearoyl-CoA desaturase (delta-9-desaturase)           | 0.1         |
| MMP13       | matrix metallopeptidase 13 (collagenase 3)             | 0.2         |
| THBS1       | thrombospondin 1                                       | 0.2         |
| DDIT4       | DNA damage inducible transcript 4                     | 0.2         |
| HIST1H1A    | histone cluster 1, H1a                                 | 0.2         |
| COMMD10     | COMM domain containing 10                             | 0.2         |
| KRT15       | keratin 15                                            | 0.2         |
| HIST1H2BM   | histone cluster 1, H2b                                 | 0.2         |
| FST         | follistatin                                            | 0.2         |
| BDKR1       | bradykinin receptor B1                                 | 0.2         |
| CAV2        | caveolin 2                                             | 0.2         |
| VSNL1       | visinin-like 1                                         | 0.2         |
| EPS8        | epidermal growth factor receptor pathway substrate 8  | 0.2         |
| ATG4C       | ATG4 autophagy related 4 homolog C (S. cerevisiae)     | 0.2         |
| PBK         | PDZ binding kinase                                     | 0.2         |
| REEP3       | receptor accessory protein 3                           | 0.2         |
| TNFSF10     | tumor necrosis factor (ligand) superfamily, member 10 | 0.2         |
| ELOVL6      | ELOVL family member 6, elongation of long chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast) | 0.2 |
| FERMT1      | fermitin family homolog 1 (Drosophila)                 | 0.3         |
| CHML        | choroideremia-like (Rab escort protein 2)              | 0.3         |
Table 3. The top 20 pathways involved in HSC-3 cells after knockdown of DDIT4.

| Ingenuity Canonical Pathways                                      | -log(p-value) |
|------------------------------------------------------------------|---------------|
| p38 MAPK Signaling                                               | 3.92          |
| PI3K/AKT Signaling                                               | 3.01          |
| Cholecystokinin/Gastrin-mediated Signaling                       | 2.97          |
| LXR/RXR Activation                                               | 2.94          |
| IL-8 Signaling                                                   | 2.71          |
| D-myo-inositol (1,4,5)-trisphosphate Degradation                 | 2.28          |
| Asparagine Biosynthesis I                                        | 2.19          |
| Semaphorin Signaling in Neurons                                  | 2.16          |
| Sphingosine-1-phosphate Signaling                               | 2.15          |
| Fatty Acid α-oxidation                                           | 2.14          |
| CD40 Signaling                                                   | 2.06          |
| IL-6 Signaling                                                   | 2.05          |
| Superpathway of D-myo-inositol (1,4,5)-trisphosphate Metabolism  | 2.02          |
| Apelin Cardiac Fibroblast Signaling Pathway                      | 2.02          |
| IL-10 Signaling                                                  | 1.99          |
| Toll-like Receptor Signaling                                     | 1.88          |
| Endocannabinoid Cancer Inhibition Pathway                        | 1.85          |
| 3-phosphoinositide Degradation                                   | 1.73          |
| N-acetylglucosamine Degradation I                               | 1.71          |

Fig. 2. Validation of microarray results of upregulated and downregulated DEGs in HSC3 cells caused by the knockdown of DDIT4. (A, B) The mRNA expression levels of RNASE7 and IL-24 in HSC-3 cells were upregulated after the knockdown of DDIT4. (C, D) The mRNA expression levels of SCD and MMP13 in HSC-3 cells were downregulated after the knockdown of DDIT4. Data shown represent means ± SD; **p<0.01. All results are representative of at least three independent experiments.
can induce apoptosis in malignant cells (14) and has immune-regulatory activities (15). PTGS2 triggers the production of PGE2 (Prostaglandin E receptor 2), which transactivates EGFR (Epidermal Growth Factor Receptor) (16), and the activation of PGE2 and EGFR can in turn activate the PI3K/AKT signaling pathway.

