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Does DYRK1A Regulate REST in Lung cancer?

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Background: Lung cancer is the most frequent cancer in the United Kingdom and the leading cause of death among cancer patients globally. There are two primary forms of lung cancer: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC, the most frequent kind of NE lung cancer, loses REST expression. Most NSCLC lack NE gene expression but maintain REST, which is expressed at varying degrees (5). REST expression is regulated at many levels in both normal physiology and illness. It has been shown that DYRK1A overexpression leads to REST level dysregulation (15). The molecular processes relating DYRK1A and REST levels remain unknown.

Objective: The purposes of this study were to examine a panel of lung cell lines for expression of DYRK1A protein and to investigate the correlation between DYRK1A and REST in normal lung and lung cancer. Additionally, to check whether DYRK1A depletion or chemical inhibition alters REST levels in lung cancer. Finally, establish if DYRK1A affects the transcription of the REST-target gene CHGB.

Methods: Western blots were carried out to identify the expression of DYRK1A and REST proteins in extracts from normal lung cell lines, SCLC, carcinoid and NSCLC. siRNA silencing, 4 oligos were used for DYRK1A and tow positive control (2 oligos for REST) and non-targeting oligo as negative control. Harmine used as specific DYRK1A inhibitor. MTS assay to tested cell viability and qRT-PCR to examine REST target gene transcript (CHGB).

Results: It found that DYRK1A expressed more in lung cancer than normal lung cell lines and there was a good correlation between DYRK1A and REST protein in normal lung cell lines. However, this correlation lost in some NSCLC cell lines. Also, DYRK1A depletion reduced REST level in H460 cell line. As well as the inhibition of DYRK1A in A549 reduce REST level. Finally, CHGB responded to DYRK1A inhibition in A549 and to DYRK1A depletion in H460 cell lines.

Conclusions:

There was a strong association between DYRK1A and REST protein in normal lung cell lines, and it was discovered that DYRK1A was expressed greater in lung cancer than in normal lung cell lines. Some NSCLC cell lines, however, lost this association. Additionally, DYRK1A removal decreased the H460 cell line's REST level. Additionally, A549's DYRK1A inhibition lowers REST levels. Lastly, CHGB reacted to DYRK1A depletion in H460 cell lines and DYRK1A inhibition in A549 cell lines.

Key-words**:** Lung cancer, REST, DYRK1A, A549 and H460

Introduction

The most frequent cancer in the United Kingdom and the leading cause of cancer-related deaths globally is lung cancer. Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) are the two

primary forms of lung cancer. Squamous cell carcinoma, adenocarcinoma, and large cell carcinoma are the three forms of non-small cell lung cancer. Compared to SCLC, NSCLC is more prevalent; both kinds have a poor prognosis, with a 7% to 9% 5-year survival rate (1). SCLC is distinguished by its neuroendocrine (NE) characteristics and rapid disease development. On the other hand, lung carcinooids, a rare kind of comparatively benign cancer, also show neuroendocrine markers (2). The neuron-restrictive silencer factor (NRSF), also referred to as the repressor element 1-silencing transcription factor (REST), is a transcriptional repressor that interacts at the promoter level with the neuron-restrictive silencer element (RE1) to limit the expression of numerous neuronal genes (3). REST has been linked to a variety of illness conditions, including Down syndrome and cancer. In several cancer types, REST has been shown to have both tumor-suppressive and carcinogenic properties. When compared to either neural progenitor cells or fully developed neurons, it has been discovered that certain medulloblastoma cell lines have higher levels of REST expression (4). The most prevalent type of NE lung cancer, SCLC, is characterized by a loss of REST expression. While REST is expressed at varying amounts, the majority of NSCLC do not express the NE gene (5). REST is a labile protein that is targeted for ubiquitin-dependent proteasomal degradation by interaction with the F-box protein SCFBTrCP through a phospho-degron in REST (6). During the G2 phase of the cell cycle, REST is degraded by the E3 ubiquitin ligase SCFBTrCP to allow transcriptional derepression of Mad2 (7). REST expression is regulated at various levels in both normal physiology and disease, including spilicing, genetic transcriptional, and post translational. In cells, REST transcription is tightly regulated, for instance by DYRK1A. DYRK1A is a member of the dual-specificity tyrosine phosphorylation-regulated kinase family, which is expressed in several tissues and activated by intramolecular tyrosine phosphorylation. Skeletal muscle expresses DYRK1B, while the brain expresses DYRK1A.The DYRK1A gene is located in the Down syndrome critical region (DSCR) of chromosome 21. DS is linked to neurobiological changes because DYRK1 is a dual specificity protein kinase that catalyzes auto phosphorylation on serine/threonine and tyrosin residues (8). In the brains of DS patients, DYRK1A kinase is overexpressed (9). Two nuclear targeting signal sequences, a protein kinase domain, a leucine zipper motif, and a very conservative 13-consecutive-histidine repeat are all present in the DYRK1A protein (figure 1).

