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Stress induced Differential Expression of THAP9 & THAP9-AS1 in the S-phase of cell cycle

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Abstract

Transposable elements (TE) function as one of the major effectors to respond to biological or environmental stress. The mobility of TEs, which is heavily controlled under normal conditions, may be activated by stress. LncRNAs are emerging as a crucial tool in the regulation of TEs.

This study focuses on the gene expression of THAP9, a domesticated transposon and lncRNA THAP9-AS1 (THAP9-antisense1), which form a sense and antisense gene pair with a promoter overlap of approximately 350bp. Under basal conditions, THAP9 is preferentially transcribed while THAP9-AS1 is heavily down-regulated. In the S-phase of the cell cycle, THAP9 expression exhibits stress-specific effects ranging from moderate enhancement to no change. On the other hand, THAP9-AS1, which has previously been reported to be upregulated in several cancers, always demonstrates enhanced expression under stress. Moreover, THAP9-AS1 is transcriptionally favoured during stress since the stress-induced fold-increase of THAP-AS1 expression is always higher than THAP9. Interestingly, the expression of both THAP9 and THAP9-AS1 exhibit a striking periodicity throughout the S-phase, reminiscent of cell cycle regulated genes. Thus, this study sets the stage to further explore the relationship between THAP9 and THAP9-AS1 and investigate THAP9-AS1's potential regulatory role during stress.

Introduction

Transposons or transposable elements (TEs) are highly dynamic sequences that can move from one genomic region to another (mobile DNA) without the need of homology between donor and target sequences. Owing to their self-propagatory nature, these sequences were labelled as 'parasitic' and 'selfish' in nature. A significant portion (3-50%) of the genomes of most species is contributed by TEs (1,2). Decades of research has led to a paradigm shift in biology where it is now a well-accepted fact that transposons are not parasitic but essential players in creating genomic diversity leading to evolution (3,4). Many TEs have been reported to be domesticated and repurposed by the host organisms for their own benefit, e.g., CENPB, Rag1, Rag2, SETMAR, THAP9 (5).

TEs were first described in 1984 by McClintock in her seminal study, reporting that the expression of TEs change in response to genomic insults and the outcomes of transpositions can

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be detrimental to the structure, function and evolution of the genome (6). To maintain cellular homeostasis, the transposon sequences are controlled from jumping around the genome by various epigenetic mechanisms (7). Heavy repressive modifications of Histone (H3K9 methylation) and DNA methylation are often observed in TE rich regions in several organisms and mutations resulting in loss of methylation causes upregulation of TEs (8,9). ATP-dependent chromatin remodelers (SWI/SNF) use their ATPase domain to hydrolyze ATP to modify nucleosomes to silence TEs (10). KRAB-ZFPs (Kruppel-associated box zinc-finger proteins), the largest class of mammalian DNA binding transcription factors, silence TEs by recruiting co-repressor proteins (11). piRNAs (PIWI interacting RNAs) carry inactive remnants of TEs and are known to repress TE activity in the germline (12).

Many lncRNAs or long noncoding RNAs (length >200 nucleotides) are located proximal to certain TEs and may contribute to their regulation (13,14). lncRNA-mediated TE repression may have evolved as a mechanism to counteract transposon-induced genomic instability (e.g., oncogenic translocations in several tumors) (15). Interestingly, about two-thirds of total human lncRNAs contain TE sequences that are inactive or remnants of active TEs (13). For example, many lncRNAs have LINE, Alu, ERVs and SINE sequences embedded in them and mutations in these regions may lead to lethal diseases (16).

Several studies have reported the activation or repression of TEs in response to stress(1). When stimulated by external stress, the cellular homeostasis gets disrupted, often leading to epigenetic changes which in turn might cause reactivation of transposons resulting in insertional mutagenesis (17). For example, under stress conditions, LINE-1 elements get activated upon release of SIRT-6 protein binding. Stress has also been reported to silence or deactivate transposons. For example, in yeast, Ty3 transposons get repressed under heat stress (18). Stress can also increase histone methylation in a tissue specific manner resulting in the silencing of various families of activated transposons (19). The number of Class II (DNA) transposons reported to be regulated under stress conditions is far less than Class I transposons (7,20).