On the other hand, the 5 most downregulated DEGs in HSC-3 cells transfected with the DDIT4 siRNA were SCD (Stearoyl-CoA Desaturase), MMP13 (Matrix Metallopeptidase 13), THBS1 (Thrombospondin 1), H1-1 (H1.1 Linker Histone Cluster Member) and COMMD10 (COMM Domain Containing 10). SCD has a function to convert lipotoxic lipid species (17), and its byproducts can reduce cytokine expression in adipocytes (18). MMPs are a group of proteolytic enzymes that are produced by tumor cells or stromal cells, and MMP13 is related to cancer vascular invasion (19). THBS1 is a tumor inhibitor that participates in many bio-processes, including angiogenesis, inflammation and cancer (20). Linker histone isoforms H1-1 and H1-2 can trigger apoptosis mediated by Bak and Bcl-XL, which regulates mitophagy (21). The COMMD family has been reported to regulate the transcription activity of NF-κB (22), cell proliferation and hypoxia response (23).

In conclusion, the results of this study provide further insights into the novel biochemical functions associated with DDIT4 other than its well-known effects on the mTORC1 pathway. DNA microarray analysis demonstrated that DDIT4 may play important roles in apoptosis, inflammation, immune responses and cancer development. However, without detailed investigation, microarray only present possibility in these aspects. And different oral squamous cells should be analyzed considering expression levels of DDIT4 might bedifferent among cell types. Additional studies are needed to further understand the role of DDIT4 related mechanism in carcinogenesis and to develop DDIT4 as a potential therapeutic target in OSCCs.

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Conflict of Interest
The authors declare that they have no competing interests.