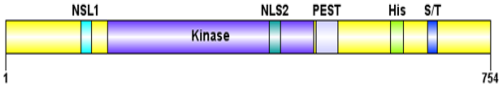

Fig 1. **DYRK1A protein structure**. (adapted from Alvarez M et al (2007))

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Numerous endocytic proteins, including dynamin 1 and amphiphysin 1, are phosphorylated by DYRK1A. Additionally, eukaryotic protein synthesis initiation factor 2B epsilon (eIF2Bε) (11) and glycogen synthase (12) are phosphorylated by DYRK1A. Moreover, DYRK1A phosphorylates several transcription factors such as nuclear factor of activated T cells (NFAT) and forkhead in rhabdomyosarcoma (FOXO1), which lead to inhibit the function of these factors (13-14). It has been shown that DYRK1A overexpression related to deregulation of REST levels (15). Moreover, It interacts with the chromatin remodeling complex REST/SWI/SNF. Gene clusters surrounding REST/NRSF binding sites have been shown to be deregulated by DYRK1A-dosage imbalance (16). Nevertheless, the molecular pathways that connect DYRK1A and REST levels are still unclear. As shown previously both overdose and DYRK1A inhibition decrease the level of REST transcription in neuronal models (15). Because the level of REST is important in lung cancer, we set out to test the effect of DYRK1A on REST level and function in lung cancer (figure2).

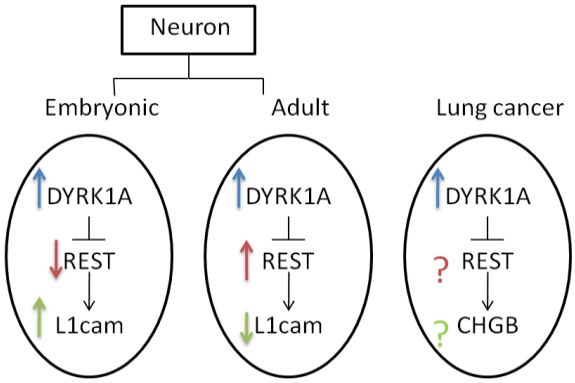

Fig 2. Effect of DYRK1A on REST and target gen L1cam in neuron (adult and embryonic) and the hypothesis of the effect of DYRK1A on REST level and target gene chromogranin B (CHGB) in lung cancer.

Aims of the study

Study objectives were to check for DYRK1A protein expression in carcinoid-derived cell lines, SCLC, NSCLC, and normal lung cells, as well as to look into how DYRK1A depletion and pharmacological inhibition affected REST levels in lung cancer.

Methods and Materials

Cell Culture. Four different kinds of human lung cell lines from the American Type Culture Collection (ATCC) were employed in this investigation. Normal lung cell lines involved: normal human bronchial epithelial cells (NHBE), normal lung fibroblasts (MRC5), SV40 transformed human bronchial epithelial cells (BEAS2B and SV40-HBE), SV40 transformed normal lung fibroblasts (MRC5VA) and SV40 transformed embryonic lung fibroblast (WI38-VA13). Small cell lung cancer cell lines used were Lu-165, GLC19, NCI-H69, NCI-H345, COR-L88, COR-L47 and U2020. A lung carcinoid line NCI-H727. Finally

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non-small lung cancers: NCI-H460, NCI-H322, NCI-H647, NCI-H2170, COR-L23 and A549.

RNAi Silencing

A549 or H460 cells were seeded into 6-well plates at a density of 60,000 cells/ml for siRNA transfections. The next day, they were transfected with 50 mols of siRNA using Lipofectamine 2000 (Invitrogene) in accordance with the manufacturer's instructions. Prior to examination, cells were cultivated for 72 hours. As shown in table 1, the following siRNA duplexes were employed in this investigation:

Table 1. siRNA duplexes.