We are interested in THAP9, a recently discovered human DNA transposase, which is homologous to the widely studied Drosophila P-element transposase (21). The THAP9 protein shares 40% similarity to the P-element transposase, and probably lacks the ability to transpose due to the absence of terminal inverted repeats and target site duplications. THAP9 is present as a single copy in the human genome and its function has not been discovered yet. Despite losing the hallmarks of a transposon, it has retained its catalytic activity (21,22). Moreover, it is not known if and how THAP9 is regulated at the cellular level.

The human THAP9 gene encodes for 6 transcripts, out of which only one encodes for the full-length transposase protein. THAP9-AS1 (THAP9 antisense) is a newly annotated lncRNA coding gene by Ensembl that encodes for 12 long non-coding RNAs (23,24). The expression of THAP9 and THAP9-AS1 is controlled by overlapping promoters on opposite strands, as curated by FANTOM database (25,26) (Fig 1). Antisense transcripts and their corresponding sense transcripts often show inverse expression; thus suggesting that one regulates the other (27,28). There have been recent reports where the THAP9-AS1 lncRNA has been implicated in pancreatic and gastric cancer, septic shock and neutrophil apoptosis (29–32). This has piqued our interests in studying the relationship between THAP9 and THAP9-AS1.

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In this study, we have investigated the effect of stress on THAP9 and THAP9-AS1 gene expression by carrying out detailed quantitative analysis in the S-phase under various stress conditions (heat shock, genotoxic, osmotic and oxidative stress).

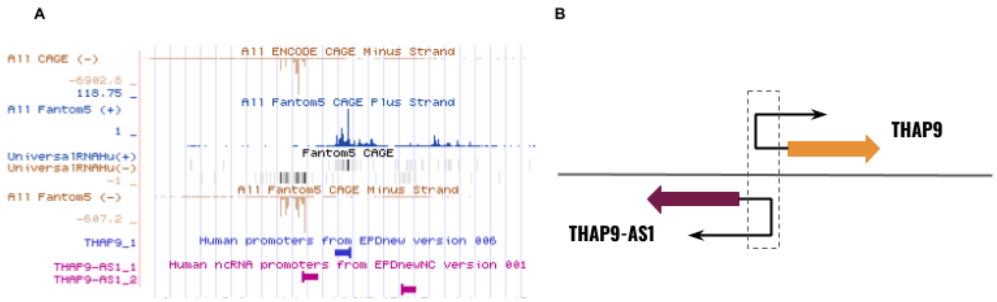

Fig 1: (A) THAP9 and THAP9-AS1 are controlled by different promoters on (+) and (-) strands. Promoter upstream of THAP9 (on (+) strand (shown in blue)) and THAP9-AS1 gene (on (-) strand (shown in pink)) as reported by CAGE data from FANTOM database (25). (B) Representative figure of overlapping promoters of THAP9 and THAP9-AS1.

Materials and methods

Reagents

HEK293T cell lines, DMEM (HyClone) supplemented with 10% Fetal Bovine Serum (Gibco), TRIzol™ Reagent (Invitrogen), High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™ 4368814), mouse anti-cyclin D1 monoclonal antibody (Invitrogen AHF0082), mouse anti-cyclin E monoclonal antibody (Invitrogen MA514336), mouse anti-cyclin A2 monoclonal antibody (Invitrogen MA1180), mouse anti-actin monoclonal antibody (Invitrogen MA1744), KAPA^{R} SYBR Fast Universal kit (Sigma KK4602)

Cell synchronization by double thymidine block

0.3 X 10^5 HEK293T cells (grown in DMEM supplemented with 10% fetal bovine serum under standard tissue culture conditions) were plated per well of 6-well plates and incubated for 12 hours at 37*C and 5% CO_2. To get a homogenous population of cells at G_1/S phase boundary, thymidine (Sigma T9250) was added to a final concentration of 2mM and incubated for 18 hours at 37*C. Cells were then washed with 1 ml pre-warmed 1X PBS and incubated with fresh media for 9 hours at 37*C. This was followed by second dose of thymidine at a final concentration of 2 mM and incubation for 18 hours at 37*C.