References
1. Ghani WMN, Ramanathan A, Prime SS, Yang Y, Razak IA, Abdul-Rahman Z, Abraham MT, Mustafa WM, Tay KK, Kallarakkal TG, Doss JG, Cheong SC, Bustam AZ, Zain RB: Survival of oral cancer patients in different ethnicities. Cancer Invest, 37: 275–287, 2019.
2. Feng Y, Song K, Shang W, Chen L, Wang C, Pang B, Wang N: REDD1 overexpression in oral squamous cell carcinoma may predict poor prognosis and correlates with high microvessel density. Oncol Lett, 19: 431–441, 2020.
3. Ellisen LW, Ramsayer KD, Johannessen CM, Yang A, Beppu H, Minda K, Oliner JD, Mckeon F, Haber DA: REDD1, a developmentally regulated transcriptional target of p63 and p53, links p63 to regulation of reactive oxygen species. Mol Cell, 10: 995–1005, 2002.
4. Brugarolas J, Lei K, Hurley RL, Manning BD, Reiling JH, Hafen E, Witters LA, Ellisen LW, Kaelin Jr WG: Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. Genes Dev, 18: 2893–2904, 2004.
5. Sofer A, Lei K, Johannessen CM, Ellisen LW: Regulation of mTOR and cell growth in response to energy stress by REDD1. Mol Cell Biol, 25: 5834–5845, 2005.
6. Si X, Xu F, Xu F, Wei M, Ge Y, Chenge S: CADM1 inhibits ovarian cancer cell proliferation and migration by potentially regulating the PI3K/Akt/mTOR pathway. Biomed Pharmacother, 123: 109717, 2020.
7. Hernández G, Lal H, Fidalgo M, Guerrero A, Zalvide J, Force T, Pombo CM: A novel cardioprotective p38-MAPK/mTOR pathway. Exp Cell Res, 317: 2938–2949, 2011.
8. Wen D, An M, Gou H, Liu X, Liu L, Ma C, Cong B: Cholecystokinin-8 inhibits methamphetamine-induced neurotoxicity via an anti-oxidative stress pathway. Neurotoxicology, 57: 31–38, 2016.
9. Shoshani T, Faerman A, Mett I, Zelin E, Tenne T, Gorodin S, Moshel Y, Elbaz S, Budanov A, Chajut A, Kalinski H, Kamer I, Rozen A, Mor O, Keshet E, Leschkowitz D, Einat P, Skaliter R, Feinstein E: Identification of a novel hypoxia-inducible factor 1-responsive gene, RTP801, involved in apoptosis. Mol Cell Biol, 22: 2283–2293, 2002.
10. Mangoni ML, McDermott AM, Zasioff M: Antimicrobial peptides and wound healing: biological and therapeutic considerations. Exp Dermatol, 25: 167–173, 2016.
11. Lai Y, Gallo RL: AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. Trends Immunol, 30: 131–141, 2009.
12. Nishikawa T, Ramesh R, Munshi A, Chada S, Meyn RE: Adenovirus-mediated mda-7(IL24) gene therapy suppresses angiogenesis and sensitizes NSCLC xenograft tumors to radiation. Mol Ther, 9: 818–828, 2004.
13. Rodriguez-Baena FJ, Redondo-Garcia S, Peris-Torres C, Martino-Echarri E, Fernandez-Rodriguez R, Plaza-Calonge M, Anderson P, Rodriguez-Manzanique J: ADAMTS1 protease is required for a balanced immune cell repertoire and tumor inflammatory response. Sci Rep, 8: 13103, 2018.
14. Lebedeva IV, Su Z, Chang Y, Kitada S, Reed JC, Fisher PB: The cancer growth suppressing gene mda-7 induces apoptosis selectively in human melanoma cells. Oncogene, 21: 708–718, 2002.
15. Miyahara R, Banerjee S, Kawano K, Efferson C, Tsuda N, Miyahara Y, Opmoodes CG, Chada S, Ramesh R: Melanoma differentiation-associated gene-7(mda-7)/interleukin(IL)-24 induces anticancer immunity in a syngeneic murine model. Cancer Gene Ther, 13: 753–761, 2006.
16. Buchanan FG, Wang D, Bargiacchi F, DuBois RN: Prostaglandin E2 regulates cell migration via the intracellular activation of the epidermal growth factor receptor. J Biol Chem, 278: 35451–35457, 2003.
17. Bódis K, Kahl S, Simon M-C, Zhou Z, Sell H, Knebel B, Strassburger K, Burkarth V, Mussig K, Markgraf D, Al-Hasani H, Szendroedi J, Roden M: Reduced expression of stearoyl-CoA desaturase-1, but not free fatty acid receptor 2 or 4 in subcutaneous adipose tissue of patients with newly diagnosed type 2 diabetes mellitus. Nutr Diabetes, 8: 49, 2018.
18. Cao H, Gerhold K, Mayers JR, Wiest MM, Watkins SM, Hotamisligil GS: Identification of a lipokine, a lip-
id hormone linking adipose tissue to systemic metabolism. Cell, 134: 933–944, 2008.

19. Etoh T, Inoue H, Yoshikawa Y, Barnard GF, Kitano S, Mori M: Increased expression of collagenase-3 (MMP-13) and MT1-MMP in oesophageal cancer is related to cancer aggressiveness. Gut, 47: 50–56, 2000.

20. Lawler J: Thrombospondin-1 as an endogenous inhibitor of angiogenesis and tumor growth. J Cell Mol Med, 6: 1–12, 2002.

21. Garg M, Ramdas N, Vijayalakshmi M, Shivashankar GV, Sarin A: The C-terminal domain (CTD) in linker histones antagonizes anti-apoptotic proteins to modulate apoptotic outcomes at the mitochondrion. Cell Death Dis, 5: e1058, 2014.

22. de Bie P, van de Sluis B, Burstein E, Duran KJ, Berger R, Duckett CS, Wijmenga C, Klomp LWJ: Characterization of COMMD protein-protein interactions in NF-kappaB signalling. Biochem J, 398: 63–71, 2006.

23. Maine GN, Burstein E: COMMD proteins: COMMing to the scene. Cell Mol Life Sci, 64: 1997–2005, 2007.