Journal of Cell Communication and Signaling (2019) 13:281–289
https://doi.org/10.1007/s12079-019-00528-4

NUTS AND BOLTS

Chronic shisha exposure alters phosphoproteome of oral keratinocytes

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Received: 9 June 2019 / Accepted: 8 July 2019 / Published online: 19 July 2019

Abstract

Shisha smoking has been epidemiologically linked to oral cancer. However, few studies have investigated the pathobiology of shisha-induced cellular transformation. We studied the effects of chronic shisha exposure (8 months) in an in vitro model using immortalized, non-neoplastic oral keratinocytes (OKF6/TERT1). Quantitative proteomic and phosphoproteomic analyses were performed on OKF6/TERT1 cells treated with shisha extract for a period of 8 months. Pathway analysis was carried out to identify significantly enriched biological processes in shisha-treated cells. Chronic shisha exposure resulted in increased cell scattering phenomenon in OKF6/TERT1 cells. Data analysis revealed differential phosphorylation of 164 peptides (fold change ≥ 1.5, $p \leq 0.0.5$) corresponding to 136 proteins. Proteins associated with mTORC1 and EIF4F complexes involved in initiating protein translation were seen to be enriched upon shisha treatment. Network analysis also highlighted downregulation of proteins involved in Type I interferon signaling in shisha-treated cells. Quantitative phosphoproteomic approach elucidated global perturbations to the molecular milieu of oral keratinocytes upon shisha exposure. Further studies are needed to validate putative targets in oral cancer patients with shisha smoking history.

Keywords Waterpipe · Hookah · Narghile · Orbitrap fusion · High-throughput

Shankargouda Patil and Pavithra Rajagopalan contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12079-019-00528-4) contains supplementary material, which is available to authorized users.

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Introduction

Shisha or hookah smoking, also known as water pipe tobacco smoking, is known to be associated with serious health risks which may be similar to those posed by cigarette smoking. Use of waterpipes has been associated with various cancers including esophageal and oral cancer. (Dar 2015; Dar et al. 2012) Mainstream smoke from 'shisha' is known to contain nicotine, tar, heavy metals and other carcinogens including carbon monoxide, both from the tobacco mixture as well as from the charcoal that is used to burn the tobacco. (Shihadeh 2003) Nicotine and cotinine levels are higher in hookah smokers compared to cigarette users. (Shafagoj et al. 2002) The harmful components in hookah tobacco are multiple folds higher than in cigarette tobacco. (Shihadeh and Saleh 2005) Even the duration and method of smoking hookah results in an increased uptake of smoke per 40–45-min session of waterpipe smoking compared to an average of 5–10 min per cigarette. Hence, awareness on the potential health risks and understanding the pathobiology of diseases associated with shisha is important.

Majority of published literature on the correlation between 'hookah' smoking and oral cancer are epidemiological in nature primarily focused on Middle Eastern regions including Egypt, Jordan and Saudi Arabia. One study discusses the presentation of squamous cell carcinoma and keratoacanthoma in three patients with known history of 'Shisha' and 'Goza' smoking. (El-Hakim and Uthman 1999) A study on Jordanian youth reports a positive correlation between 'narghile' smoking and development of oral cancer at a younger age. (Al-Amad et al. 2014) A meta-analysis published on the health outcomes of waterpipe tobacco smoking (WTS) lists oral cancer as one of the adverse health outcomes seen to be significantly associated with WTS. (Waziry et al. 2017) To the best of our knowledge, there are no studies documenting molecular alterations in oral cells due to chronic shisha smoke exposure.

Phosphorylation is an important post translational mechanism regulating protein activity in cells. Perturbation in kinases resulting in aberrant phosphorylation of oncoproteins and tumor suppressor proteins is frequently reported in many cancers. (Laulajainen et al. 2011) High throughput techniques such as mass spectrometry-based phosphoproteomic analyses have aided in the identification of promising biomarkers in various cancers. A few studies have elucidated altered signaling and identified potential therapeutic targets in oral cancer. (Winck et al. 2014) High throughput studies have also been carried out to study tobacco smoke-mediated global signaling alterations. Previous studies on established lung cancer cell lines have shown that chronic exposure to cigarette smoke result in differential phosphorylation of several kinases such as PAK6, EPHA4 and LYN and subsequent alterations to their downstream signaling cascades. (Solanki et al. 2017) Another study on pancreatic cancer cell lines treated with tobacco components, showed altered phosphorylation in nuclear and transcriptional proteins upon treatment. (Paulo et al. 2015) Our team has previously investigated the detrimental effects of chronic tobacco smoke exposure in oral keratinocytes using a quantitative phosphoproteomic approach and identified protein kinase N2 (PKN2) as a key player in transforming oral cells upon smoke exposure. (Rajagopalan et al. 2018) These studies indicate that mass spectrometry-based quantitative phosphoproteomics is valuable to study signaling alterations in transformed cells as a consequence of xenobiotic insults.

In this study, we carried out phosphoproteomic and global proteomic analyses of non-neoplastic human oral keratinocytes (OKF6/TERT1) chronically treated with shisha extract. Using quantitative proteomics approach, we elucidated differentially regulated signaling events in shisha treated oral cells. This study serves as a useful resource to better understand how chronic exposure to shisha leads to global signaling alterations.