Assessment of cell synchrony

The cell lysates collected at 0h, 2h, 4h, 6h, 8h in the S-phase were run on 10% denaturing SDS PAGE in a running buffer (1X Tris- Glycine SDS). PageRuler™ Prestained Protein Ladder (10 to 180 kDa, ThermoFisher Scientific; 26616) was used as a molecular size marker and the samples were transferred to PVDF membrane by electroblotting using a standard gel transfer system. The membrane was blocked to remove nonspecific binding using 3% (w/v) skimmed milk in Tris-Buffered Saline with Tween-20 (TBST). Membranes were washed with 1X TBST

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and incubated overnight at 4°C with 1:1000 dilution of primary cyclin-D, 2ug/ml cyclin-E, 2ug/ml cyclin-A and 1:1000 beta-actin primary antibody followed by incubation with 1:5000 dilution of HRP- coupled secondary antibodies for 2h at room temperature and detected using enhanced chemiluminescence (Pierce, PI32106) on a Bio- Rad Gel Documentation system.

Stress Treatments

Heat stress: After the second thymidine block, the cells were subjected to heat at 42-43 degrees for 20 min to induce heat shock. After 20 minutes, the media was changed and cells were collected at 0-hour, 2 hour, 4 hour, 6 hour, 8 hour to cover the entire span of S-phase.

Genotoxic stress: Cells were kept under UV light for 20 min after double thymidine block. This was followed by addition of fresh media and collection of cells at 0h, 2h, 4h, 6h, 8h.

Osmotic stress: Different concentrations of DMEM were prepared by using dissolving powdered form of the medium (Himedia AT186) to prepare 10X and 0.1X solutions supplemented with 10% fetal bovine serum and 1% antibiotic (Penicillin-Streptomycin-Glutamine Gibco 10378-016). After the double thymidine block, cells were grown in different osmotic conditions for 30 min followed by replacement with fresh media and collection of cells at 0h, 2h, 4h, 6h, 8h.

Oxidative stress: Hydrogen peroxide (Merck 18304) was used in varying concentrations (final concentrations of 10uM, 25uM and 50uM) to induce oxidative stress in HEK293T cells. Following the double thymidine block, cells were incubated in media containing hydrogen peroxide for 30 min. After incubation fresh media was added to the cells and cells were collected at 0h, 2h, 4h, 6h and 8h.

RNA Isolation, cDNA preparation:

After the respective stress treatments, cells were isolated at their specific time points (0h, 2h, 4h, 6h, 8h) by scraping each well in 1ml Trizol after a wash with 1X PBS. The cell lysate was mixed thoroughly and transferred to the eppendorf tube and incubated for 5 minutes. 250ul of chloroform (Sigma 2566) was added and vortexed for 15-20 secs and kept at room temperature for 5 min, It was centrifuged at 10,000rpm for 5 min. The top aqueous layer was transferred carefully to a fresh tube and 550ul of isopropanol (Fisher scientific 26897) was added and mixed by gently inverting the tube 5-6 times and kept at room temperature for 5 min. The tubes were centrifuged at 14,000rpm for 30 min. Supernatant was decanted and 1ml of 75% ethanol prepared in DEPC (Sigma D5758) treated water was added, mixed gently and centrifuged at 9,500rpm for 5 min. The pellet was air- dried and resuspended in 15-20ul of DEPC water. The RNA was quantified and the samples with optical density 260/280 greater than to 1.8 were used for cDNA preparation. cDNA synthesis was carried out as per manufacturer's protocol using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™ 4368814). The synthesized cDNA was quantified and diluted to a final working concentration of 50ng for qPCR.

qPCR

qPCR reaction was set up as per manufacturer's instructions (Applied Biosystems™ 7500) with 50ng cDNA. The primers (F= forward, R=reverse) used for amplification are as follows:

THAP9 (F:5'-GGGGTTTATGTGCTTTGGTCTTGGAAAAC-3', R:5'-GCAATCTGTTGACTAGAAG-3')
THAP9-AS1 (F:5'-CACAATCTGGCGCCATCG-3', R:5'-CCTTATTTCCTTCATTGTGGCAAAG-3')
P53 (F:5'-CCTCAGCATCTTATCCGAGTGG-3', R:5'-TGGATGGTGGTACAGTCAGAGC-3')
HSP70 (F:5'-ACCTTCGACGTGTCCATCCTGA-3', R:5'-TCCTCCACGAAGTGGTTCACCA-3')

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SOD1 (F:5'-GTAGTCGCGGAGACGGGGTG-3', R:5'-GAGGCCTGGCGGGCGAC-3')
HPRT1 (F:5'-CATTATGCTGAGGATTTGGAAAGG-3', R:5'-CTTGAGCACACAGAGGGCTACA-3')

Data Analysis

Fold change of each gene was measured by comparative C_T method (also known as $2^{-\Delta\Delta Ct}$ method) using following equation (33):

$\Delta\Delta C_T$ = *[(C_T gene of interest - C_T internal control) Stressed sample - (C_T gene of interest - C_T internal control) Normal sample]*

HPRT1 gene is used as internal control in this study.

Gene expression under normal conditions was calculated as $2^{-\Delta Ct}$ where ΔC_T is the difference between C_T values of gene of interest and HPRT.
Two-tailed, unpaired Student's t test was used to evaluate expression differences between gene of interest and internal control gene (HPRT1). P values of <0.05 were considered statistically significant.

Results

External or internal stress is known to stimulate changes in the activity of transposable elements(1). No information is available for the stress response of THAP9 and THAP9-AS1. Approximately 1100 lncRNAs are known to be enriched in S phase (34). Although the exact role of this upregulation of lncRNAs in S phase remains undiscovered, the most prevalent hypothesis is that they express in response to DNA damage during the cell cycle (35).

Thus, we decided to focus our investigations of possible stress-induced changes of THAP9 and THAP9-AS1 gene expression to the S-phase of the cell cycle. This was achieved by synchronizing cells at the G_1/S phase border of the cell cycle prior to stress exposure. To ensure that the cells are in S-phase we probed them for the expression of S-phase specific cyclins (cyclin A and cyclin E) (Fig 2). The S-phase typically lasts for 6-8 hours in mammalian cells and thus measurements were made in early S-phase (0-4h), mid S-phase (4-6h) and late S-phase (6-8h).

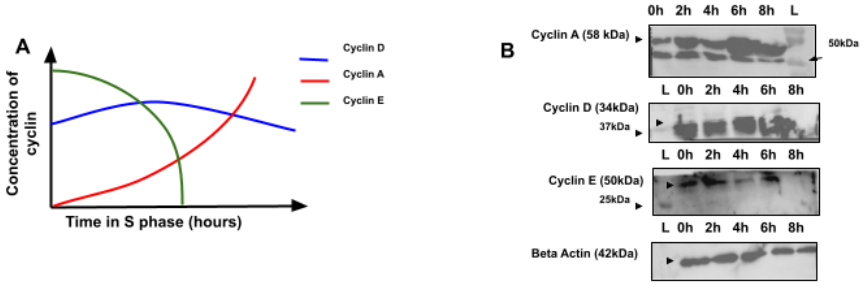

Fig 2: Assessment of cell synchronization by immunoblotting. (A) Representative figure of cyclin expression in the S-phase of cell cycle. Cyclin A (red) increases in concentration as the cell progresses through the S-phase. Cyclin E (green) declines in the S-phase after activation of cyclin A. Cyclin D remains constant throughout the S-phase. (B) Western blots showing the time course of cyclin A, D, E expression in HEK293T

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cells progressing through S-phase after release of double thymidine block. Beta actin (42 kDa) is shown as a loading control.