GENE SYMBOL	Target Gene	OLIGO TYPE
DYRK1A	NM_130438	siGENOME 1,2,4 and 17 (pool)
DYRK1A	NM_130438	siGENOME 1
DYRK1A	NM_130438	siGENOME 2
DYRK1A	NM_130438	siGENOME 4
DYRK1A	NM_130438	siGENOME 17
n/a	Non-targeting	siGENOME siCON2
REST	NM_005612	siGENOME REST 1
REST	NM_005612	siGENOME REST 5

Inhibitors

At room temperature, harmine (Fluka, Buchs, Switzerland) was dissolved in ethanol with continuous stirring to create a 10 mM solution, which was further diluted with 50% (v/v) ethanol to a concentration of 0.5 mM. In order to treat the cultivated cells, this stock solution was further diluted to the working concentrations using the necessary culture medium.

Cell Viability assay

The MTS assay was used to assess how inhibitors affected cell viability. On 96-well plates, A549 or H460 cells were planted in 100ul medium at a density of 5000 cells per well. Following an overnight culture, the cells were cultivated for 48 hours without media changes using successive dilutions of harmine in culture medium. The MTS test was carried out by adding 20ul of CellTiter 96® AQueous one solution

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reagent (promega), and the absorbance was measured at 490 nm.

Protein extraction:

After washing cells in PBS, they were lysed in 1x Laemmli buffer (50 mM Tris pH 6.8, 2% SDS, 10% glycerol) and heated to 1100C for 10 minutes, vortexing every 2 minutes to create whole cell extracts. They were then diluted in Laemmli buffer 1:5. Cells were scraped into 1 milliliter of ice-cold phosphate-buffered saline (PBS) to extract nuclear and cytoplasmic protein fractions. The pellet was then collected by centrifugation and resuspended in 150 microliters of SU buffer (0.5% NP-40, 25 milliliters of Tris pH 7.5, 150 milliliters of NaCl, and proteas inhibitors). The samples were vortexed and incubated on ice for ten minutes. Samples were centrifuged at 16,000 xg for 5 minutes in order to pellet nuclei, and the cytoplasmic fraction was obtained from the supernatant. After resuspending the cell pellet in 15 µl of Dignam buffer (20 mM HEPES pH 7.9, 25% glycerol, 0.42 M Nacl, 1.5 mM EDTA, 0.5 mM DTT, and protease inhibitors), the nuclear extract was obtained by incubating it in a cold environment for 40 minutes while continuously vortexing it at 2000 rpm. The samples were then centrifuged for 10 minutes at 16,000 xg. The nuclear fraction was obtained from the supernatant. The BCA test was used to quantify each protein extract.

Western Blotting: The expression of DYRK1A and REST proteins in extracts from normal lung cell lines, SCLC, carcinoid, and NSCLC was examined using Western blots.Using 8% polyacrylamide gel, sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was used to separate 10 µg of whole cell protein extract, 5 µg of cytoplasmic protein fraction, or 2.5 µg of nuclear protein fraction. Gels were operated at 200 V for an hour. To standardize size, Perfect Protein and Rainbow molecular weight markers were put into the first and last lanes of every gel. For 75 minutes at 24 V, proteins were moved to Schleichr & Schuell nitrocellulose membranes in transfer buffer (14.4 gm glycine, 3.03 gm Tris, 0.2 vol methanol). Three sections of membranes were cut at the 75 kDa and 150 kDa markers. Tubulin and anti-actin were used to blot the first component, which was between 20 and 75 kDa. The anti-DYRK1A was used to blot the second portion from the 75-150 kDa. Anti-REST was used to blot the third component, which was 150–225 kDa in size. In TBST (TBS 0.1% Tween 20), 5% nonfat dried milk (NFDM) was used to inhibit the nitrocellulose membranes of DYRK1A. To prevent nonspecific sites, 3% NFDM in phosphate-buffered saline (PBS) was added to the REST protein.The primary antibodies mouse monoclonal anti-Tubulin (1:10000, Sigma) and mouse monoclonal anti-Beta-Actin (1:10000, Abcam) were then used to incubate the blots. Millipore supplied the polyclonal rabbit anti-REST (1:2000) and anti-DYRK1A (1/1000, cell signaling). Millipore supplied the polyclonal rabbit anti-REST (1:2000) and anti-DYRK1A (1/1000, cell signaling). After being diluted in TBST with 5% NFDM, the DYRK1A primary antibody was incubated at 40C for the entire night. The REST antibody was incubated at 40°C for the entire night

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after being diluted in TBS with 3% NFDM.Odyssey® Infrared Imaging was used to quantify every protein. Actin served as a reference for all proteins.