Materials and methods

Cell culture and adaptation of oral keratinocytes to shisha extract

Non-neoplastic human oral keratinocytes, OKF6/TERT1, used in this study were a kind gift from Dr. James Rheinwald (Brigham and Women's Hospital, Boston, MA). OKF6/TERT1 were grown and maintained in keratinocyte serum free medium (KSFM) (supplemented with bovine pituitary extract (25 μg/ml), epidermal growth factor (EGF) (0.2. ng/ml) (ThermoFisher Scientific, MA)), 1% penicillin/streptomycin and calcium chloride (0.4. mM). The cells were cultured at 37 °C in a humidified air incubator with 5% CO_2. Shisha extract was prepared and used to treat OKF6/TERT1 cells chronically for up to a period of 8 months as described previously. (Patil et al. 2019) OKF6/TERT1 cells exposed to shisha extract (0.5.%) for 8 months will be referred to as "OKF6/TERT1-Shisha" and untreated parental cells grown for the same duration will be referred to as OKF6/TERT1-Parental.

Colony formation assays

Colony formation assays were carried out as described previously. (Rajagopalan et al. 2018) Briefly, OKF6/TERT1-Parental and OKF6/TERT1-Shisha cells were seeded in triplicate at a density of 3×10^3/well in sterile 6-well plates and cells were monitored for 8 days. Cells were fixed with methanol, stained with 4% methylene blue and resulting cell

scattering phenomenon was visualized for ten randomly selected viewing fields. Representative images were photographed at 3x magnification. All experiments were performed in triplicate.

Sample preparation for mass spectrometry analysis

OKF6/TERT1-Parental and OKF6/TERT1-Shisha cells were grown to 80% confluence followed by serum starvation for 8 h. The cells were washed with 1X PBS thrice and harvested in lysis buffer (2% SDS, 5 mM sodium fluoride, 1 mM β-glycerophosphate, 1 mM sodium orthovanadate in 50 mM Triethyl ammonium bicarbonate (TEABC)). The cell lysates were sonicated, centrifuged and protein concentration was determined by BCA (Thermo Scientific, Bremen, Germany).

In-solution digestion and TMT labeling

In-solution digestion and TMT labeling of samples are described in detail in Supplementary Methods.

Basic reversed-phase liquid chromatography (bRPLC) and phosphopeptide enrichment using IMAC

TMT labelled peptides were pooled and subjected to basic pH reverse phase chromatography (bRPLC), as previously described. (Patil et al. 2019) The 96 fractions obtained were concatenated into 6 fractions. From the pooled fractions, one-tenth volume equivalent peptides were taken for total proteome analysis. The remaining samples were subjected to immobilized metal affinity chromatography (IMAC) based phosphopeptide enrichment. (Thingholm and Larsen 2016) The enriched samples were desalted and used for mass spectrometry analysis.

LC-MS/MS analysis and data analysis

Parameters for LC-MS/MS analysis and protein identification and quantification are described in Supplementary Methods.

Proteomic data access

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Vizcaino et al. 2016) partner repository with the dataset identifier PXD011318.

Bioinformatics analysis

Welch's t-test of all quantified proteins and phosphopeptides in OKF6/TERT1-Parental and OKF6/TERT1-Shisha cells was calculated using Perseus (Version 1.6.). (Tyanova et al. 2016) Heatmaps were generated by supervised hierarchical clustering method based on Euclidean distance and average linkage using Morpheus (version 3.0.2.0.6) software (https://software.broadinstitute.org/morpheus/). Interaction network analysis for dysregulated and differentially phosphorylated proteins was performed using STRING v10. (Szklarczyk et al. 2015) Pathway analysis was performed using DAVID Bioinformatics Resources 6.8. (da Huang et al. 2009).

Results

We developed a cellular model mimicking chronic shisha smoke exposure (8 months) using immortalized, non-neoplastic human oral keratinocytes. The treated cells were then employed to understand cellular and molecular alterations associated with chronic exposure to shisha extract.

Chronic treatment with shisha extract induces cellular transformation in oral keratinocytes

In a previous study, we evaluated the phenotypic changes in OKF6/TERT1-Shisha cells compared to OKF6/TERT1-Parental cells. (Patil et al. 2019) Chronic treatment with shisha extract resulted in increased proliferative rates in OKF6/TERT1 cells. In addition, the cells showed increased invasiveness. Increased cell scattering is a phenomenon associated with cancer cell-like phenotype. In conjunction with previous data, we observed a drastic increase in cell scattering ability of OKF6/TERT1 cells chronically treated with 0.5% shisha extract for 8 months (Fig. 1a).

Phenotypic alterations in chronic in vitro exposure models are usually associated with corresponding molecular changes. Hence, we investigated global phosphoproteomic alterations in OKF6/TERT1 cells chronically treated with shisha extract for duration of 8 months.