Genotoxic stress increases THAP9 and THAP9-AS1 expression throughout S-phase. UV irradiation was administered to induce genotoxic stress response in the S-phase. P53, which is known to show an oscillatory pattern of expression throughout S-phase after genotoxic stress, was used as a positive control (36). Both THAP9 and THAP9-AS1 were upregulated under UV stress and followed a similar pattern to that of P53, wherein the expression of both the genes falls at 2h and then increases consistently until the end of the S-phase ($p<0.01$) (Fig. 3B). The highest expression of both genes was recorded at late S-phase (8 hours); THAP9-AS1 expression was approximately 8-fold higher while THAP9 expression was 5-fold higher than the corresponding recordings at 0 hour ($p<0.01$) (Fig. 3B).

THAP9-AS1 gene expression increases after heat shock

To investigate whether heat has any effect on the gene expression of THAP9-AS1 and THAP9, we subjected synchronised HEK293T cells to heat shock. We observed no significant change of THAP9 mRNA expression throughout the S-phase with a sudden increase in late S-phase (8 hours). On the other hand, THAP9-AS1 transcript expression increases continuously from 2 to 8 hours in S-phase to a final 10-fold increase ($p < 0.001$) (Fig. 3C). HSP70 (Heat shock protein) which is a positive biomarker for heat stress also upregulates from mid to late S-phase (Fig. 3C).

Osmotic stress leads to differential expression of THAP9-AS1 and THAP9. THAP9-AS1 exhibits differential gene expression under different types of osmotic stress. It is highly downregulated under hypo-osmotic conditions throughout the S-phase ($p<0.001$), with maximum downregulation in early S-phase (more than 5-fold decrease). Conversely, under hyper-osmotic conditions, the expression of THAP9-AS1 increases to ~10 fold by late-S phase (6-8 hour), after an initial downregulation in early S-phase (0 h) ($p<0.0001$). This pattern of expression is similar to that of P53 ($p<0.0001$), which was used as a positive marker for osmotic stress and maximum increase in its expression was observed in early S-phase (2 hour) ($p<000.1$).

On the other hand, THAP9 expression does not appear to change under hypo-osmotic stress, except for a slight increase in mid-S phase and an approximately two-fold increase in late S-phase ($p<0.001$). Under hyper-osmotic stress, THAP9 is highly downregulated in early and mid S phase but its expression surges at the end of S-phase ($p<0.0001$).

Oxidative stress triggers an increase in the expression of THAP9-AS1

To induce oxidative stress, the cells were exposed to three different concentrations of hydrogen peroxide (H_2O_2), i.e., 10uM, 25uM and 50uM. At the lowest concentration of H_2O_2 (10uM), THAP9-AS1 and THAP9 are downregulated throughout the S-phase and increase to ~7 fold and ~5 fold respectively in the final stages of the S-phase ($p<0.0001$). When the concentration of H_2O_2 was raised to 25uM, both THAP9-AS1 and THAP9 were upregulated after 2 hours in S phase. The expression of THAP9-AS1 stays higher than THAP9, although both the genes show a decrease in expression in the middle of the S-phase (4 hour). The highest recorded transcript levels were at 8 hours for THAP9-AS1 and at 6 hours for THAP9.

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At the highest concentration of H_2O_2 (50 uM), we observed that THAP9-AS1 exhibits an oscillatory pattern of expression. It decreases until the mid-S phase followed by an increase in expression until the end of the S-phase ($p<0.001$). THAP9, on the other hand, increases to ~2 fold in early-S phase and then downregulates at 6 hours followed by ~5-fold increase at 8 hours. Superoxide dismutase-1 (SOD1) which gets activated in response to oxidative stress was used as a positive biomarker. We observe that with increase in the concentration of H_2O_2, the upregulation of SOD1 gets triggered in early S-phase (0-2 hours) in comparison to the lower concentration of H_2O_2 (10uM) which leads to the highest expression of SOD1 at the end of the S-phase (8 hour).