RNA extraction and cDNA preparation

Following the manufacturer's recommendations, total RNA was extracted from roughly 6×104 cells using the RNeasy plus Mini Kit (Qiagen). Before being applied to the spin column membranes, cells were briefly disrupted by adding RLT buffer with β-mercaptoethanol and precipitated with 70% ethanol. After washing the columns with RWI and RPE buffers, 30 µl of RNase-free water was used for the final elution of the total RNA. Samples of total RNA were kept at -80°C. Using a NanoDrop (The NanoDrop® ND-1000 UV-Vis Spectrophotometer) to measure absorbance at 260 and 280 nm, the concentration of total RNA was determined for each sample. cDNA synthesis was carried out using the Reverse Transcription System (Promega) and RevertAid H minus M-MuLV Reverse Transciptase (MBI Fermentas). For five minutes, 1 ml of oligodT primer and 1 µg of RNA were incubated at 70°C. A mixture of PCR nucleotide mix, 5x reverse transcription buffer (MBI), RNasin (Promega), and nuclease-free ddH2O was then added to the RNA and incubated for 5 minutes at 37°C. After adding 0.1µl of reverse transcriptase, the mixture was incubated for one hour at 42°C and then for ten minutes at 70°C. Ultimately, 100 µl of diluted cDNA was used.

QRT-PCR

An IQ5 real-time PCR detection system (Bio-Rad) was used to perform quantitative real-time RT-PCR (qRT-PCR) in triplicate using 1 µl of cDNA, forward and reverse primers, and IQ SYBR Green Supermix. The samples were amplified 40 times at 95°C (30 s) and 60°C (30 s). The quantification cycle (Ct) values for test genes were standardized to the reference gene β-actin (ACTB) using the 2-ΔΔct equations, and melt curve analysis was performed at the conclusion of each experiment.

Results

Expression of DYRK1A protein in cell lines derived from normal lung and sub-types of lung cancer

DYRK1A have not previously tested on lung cancer. DYRK1A protein expression in lung cancer and normal lung cell lines was examined using whole cell protein extracts from 20 distinct lung cell lines: one lung carcinoid cell line, seven SCLC, six NSCLC, and six normal cell lines (three epithelial and three fibroblast cell lines) as presented in figure 3.

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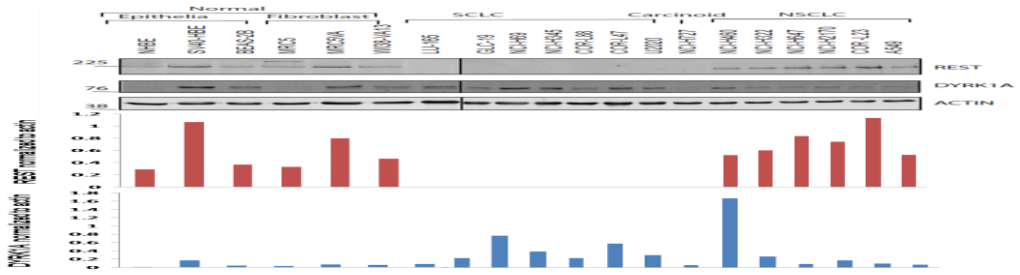

Fig 3. Co-expression of DYRK1A and REST proteins in normal cell line, sub-types of lung cancer cell lines. Immunoblot analysis of DYRK1A and REST proteins in whole cell extracts from 20 cell lung cell lines. Blots reveal REST and DYRK1A proteins in normal epithelial and fibroblast, SCLC, carcinoid and NSCLC. All proteins were measured using Odyssey and normalized to actin. Data as exhibited for REST protein (red) and DYRK1A (blue).

REST protein was detected in both normal and NSCLC, while it was absent in all SCLC and carcinoid lung cell lines DYRK1A was expressed at relatively low levels in untransformed normal lung cell lines (NHBE AND MRC5) but more highly expressed in transformed normal cell lines (SV40-HBE, MRC5VA, BEAS-2B and WI38-VA13). In NSCLC also increased relative to untransformed normal, but more variable.

It was calculated the means DYRK1A and REST protein expression in normal cell lines and the two major sub-types of lung cancer. Normal cell lines showed more REST protein and less DYRK1A protein as shown in figure 4A. NSCLC expressed higher DYRK1A and less REST compared to normal cell lines. SCLC expressed DYRK1A but undetectable REST protein.