Shisha exposure results in global proteomic and phosphoproteomic changes in oral keratinocytes

We employed quantitative Tandem Mass Tag (TMT)-based proteomic and phosphoproteomic approaches to elucidate molecular alterations induced by shisha in oral keratinocytes. Proteomic analysis resulted in quantification of 4,755 proteins of which 86 proteins were differentially expressed with a fold change of 1.5. (p value <0.05). Quantitative phosphoproteomic analysis resulted in identification and quantitation of 4,621 phosphosites corresponding to 2,042 proteins. Employing a 1.5-fold cut-off (p value ≤ 0.05), we identified 127 hyperphosphorylated and 37 hypophosphorylated phosphopeptides corresponding to 136 proteins. Amongst hyperphosphorylated proteins, 7 hyperphosphorylated (≥ 1.5 fold) kinases were identified including protein kinase C delta (PRKCD).

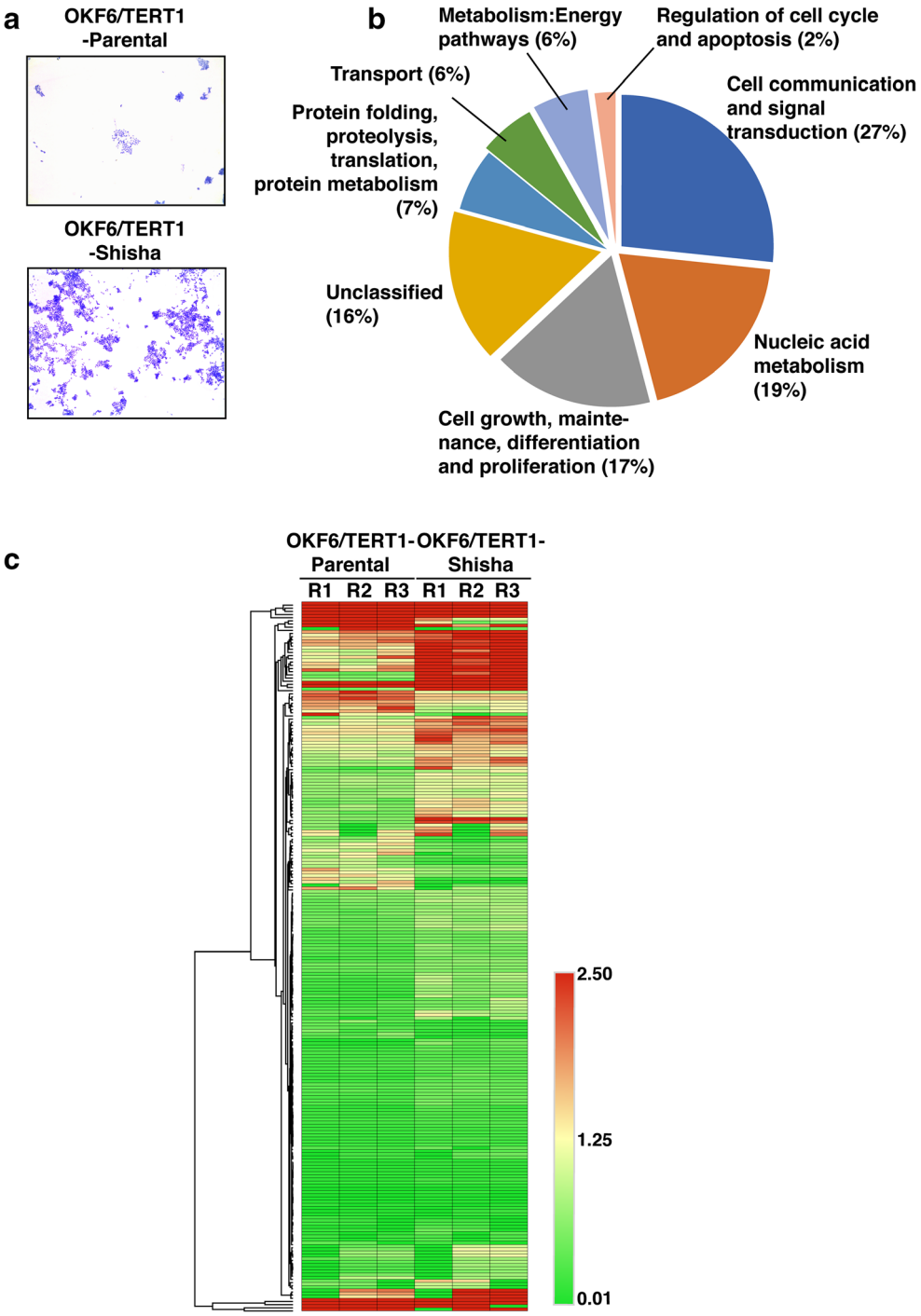

Fig. 1 Chronic shisha treatment alters non-neoplastic, non-transformed oral keratinocytes and induces global phosphoproteomic changes. **a.** Colony formation assay of shisha-treated and parental cells visualized (3X magnification) after staining with methylene blue. **b.** Gene Ontology-based functional annotation of differentially phosphorylated proteins (≥1.5 fold) in OKF6/TERT1-Shisha cells. All depicted annotation terms were obtained from Human Protein Reference Database (http://www.hprd.org). **c.** Heat map depicting the expression pattern of phosphopeptides quantified ($p < 0.05$) in all three replicates of OKF6/TERT1-Parental and OKF6/TERT1-Shisha cells