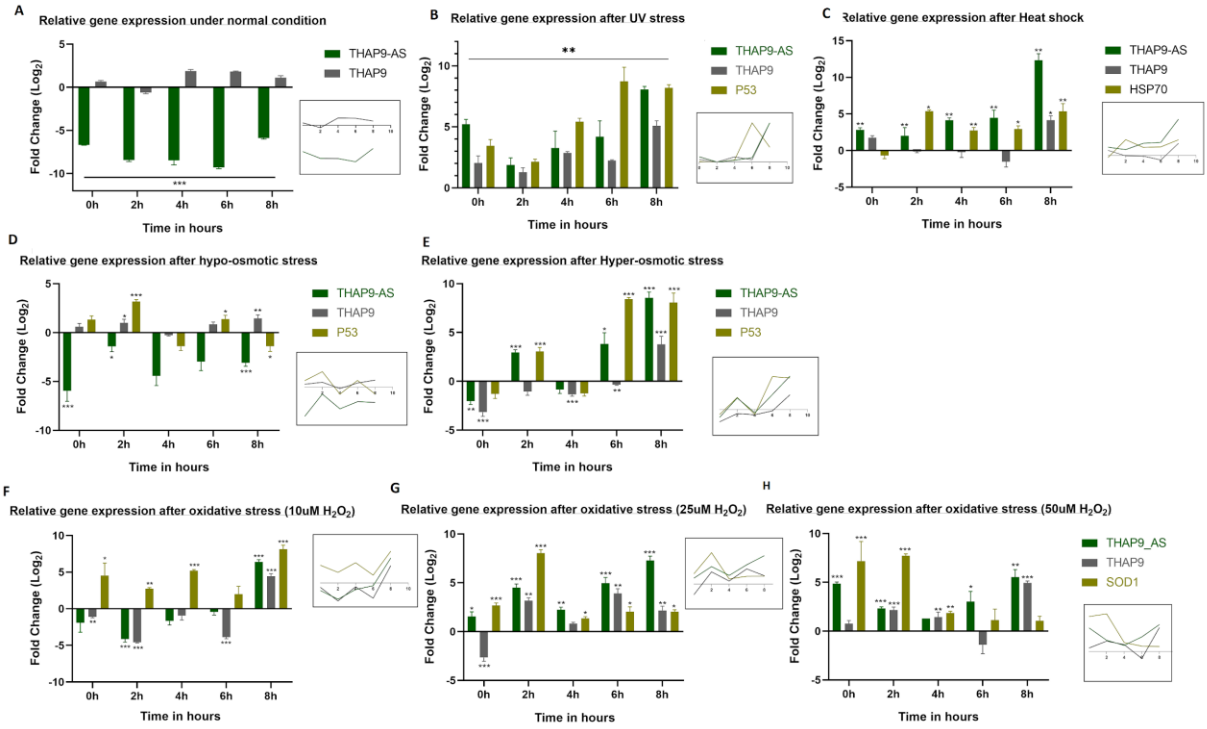

*Fig 3: RT-qPCR analysis of THAP9 and THAP9-AS1 under normal (A) and stress (B-H) conditions in the S-phase. Relative gene expression data (fold change from basal state) represented as mean±SEM (n=4, n=3 for oxidative stress) from 2 independent experiments. Data is normalized with HPRT1 gene expression data and represented as Log_2 of fold change on y axis, time of sample collection on x axis. Upregulation of genes is represented on the positive y-axis and downregulation is shown on negative y-axis. Statistical significance was determined by two-tailed unpaired Student's t test (*p<0.01, **p<0.001, ***p<0.0001). The insets show the same trend of relative expression but in the form of line plots. Y axis depicts Log_2 of fold change and x axis denotes time of sample collection.*

Conclusions: Overall, we show that THAP9-AS1 which is downregulated under basal conditions (Fig. 3A), appears to be highly upregulated (Fig. 3B-H) during stress. It is interesting to note that diverse stress conditions all lead to increased expression of this lncRNA transcript. THAP9 on the other hand, exhibits a stress-specific expression pattern, wherein certain stress conditions either

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increase (genotoxic, oxidative, heat) or do not affect (hypo-osmotic) its transcription. Furthermore, stress induces an oscillatory expression pattern of both THAP9 and THAP9-AS1 throughout the S-phase.