As DYRK1A may regulate REST, the probable correlation between DYRK1A and REST proteins in normal and NSCLC cell lines were tested. It had found that, whilst there a good correlation between these proteins in normal lung cell lines (R^2=0.759) in some NSCLC this correlation was lost in others (figure 4B). Therefore, one cell line (A549) was selected, where correlation was seen and H460, that did not demonstrate correlation to study the effect of DYRK1A depletion on REST protein. Next, we tested the sub-cellular location of DYRK1A in these cell lines using immunoblot analysis of protein lysates from A549 and H460 cell lines. It was Actin blotted to correct for protein loading as this is expressed in nuclear and cytoplasm and tubulin to check fractionation as this is only localized in cytoplasma.it was found that both DYRK1A and REST predominantly localized in nuclear fractions as illustrated in figure 4C.

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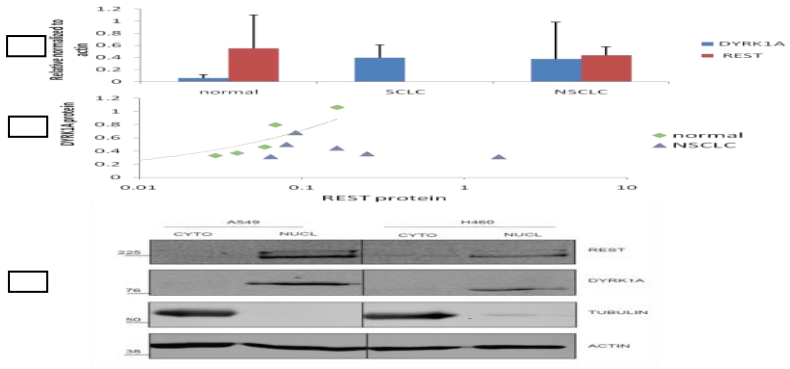

Fig 4. The expression of DYRK1A. (A) Mean expression of DYRK1A and REST in normal, SCLC and NSCLC. Error bars show stand deviation. (B) The association among DYRK1A and REST proteins in normal and NSCLC cell lines. (C) Immunoblot analysis of the sub-cellular location of DYRK1A and REST in NSCLC (A549 and H460) cell lines.

Effect of DYRK1A depletion on REST protein

First, the effect of DYRK1A depletion in the A549 cell line was tested by transfecting cells with 4 oligos targeting DYRK1A (oligo1, oligo2, oligo4 and oligo17) either individually or as a pool. As a positive control deplete RES using the oligo siREST1. siCON2 is a non-target siRNA was used as control. The transfection was performed for 72 h. Immunobloting was used to detect the expression of DYRK1A and REST. Proteins were quantified by Odyssey and regulated to siCON2. As publicized previously A549 had very low expression of DYRK1A protein (figure 3) and there was no evidence for depletion of DYRK1A in this cell line (figure 5).

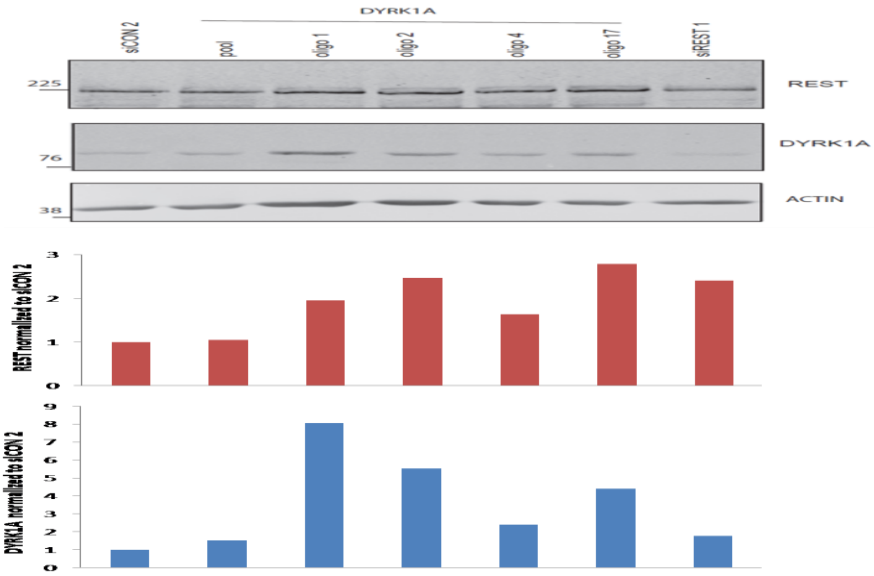

Fig 5. The effects of DYRK1A depletion and REST depletion in A549 cells. Immunoblot analysis of depletion of DYRK1A and REST proteins in nuclear fractions from A549 cell lung cell lines 72 h transfection. Blots demonstrate REST and DYRK1A proteins. Actin protein was used as control to correct for protein loading. REST (red) and DYRK1A (blue) proteins were measured using Odyssey and standardized to actin.