Chronic shisha exposure enriches for unique cellular processes in oral keratinocytes

Biological process-based enrichment analysis was performed for differentially phosphorylated proteins in OKF6/TERT1-Shisha cells using HPRD (www.hprd.org). As seen in Fig. 1b, most dysregulated proteins are involved in cell communication and signal transduction (27%), followed by nucleic acid metabolism (19%) and cell growth, maintenance, differentiation and proliferation (17%). Bioinformatics analysis of phosphoproteomic data for OKF6/TERT1-Shisha cells compared to parental cells highlighted distinct patterns of expression upon shisha treatment (Fig. 1c). Network analysis of proteins dysregulated and/or differentially phosphorylated in OKF6/TERT1-Shisha compared to parental cells revealed enrichment of unique cellular processes, including cell movement and migration, interferon response and translation initiation,

amongst others (Fig. 2). Activation of specific cellular processes such as cell movement and cell migration upon shisha treatment is in concordance with cellular assays where we identified increased cell scattering in shisha treated cells.

We observed overexpression and/or hyperphosphorylation of a number of proteins known to be associated with regulation of actin cytoskeleton and cell motility (Fig. 3a). Filamin B (FLNB) is a member of a family of actin cross-linking proteins which was seen to be overexpressed (1.8 fold) in shisha treated cells compared to parental cells. Myosin regulatory light chain 12A (MYL12A) hyperphosphorylation at one of the di-phosphorylation sites, serine 25 (2.9 fold) was observed in our data. This site is known to regulate its activity. (Rovner et al. 1995).

We also identified hyperphosphorylation of integrin subunit beta 4 (ITGB4) at threonine-1832 (3.5 fold) which is associated with hemidesmosomal disassembly. (Frijns et al. 2012) PRKCD-mediated serine phosphorylation of integrin beta 4 has been linked to decreased keratinocyte attachment to underlying matrix. (Alt et al. 2001) Although we did not identify serine phosphorylation sites for ITGB4 in our data, we observed hyperphosphorylation of PRKCD at multiple sites including, serine-320 (2.3 fold), tyrosine-329 (2.3 fold) and serine-674 (5.1 fold). We also observed a 1.5-fold hyperphosphorylation of beta-catenin (CTNNB1) at serine-552 upon chronic shisha treatment in oral keratinocytes. Phosphorylation at this site is mediated by Akt and is known to promote tumor cell invasiveness in A431 human epidermoid carcinoma cells. (Fang et al. 2007) Overall, our data suggests an increase in cell motility-associated processes in shisha treated oral keratinocytes.

Interestingly, a number of proteins associated with interferon signaling and response were seen to be down-regulated in our study (Fig. 2). Inflammatory response-