With emerging knowledge of sense-antisense gene pairs as a regulatory phenomenon in mammalian cells, it would be interesting to explore the relationship between THAP9 and THAP9-AS1. It is tempting to speculate that THAP9-AS1 lncRNA, which is heavily downregulated under basal conditions, is preferentially expressed in stressed cells to regulate THAP9's expression and subsequent downstream responses including possible chromosomal rearrangements via transposition. This is further corroborated by the observation that THAP-AS1 has consistently higher overall expression than THAP9, in both stressed (this study) and cancer cells (30,31). Although the function of both these genes remains elusive, this study has paved the way for exploring the function of the THAP9-THAP9-AS1 gene-pair in the context of DNA damage response.

Discussion

Cells respond to external and internal stress via elaborate, multi-faceted systems and pathways. Most intrinsic stresses cause DNA lesions that are surveyed at DNA damage checkpoints during the cell cycle (37). Many transposons or 'jumping genes' are known to be activated under stress conditions which can lead to insertional mutagenesis and genomic instability (7,38,39).

Cancer cells exhibit an intrinsic stress due to altered metabolic activities (40). For example, a majority of tumor cells exhibit high oxidative stress to maintain high cell proliferation and facilitate the process of EMT (epithelial-mesenchymal transition) (41). Genome-wide studies of different types of cancers demonstrate that transposons are often responsible for oncogenic translocations (38,39,42). As a safety measure, transposons are often epigenetically silenced by repressive histone modifications or regulated post-transcriptionally by lncRNAs to counter ectopic expression of these dynamic sequences (7).

The activation of TEs under stress can be both beneficial or deleterious for the host (7). This is because the actual relationship between stress and TE expression is quite complex and varies depending upon the type of stress, type of organism and the epigenetic/genetic/metabolic changes drawn out by the stress (7). The regulation may be temporal or spatial wherein certain TEs can be turned on at specific stages of embryonic development or in specific cell types or tissues (7). Since stress induces both the defence response and the activation/deactivation of certain TEs within a host, it is hard to decipher the cause-effect relationship between the TEs and stress. Stress often results in double stranded DNA breaks which are detected and repaired at the DNA-integrity checkpoints of the cell cycle, by NHEJ (Non homologous end joining) when they occur in early S/G_1 phase or Homologous repair when they occur in late S/G_2 phase (43). Interestingly, p53, which is known for its role in maintaining genomic stability during external stress and is mutated in almost all cases of cancer (38,42,44), may directly repress certain transposons (44).

LncRNAs which were long considered "junk" are now emerging to be important players in almost all aspects of cellular regulation (35,45,46) including cell proliferation in the cell cycle S-phase (34,35,46,47) and regulation (e.g., by PANDA, lncRNA p21) of genes crucial to DDR (DNA

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damage response) (48–51). lncRNAs have been implicated in several diseases including cancer, diabetes and neurodegenerative disorders (52). Several lncRNAs have been identified as oncogenes or tumor suppressors which show cancer-specific upregulation (53). LncRNAs can interact with proteins and serve multiple roles like, decoys, scaffolds and guides (54). However, the role of several lncRNAs is poorly understood because their expression is highly specific to cell type, cellular stage, stimuli, experimental conditions (45,55–58).

THAP9-AS1 is a recently annotated lncRNA and has been shown to be upregulated in pancreatic cancer and neutrophil apoptosis and downregulated in case of septic shock (29–32). In this study, we observe that THAP9-AS1 transcript expression is highly downregulated under basal conditions (Fig. 3A) but consistently upregulated by various stress conditions (Fig. 3B-H) in the S-phase of the cell cycle.

Ultraviolet rays are highly genotoxic and cause severe DNA damage leading to cell death. Genotoxic agents (like chemotherapy) are also used to treat cancer but therapy-resistant cancer cells are able to evade the damage by inhibiting tumor suppressor genes, increasing cellular growth factors and eluding cell cycle checkpoints (59). Several lncRNAs (NONHSAT1010169, GUARDIN, NEAT1) show changes in gene expression under genotoxic stress and are known to contribute to drug resistance in therapy-resistant cancer (47). P53 is responsible for genome defense in case of DNA damage and is known to be regulated by several lncRNAs (linc-p21, MEG3, TUG1, PANDA, PRAL and LED) in cancer and stress (47,50,53,60,61). We observed that genotoxic stress induces steady increase of THAP9-AS1 expression to a final ~10-fold upregulation by the end of S-phase (Fig 3B). Although THAP9-AS1's function is unclear, its reported upregulation in pancreatic and gastric carcinoma suggests a possible role in carcinogenesis and DNA damage response.