Next, the same experiment in the H460 cell line, which has more DYRK1A protein than A549 was

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performed. In this case, a decrease of DYRK1A was evident with all targeting siRNA especially with pool, oligo2 and oligo4. In each case the decrease in DYRK1A was associated with a decrease in REST although this was less than depletion of REST with siREST depletion. Interestingly, REST depletionalso resulted in a decrease in DYRK1A. Also, there was a decrease of DYRK1A when REST depletion (figure 6)

Because oligo2 and oligo4 were the most effective oligos on DYRK1A and because we saw an effect of siREST 1 on DYRK1A level, we selected these oligos for DYRK1A with two oligos for REST (oligo1 and oligo5) to confirm these effects as shown in figure 7.

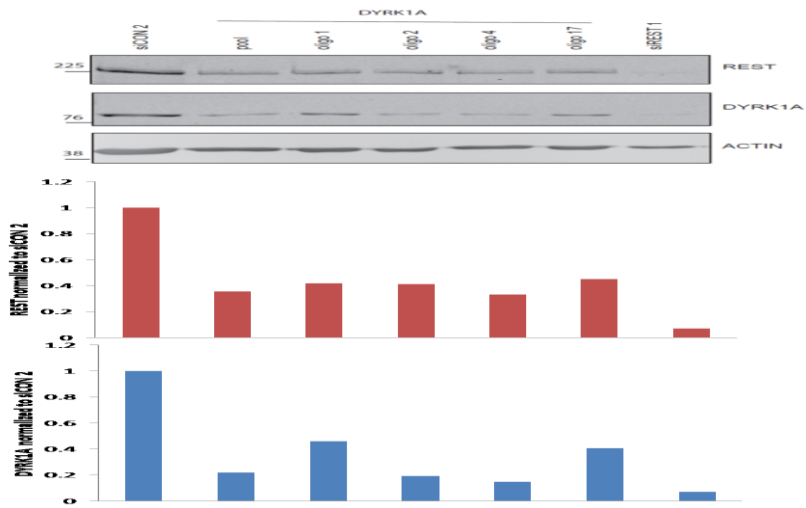

Fig 6. The effect of DYRK1A depletion and REST depletion on H460 cells. Immunoblot analysis of depletion of DYRK1A and REST proteins.To account for protein, actin protein was utilized as a control loading.Using Odyssey, the REST (red) and DYRK1A (blue) proteins were measured and normalized to actin.

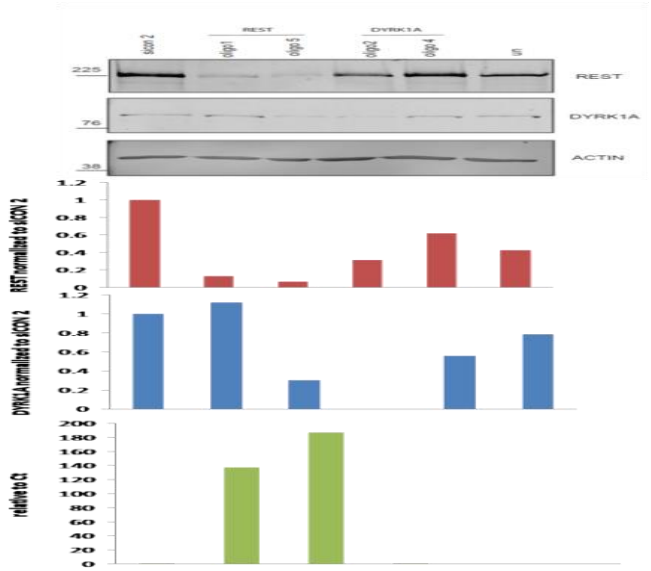

Fig 7. The effect of DYRK1A depletion (oligo2 and 4) and REST depletion (oligo1 and 5) in H460 cells. Immunoblot analysis of depletion of DYRK1A and REST proteins were tested. REST (red) and DYRK1A (blue) Odyssey was used to quantify the proteins and standardize the results to siCON 2. QPCR was used to detect the effect of this depletion on chromogranin B (green).