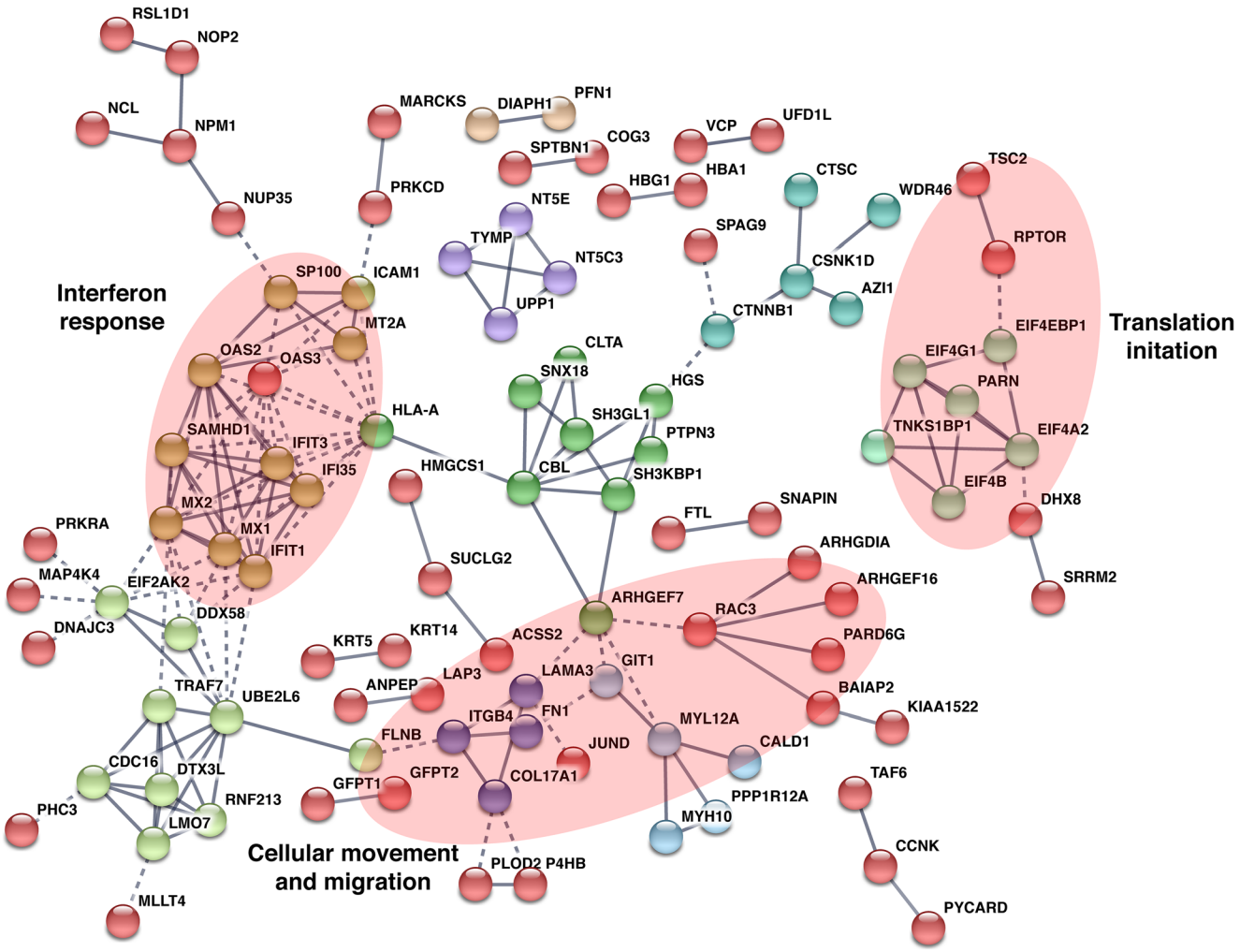

Fig. 2 Biological processes affected in response to chronic shisha exposure in oral keratinocytes. STRING v10 was used for analysis of enriched networks in oral cells chronically treated with shisha extract compared to untreated parental cells

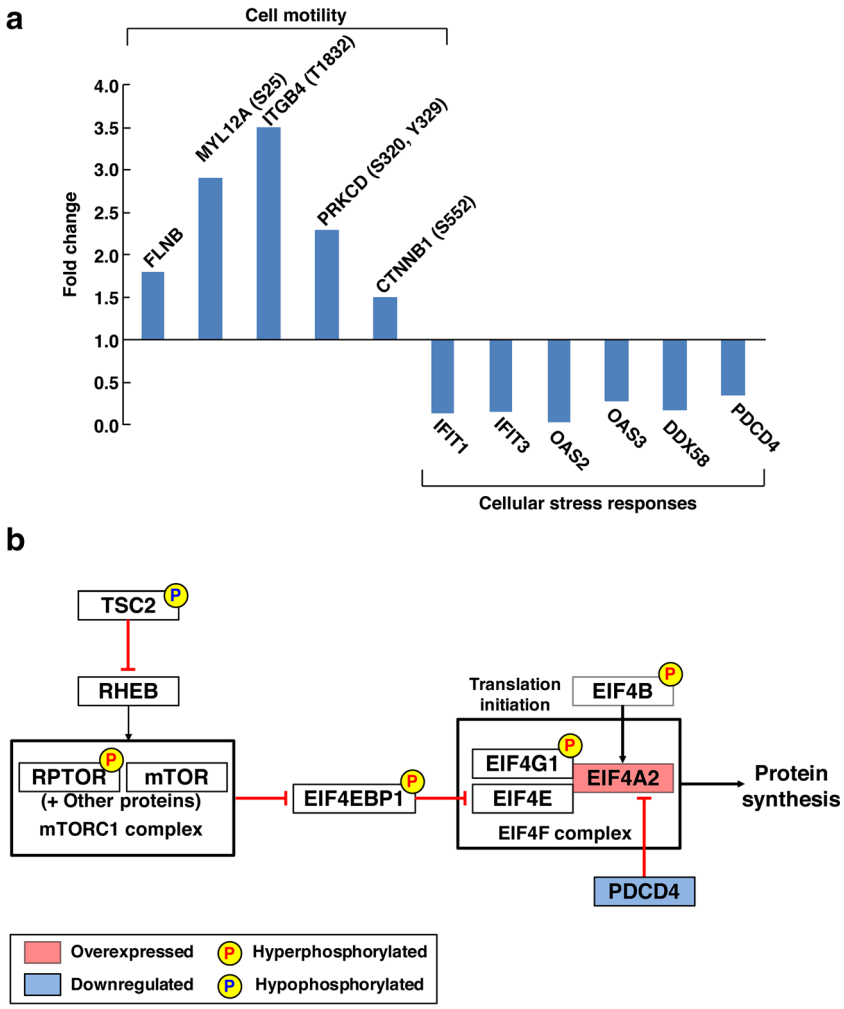

Fig. 3 Proteins involved in cellular motility, cellular stress response and protein synthesis are dysregulated upon chronic shisha exposure. **a.** Histogram depicting fold change levels of dysregulated proteins or differentially phosphorylated sites **b.** Proteins associated with mTOR signaling axis and their expression and phosphorylation levels

associated proteins such as interferon-induced proteins with tetratricopeptide repeats 1 and 3(IFIT1, IFIT3) were found to be downregulated in shisha treated cells. Chronic shisha treatment also resulted in downregulation of proteins associated with interferon signaling such as DExD/H-box helicase 58 (DDX58) and members of the 20–50-oligoadenylate synthetase family including OAS2 and OAS3.