LncRNAs also play an integral role in heat shock response. HSR1 (Heat shock RNA -1) plays an accessory role in forming the trimeric complex of HSF1, a heat shock protein for binding to DNA (62). Additionally, lncRNAs also regulate transcription of heat shock response genes (63). Here we report heat-induced upregulation of THAP9-AS1. On the other hand, THAP9 is downregulated after a brief heat shock (Fig 3B); this might be a result of repression of general transcription under heat stress (63).

Osmotic stress affects the volume and tonicity of the cell and creates DNA lesions thereby eliciting a DNA damage response similar to that of UV stress (64). Hyper-osmotic stress is known to increase the expression of DNA damage responsive genes but not much is known about the participation of non-coding RNA in osmotic response in mammalian cells (65). In this study we have observed that hyperosmotic stress induces upregulation of THAP9-AS1 expression (Fig 3). A recent study suggests that hypo-osmotic stress cannot be linked to the misregulation of the cell cycle (66). This stands congruent to our findings that demonstrate that neither THAP9 nor THAP9-AS1 expression is affected by hypo-osmotic stress.

Mitochondria, a key organelle involved in stress response, play a crucial role in maintaining cellular redox homeostasis by production of ROS (Reactive oxygen species). Oxidative stress, which occurs when ROS production surpasses the antioxidant defences, is known to damage DNA and disrupt mitochondrial function, as observed in cancer cells (67) as well as age related pathologies and cardiovascular diseases (68). Interestingly, THAP9 localizes in mitochondria, and both its expression as well as that of THAP9-AS1 (Fig. 3 G, H) is enhanced

when subjected to oxidative stress. lncRNAs (MALAT1, NEAT1, H19, HULC) are upregulated in response to oxidative stress (47,69).

The human THAP9 gene and its antisense transcript, THAP9-AS1 are controlled by overlapping promoters on opposite strands (Fig 1). In most reported sense-antisense gene pairs, the antisense gene can regulate the expression of the sense gene by RNA interference or by transcriptional interference, i.e., by competing for the enzymes essential for transcription on the shared promoter sites (27). Stress may induce the emergence of *de novo* transcripts, which often arise from or overlap with regions that already contain genes in the opposite orientation (70). Given the overlapping promoters of THAP9 and THAP9-AS1, it can be hypothesized that the two genes may regulate each other's expression or that they share a common switch that contributes to the expression bias under certain stress conditions. Under basal conditions it is observed that THAP9 is preferentially transcribed whereas under stress conditions, THAP9-AS1 is transcriptionally favoured. This leads us to speculate that there may be stress-specific transcription regulators which enable the switching to favoured THAP9-AS1 expression during stress. This also might explain the upregulation of THAP9-AS1 in cancer cells which are naturally stressed (29–31). It is to be noted though that in most stress conditions, the expression of both transcripts increase. Thus, it is possible that both these genes play an unknown role in DNA repair. Also, interestingly, the stress-induced fold-increase of THAP-AS1 expression is always higher than THAP9. This expression difference could be explained by the smaller length of the THAP9-AS1 transcript in comparison to the THAP9 transcript (71) or due to the less time required for a lncRNA (THAP9-AS1) to respond to stress in comparison to a protein (THAP9) which requires additional steps of translation and folding.

We also observe a periodicity in the expression of both THAP9 and THAP9-AS1 throughout the S-phase of the cell cycle, wherein, the amount of transcripts first decreases and then increases and vice versa (Fig. 3B, 3D, 3F, 3G, 3H). The periodic gene expression of THAP9 and THAP9-AS1 resembles the periodicity pattern reported for the S-phase regulated expression cluster (72). The upregulated genes in S phase are also known to be involved in DNA damage response (72).

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