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Moreover, the functional effect of this depletion using QPCR was used to measure chromogranin B mRNA. It have found that there was a decrease of REST protein with both oligos for REST, but only oligo5 had an effect of DYRK1A protein. Only oligo2 of DYRK1A had a good knockdown and had some effect on REST and there was a response of CHGB to depletion of REST, and correspondingly oligo5 had the most effect (figure 7).

Because the depletion of DYRK1A was not very efficient or reproducible, we used an alternative approach. We used harmine, which was previously identified as inhibitor of DYRK1A (17). We first examined harmine's impact on cell viability, as it has been described as a cytotoxic agent in the past (18). Viability tests using H460 cells revealed that harmine exhibits negligible toxicity at concentrations as low as 10 µM. In contrast, harmine exhibited no toxicity at dosages up to 1 µM, but at 5 µM and 10 µM, toxicity increased, according to viability tests using A549 cells (figure 8).

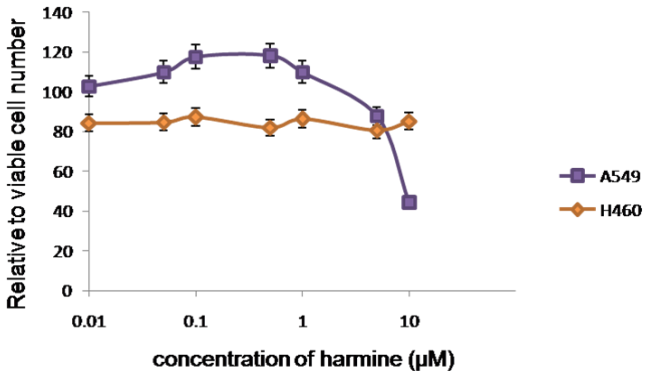

Fig 8. Dose dependence curve of harmine Cytotoxicity of harmine. A549 cells and H460 cells were incubated for 48 hours at different harmine concentrations (0.05, 0.01, 0.1, 0.5, 5, and 10 µM). The vitality of the cells was assessed using MTS assay and normalized to untreated cells. The graph shows the means of 6 replicates, Error bars shown std devition.

Based on the cytotoxiaty and quality IC50 of harmine for DYRK1A of 33 nM (17), we selected five concentrations of harmine 0.05, 0.1, 0.5, 1 and 5 µM to inhibit DYRK1A in A549 and H460 cell lines and tested the effect on REST protein levels and REST function. There was a decrease of REST when we inhibited A549 cells with harmine (figure 9A). Interestingly, there was an increase of chromogranin B transcript as a result of decrease REST level, however, this peaked at 0.1 µM and then declined. No decrease of REST was seen in H460 cell line (figure 9B) although; CHGB transcript was again induced by 0.1 µM harmine.

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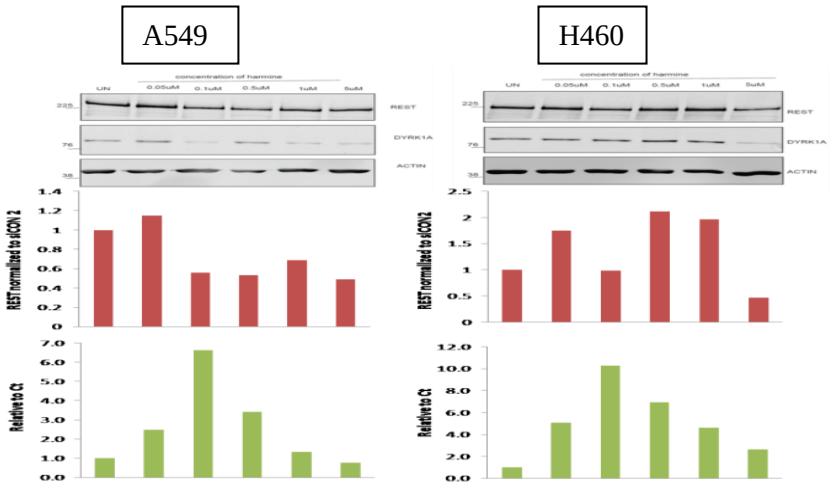

Fig 9. The effect of DYRK1A inhibitor hrmine on REST expression and function. (A) A549 cell line. (B) H460 cell line. Immunoblot study the effect of harmine at diverse concentration on DYRK1A and REST proteins. The quantification of the DYRK1A inhibition on REST protein (red) was normalized to siCON 2. The effect of harmine on the REST-restrictied target gene on chromogranin B (green) was relative to untreated cells.