In addition, we observed downregulation of proteins associated with cellular response to stress such as eukaryotic translation initiation factor 2 alpha kinase 2 EIF2AK2 (0.3 fold). EIF4AK2 or PERK expression is essential for phosphorylation and subsequent nuclear translocation of NRF2, a key player in cellular stress response. NRF2 translocates to the nucleus and activates transcription of antioxidant response element (ARE) containing genes. As a consequence of chronic shisha treatment, we observe downregulation of a number of ARE-related genes including NT5E, MT2A, FTL, LCP1 and MYH10. Chronic shisha exposure may therefore adversely affect cellular stress responses in oral keratinocytes resulting in cellular transformation.

Chronic shisha exposure alters signaling processes associated with mTORC1 and translation initiation in oral keratinocytes

We performed pathway analysis to investigate signaling pathways altered upon chronic shisha exposure in oral keratinocytes. The pathway with the highest fold enrichment in shisha treated cells was the mTOR signaling pathway (Fig. 3b). We identified differential phosphorylation of a number of key players involved in the formation and regulation of the mTORC1 complex. TSC complex subunit 2 (TSC2) is a known tumor suppressor gene which controls the mTORC1 signaling axis by inhibiting the protein Ras homolog, mTORC1 binding (RHEB). We observed a significant hypophosphorylation (0.3 fold) at serine-1483 in TSC2 which is a site known to control mTOR signaling. (Jacobs et al. 2017) Downstream of TSC2, we observed increased phosphorylation at the activation sites (S859 and S863; 1.86-fold) of regulatory associated protein of MTOR complex 1 (RPTOR) protein which directly interacts with mTOR to form the mTORC1 complex. This active mTORC1 complex is known to

phosphorylate the eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1) at threonine-70 and thereby inhibit its activity. (Chen et al. 2015) In conjunction with previous literature, we observe an increased phosphorylation at the inhibitory T70 site (1.7 fold) for EIF4EBP1 in our data.

EIF4EBP1 or 4E-BP1, is known to inhibit eukaryotic translation initiation factor 4E (EIF4E) which is a component of the multimeric EIF4F complex involved in translation initiation. We identified hyperphosphorylation of some of the other components of EIF4F complex including eukaryotic translation initiation factor 4B (EIF4B) (S93, S450; >2 fold) and eukaryotic translation initiation factor 4 gamma 1(EIF4G1) (S1084; 2.8-fold). In addition, overexpression of eukaryotic translation initiation factor 4A2 (EIF4A2) by 1.9. fold was observed in shisha treated cells. This is in concordance with our observation of increased phosphorylation of eukaryotic translation initiation factor 4B (EIF4B) at S93 which promotes activity of EIF4A2 and overall reduced expression (0.3 fold) of programmed cell death 4 protein (PDCD4). Figure 3b depicts the regulation of the translation initiation machinery by the mTOR complex, highlighting the possible adverse effects of shisha exposure on cellular translational machinery and increased protein synthesis in transformed cells.

Discussion

The majority of literature that link shisha and development of precancerous lesions or oral cancer are epidemiological in nature. There are few molecular studies that investigate the adverse effects of shisha exposure in oral cells. Chronic rather than acute exposure to carcinogens in vitro best mimics long-term exposure effects seen in patients. We therefore developed a cellular model to investigate the effect of chronic shisha exposure in oral cells using immortalized, non-neoplastic oral keratinocytes.

High-throughput approaches are unique tools to address global signaling alterations occurring upon carcinogen exposure. We employed TMT-based proteomic and phosphoproteomic analyses and identified signaling changes associated with cellular transformation in shisha-treated oral keratinocytes. Proteins involved in cytoskeletal reorganization and cell motility such as FLNB, MYL12A, ITGB4 and CTNNB1 were dysregulated upon chronic shisha exposure. Filamin B is known to enhance cancer cell invasiveness through diphosphorylation of myosin regulatory light chain 12A (MYL12A) and phosphorylation of focal adhesion kinase (FAK). (Iguchi et al. 2015) Active MYL12A is also known to induce cell spreading and migration in breast cancer cell lines. (Betapudi et al. 2006) Disassembly of hemidesmosomes involving integrin alpha 6 beta 4 has been previously shown to occur transiently in keratinocytes during carcinoma invasion. (Walko et al. 2015).

We also identified downregulation in interferon signaling proteins, including IFIT1, IFIT3, OAS2, OAS3 and DDX58. Interferon-stimulated genes (ISGs) can be induced by many cellular stresses and IFN signaling is implicated in suppression of oncogenic transformation. Downregulation of proteins involved in IFN signaling in shisha treated cells highlights the possibility that prolonged shisha exposure transforms oral keratinocytes.

Pathway analysis identified activation of pathways such as mTOR signaling pathway, epithelial cell invasion, actin cytoskeletal reorganization and focal adhesion and tight junction in shisha exposed cells compared to parental cells. We observed hyper or hypophosphorylation of a number of key players involved in the formation and regulation of the mTORC1 complex and translation initiation such as TSC2, RPTOR, EIF4EBP, components of EIF4F complex and PDCD4. Interestingly, PDCD4 is known to have tumor suppressor activity and inhibits the helicase activity of EIF4A2, thereby inhibiting protein synthesis. (Yang et al. 2003) EIF4F complex initiates translation at 5′ end of mRNA, especially those with highly structured 5'UTR sequences. This sets the stage for "translation discrimination" as mRNA coding for oncogenes and other proteins important for growth and proliferation possess large putative highly structured 5'UTRs. (Gingras et al. 2001).

To the best of our knowledge, this is the first study of its kind that employs quantitative phosphoproteomics to investigate effects of chronic shisha exposure in oral cells. The observations discussed here can serve as a reference for future clinical studies in oral cancer patients with a history of shisha smoking.

General awareness on the ill-effects of waterpipe tobacco or shisha smoking is low. Common perception amongst users is that it is less harmful than cigarette smoke. This is believed to be due to the 'filtration' of the harmful compounds of the product by the water through which the smoke is bubbled. (Patil et al. 2019) Our data reveals global molecular alterations upon chronic exposure to aqueous extract of shisha (0.5%) over a period of 8 months. We have previously observed significant phenotypic changes in transformed oral keratinocytes chronically treated with shisha extract. (Patil et al. 2019) Overall, our phosphoproteomic and proteomic data highlight distinct adverse effects of chronic shisha exposure in an in vitro model using non-neoplastic oral keratinocytes. We observed dysregulation and differential phosphorylation of proteins involved in key cellular processes including cell

movement, cellular stress response and protein translation. A number of these proteins and signaling cascades are known to be altered in oncogenic transformation in various cancers.

Our study highlights the importance of employing high throughput quantitative approaches to investigate the adverse effects of long-term exposure to xenobiotic stresses such as those developed from shisha smoking. Further functional studies are essential to better elucidate the key molecular players involved in shisha smoke-associated oncogenic transformation.

Acknowledgements We thank the Department of Biotechnology (DBT), Government of India for research support to the Institute of Bioinformatics. IOB is supported by DBT Program Support on Neuroproteomics and infrastructure for proteomic data analysis (BT/01/COE/08/05). Krishna Patel and Niraj Babu are recipients of Senior Research Fellowships from the Council for Scientific and Industrial Research (CSIR), Government of India. We thank Dr. Anita Mahadevan of National Institute of Mental Health and Neurosciences (NIMHANS) for providing use of microscope facility.

Author's contributions AC, HG and SP participated in study conception and study design. PR, TS, NB and HS were involved in cell culture and performed all assays and experiments. SVM and GS carried out fractionation and mass spectrometric analysis of samples. PR and KP prepared the manuscript and manuscript figures. PR, KP and JA were involved in data analyses and interpretation. AC, HG, SP, DS, SB and MF edited, critically read and revised the manuscript. All the authors have read and approved the final manuscript.

Compliance with ethical standards

Approved by ethics committee/institute Not applicable.

Conflict of interest The authors declare that there are no conflicts of interest.

References

Al-Amad SH, Awad MA, Nimri O (2014) Oral cancer in young Jordanians: potential association with frequency of narghile smoking. Oral Surg Oral Med Oral Pathol Oral Radiol 118(5): 560–565

Alt A, Ohba M, Li L, Gartsbein M, Belanger A, Denning MF, Kuroki T, Yuspa SH, Tennenbaum T (2001) Protein kinase Cdelta-mediated phosphorylation of alpha6beta4 is associated with reduced integrin localization to the hemidesmosome and decreased keratinocyte attachment. Cancer Res 61(11):4591–4598

Betapudi V, Licate LS, Egelhoff TT (2006) Distinct roles of nonmuscle myosin II isoforms in the regulation of MDA-MB-231 breast cancer cell spreading and migration. Cancer Res 66(9):4725–4733

Chen L, Xu B, Liu L, Liu C, Luo Y, Chen X, Barzegar M, Chung J, Huang S (2015) Both mTORC1 and mTORC2 are involved in the regulation of cell adhesion. Oncotarget 6(9):7136–7150

da Huang W, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4(1):44–57

Dar NA (2015) Narghile smoking is associated with the development of Oral Cancer at early age. J Evid Based Dent Pract 15(3):126–127

Dar NA, Bhat GA, Shah IA, Iqbal B, Makhdoomi MA, Nisar I, Rafiq R, Iqbal ST, Bhat AB, Nabi S, Shah SA, Shafi R, Masood A, Lone MM, Zargar SA, Najar MS, Islami F, Boffetta P (2012) Hookah smoking, nass chewing, and oesophageal squamous cell carcinoma in Kashmir, India. Br J Cancer 107(9):1618–1623

El-Hakim IE, Uthman MA (1999) Squamous cell carcinoma and keratoacanthoma of the lower lip associated with "Goza" and "Shisha" smoking. Int J Dermatol 38(2):108–110

Fang D, Hawke D, Zheng Y, Xia Y, Meisenhelder J, Nika H, Mills GB, Kobayashi R, Hunter T, Lu Z (2007) Phosphorylation of beta-catenin by AKT promotes beta-catenin transcriptional activity. J Biol Chem 282(15):11221–11229

Frijns E, Kuikman I, Litjens S, Raspe M, Jalink K, Ports M, Wilhelmsen K, Sonnenberg A (2012) Phosphorylation of threonine 1736 in the C-terminal tail of integrin beta4 contributes to hemidesmosome disassembly. Mol Biol Cell 23(8):1475–1485