Discussion

DYRK1A phosphorylates several transcription factors during the last few years such as NFAT and FOXO1 (14). Previous studies showed that DYRK1A perturbs REST levels in stem cell fate in DS (15). It has been shown that DYRK1B binding PCDH expressed in colon carcinoma but not in normal tissues (19). This study examined how DYRK1A affected REST in lung cancer. Expression of DYRK1A has been tested in normal and sub-types lung cancer. For the first time, we demonstrated that lung cancer had higher levels of DYRK1A than did healthy lung cell lines. Moreover, there was a good correlation between DYRK1A and REST proteins in normal lung cell lines. On the other hand, this correlation was lost in lung cancer cell lines. Therefore, we asked whether DYRK1A alter REST expression of function in lung cancer. Also, it was established the subcellular localization of DYRK1A in lung cancer comparing two cell lines A549 and H460. It has been found that DYRK1A protein was exclusively in the nuclear fraction in both cell lines. According to reports, DYRK1A is primarily a nuclear kinase, it was discovered that DYRK1A accumulated in the HEK293 and transfected COS-7 cells' nuclei (20**)** For instance, caspase 9 and DYRK1A were found to be co-localized in the nucleus, and in the nuclear compartment, DYRK1A phosphorylates caspase 9 (21).The co-localized of REST and DYRK1A in lung cancer cells suggests REST may be also be a target of this kinase and would be consistent with a role of DYRK1A in regulating REST transcription. Next, the effect of DYRK1A depletion on REST level in A549 or H460 cell lines (NSCLC) was tested. We could not see DYRK1A knockdown in A549, however, when we treated A549 cell line with harmine, there was 50% decline in REST protein levels. This may due to the small amount of DYRK1A that is expressed in A549 cell line. In contrast, there was some evidence of reduction of

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REST level when we successfully knockdown DYRK1A in H460 cell line. However, decline of REST level was not seen when we treated these cells with harmine. According to reports, DYRK1A overexpression alters REST levels for instance, in embryonic neuron REST level was decreased and increased REST expression in adult neurons (16). Moreover, there was decrease of DYRK1A protein when A549 cell line inhibited with harmine. This suggested that DYRK1A may regulate the transcription factor (REST) and possibly as DYRK1A has an RE1 motif to target REST. However, only we saw that in halve oligos so may be off target. Previously it has shown that DYRK1A interacts with SWI/SNF that interacts with REST (16). It has been demonstrated that blocking the DYRK1A gene in neurons lowers the level of REST (15). As well as the reduction of REST protein as a result of DYRK1A depletion in harmine, that identified as inhibitor for DYRK1A (20-21), the effect on REST was investigated by RT-PCR using chromogranin B as REST-target gene, which is known to respond rapidly to decreased REST level. It has been found that CHGB increased with decreased REST and was more responsive to siREST5 than siREST1. However, CHGB did not respond to DYRK1A depletion whereas it responded with harmine. This result can’not rule out the possibility that other kinase genes may have compensation role with DYRK1A and its effect on the REST levels function in lung cancer. This may suggest that DYRK1B also inhibited by harmconcentration. In order to uncover the involvement of DYRK1A at this influence that may have on the REST levels in malignancies, more research will be required. Finally, further work will be necessary to confirm these results. There are some suggestion to do that such as improve reproducibility and efficiency of DYRK1A knockdown and Investigate DYRK1B expression and regulation of REST as well as Compensation between DYRK1s by double-knockdown DYRK1A and DYRK1B and Titrate harmine response in H460 and Test DYRK1A on normal lung where there was correlation between DYRK1A and REST

Conclusions

It was discovered that normal lung cell lines showed a strong link between DYRK1A and REST protein, while lung cancer cell lines showed higher expression of DYRK1A. Nevertheless, in several NSCLC cell lines, this association disappeared. Additionally, DYRK1A removal decreased the H460 cell line's REST level. Additionally, A549's DYRK1A inhibition lowers REST levels. Lastly, CHGB reacted to DYRK1A depletion in H460 cell lines and DYRK1A inhibition in A549 cell lines.

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