Gingras AC, Raught B, Sonenberg N (2001) Regulation of translation initiation by FRAP/mTOR. Genes Dev 15(7):807–826

Iguchi Y, Ishihara S, Uchida Y, Tajima K, Mizutani T, Kawabata K, Haga H (2015) Filamin B enhances the invasiveness of Cancer cells into 3D collagen matrices. Cell Struct Funct 40(2):61–67

Jacobs BL, McNally RM, Kim KJ, Blanco R, Privett RE, You JS, Hornberger TA (2017) Identification of mechanically regulated phosphorylation sites on tuberin (TSC2) that control mechanistic target of rapamycin (mTOR) signaling. J Biol Chem 292(17): 6987–6997

Laulajainen M, Muranen T, Nyman TA, Carpen O, Gronholm M (2011) Multistep phosphorylation by oncogenic kinases enhances the degradation of the NF2 tumor suppressor merlin. Neoplasia 13(7):643–652

Patil S, Subbannayya T, Mohan SV, Babu N, Advani J, Sathe G, Rajagopalan P, Patel K, Bhandi S, Solanki H, Sidransky D, Gowda H, Chatterjee A, Ferrari M (2019) Proteomic changes in oral keratinocytes chronically exposed to shisha (water pipe). OMICS 23(2):86–97. https://doi.org/10.1089/omi.2018.0173.

Paulo JA, Gaun A, Gygi SP (2015) Global analysis of protein expression and phosphorylation levels in nicotine-treated pancreatic stellate cells. J Proteome Res 14(10):4246–4256

Rajagopalan P, Nanjappa V, Patel K, Jain AP, Mangalaparthi KK, Patil AH, Nair B, Mathur PP, Keshava Prasad TS, Califano JA, Sidransky D, Gowda H, Chatterjee A (2018) Role of protein kinase N2 (PKN2) in cigarette smoke-mediated oncogenic transformation of oral cells. J Cell Commun Signal 12(4):709–721

Rovner AS, Freyzon Y, Trybus KM (1995) Chimeric substitutions of the actin-binding loop activate dephosphorylated but not phosphorylated smooth muscle heavy meromyosin. J Biol Chem 270(51):30260–30263

Shafagoj YA, Mohammed FI, Hadidi KA (2002) Hubble-bubble (water pipe) smoking: levels of nicotine and cotinine in plasma, saliva and urine. Int J Clin Pharmacol Ther 40(6):249–255

Shihadeh A (2003) Investigation of mainstream smoke aerosol of the argileh water pipe. Food Chem Toxicol 41(1):143–152

Shihadeh A, Saleh R (2005) Polycyclic aromatic hydrocarbons, carbon monoxide, "tar", and nicotine in the mainstream smoke aerosol of the narghile water pipe. Food Chem Toxicol 43(5):655–661

Solanki HS, Advani J, Khan AA, Radhakrishnan A, Sahasrabuddhe NA, Pinto SM, Chang X, Prasad TSK, Mathur PP, Sidransky D, Gowda H, Chatterjee A (2017) Chronic cigarette smoke mediated global changes in lung Mucoepidermoid cells: a Phosphoproteomic analysis. OMICS 21(8):474–487

Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ, von Mering C (2015) STRING v10: protein-

protein interaction networks, integrated over the tree of life. Nucleic Acids Res 43(Database issue):D447–D452

Thingholm TE, Larsen MR (2016) Phosphopeptide enrichment by immobilized metal affinity chromatography. Methods Mol Biol 1355:123–133

Tyanova S, Temu T, Sinitcyn P, Carlson A, Hein MY, Geiger T, Mann M, Cox J (2016) The Perseus computational platform for comprehensive analysis of (prote)omics data. Nat Methods 13(9):731–740

Vizcaino JA, Csordas A, del Toro N, Dianes JA, Griss J, Lavidas I, Mayer G, Perez-Riverol Y, Reisinger F, Ternent T, Xu QW, Wang R, Hermjakob H (2016) 2016 update of the PRIDE database and its related tools. Nucleic Acids Res 44(D1):D447–D456

Walko G, Castanon MJ, Wiche G (2015) Molecular architecture and function of the hemidesmosome. Cell Tissue Res 360(2):363–378

Waziry R, Jawad M, Ballout RA, Al Akel M, Akl EA (2017) The effects of waterpipe tobacco smoking on health outcomes: an updated systematic review and meta-analysis. Int J Epidemiol 46(1):32–43

Winck FV, Belloni M, Pauletti BA, Zanella Jde L, Domingues RR, Sherman NE, Paes Leme AF (2014) Phosphoproteome analysis reveals differences in phosphosite profiles between tumorigenic and non-tumorigenic epithelial cells. J Proteomics 96:67–81

Yang HS, Jansen AP, Komar AA, Zheng X, Merrick WC, Costes S, Lockett SJ, Sonenberg N, Colburn NH (2003) The transformation suppressor Pdcd4 is a novel eukaryotic translation initiation factor 4A binding protein that inhibits translation. Mol Cell Biol 23(1):26–37

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