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RESEARCH ARTICLE

Hypoxia-mediated Up-regulation of Metastatic Genes in Human Tongue Oral Squamous Cell Carcinoma: [Part I] Urokinase Plasminogen Activator [uPA]

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Abstract

Background: Most human tumors develops regions of chronically or transient hypoxic cells during growth. The effect of hypoxia on tumor tissue has a strong impact on tumor cell migration and metastatic behavior. uPA a major member plasminogen activator system that is involved at different stages of metastasis. **Aim:** To compare uPA expression in three oral tongue squamous cell carcinoma (OTSCC) and normal human oral keratinocytes (NHOK) cell lines and study the effect of hypoxia on that expression. **Methods:** Three Oral Tongue Squamous Cell Carcinoma (OTSCC) and three Normal Human Oral Keratinocytes (NHOK) cell lines were analyzed under normoxia and hypoxia for uPA mRNA concentration with northern blot analysis and uPA protein expression with western blot analysis, the expressed protein was further examined for active component with fibrin overlay zymography, and immunocytochemistry were utilized to confirm uPA expression. **Results:** uPA was expressed under normoxia condition in all three OTSCC and NHOK cell lines with much higher basal level in tongue cancer. Hypoxia highly upregulated uPA mRNA expression in OTSCC compared to NHOK (P= 0.00012) and uPA protein as well (P= 0.029), and active uPA was also upregulated (P= 0.008). **Conclusion:** uPA genetic expression is an important regulator for metastatic behavior and invasiveness of oral tongue squamous cell carcinoma (OTSCC), and hypoxia plays an essential role for its upregulation.

Introduction:

Most human tumors develops regions of chronically or transient hypoxic cells during growth [(1)]. The effect of hypoxia on tumor tissue, not only counts for tissue necrosis but also a strong impact on tumor cell biology [(2)].

Hypoxic tumor regions may show increased expression of many genes because of hypoxia-induced activation of DNA transcription factors [(3)].

Hypoxia may also lead to increased gene expression in tumor tissue by inducing amplification, rearrangements, translocations, and genomic instability [(3-6)]. Many hypoxia-inducible genes are controlled by a common transcription factor, HIF-1, composed of two subunits, HIF-1α and HIF-1β [(7)]. The post-transcriptional hypoxia-mediated control of the expressed genes results from increased transcription of HIF-1α and HIF-1β and decreased HIF-1α protein degradation in the proteome of hypoxic cells [(8-10)]. We have previously shown in previous experiment that hypoxia up-regulated both HIF-1α and VEGF in human oral tongue (OTSCC) cell lines, and the up-regulation of HIF-1α correlated with that of VEGF expression [(11)].

Several of the gene products that are induced or up-regulated under hypoxic conditions may play an important role not only through induction of VEGF but also through induction of proteolytic enzymes [4, 12]. Therefore, it has been suggested that hypoxia may promote the development of metastatic disease in human cancer [5, 6].

Interestingly, even after VEGF production and neovascularization oxygen supply generally stays beyond tumor demands, and thus, hypoxia remains a constant feature of these tumors [12, 13].

The oral cancer invasion process and metastasis requires the extensive production and braking of cell-cell and cell/ECM contacts as well as the degradation of ECM which represents the physical barrier in the direction of migration/invasion that is crucial in order for the tumors to degrade ECM proteins and invade the surrounding normal tissue [14].

The matrix degradation is catalyzed by several proteases, expressed by the cells, including PA system and matrix metalloproteinases (MMP) such as collagenases and stromelysins [15, 16]. The activity of these proteases is localized and regulated by their interactions with ECM components and a variety of specific inhibitors and cell surface receptors [14].

The PA system is a cascade reaction, leading to the generation of a broad-spectrum proteinase plasmin, which is able to degrade many extracellular proteins, either directly or through activation of other matrix-degrading proteases including members of MMP gene family [17, 18].

Plasmin is generated from its abundant proenzyme plasminogen by plasminogen activators, urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA), both of which are serine proteases [19]. uPA is thought to be more involved in tissue remodeling while tPA in vascular fibrinolysis due to its fibrin binding capacity [20, 21].

The proteolytic activity of uPA is associated with cell migration, angiogenesis and tissue degradation in both normal and pathological conditions including cancer invasion and is tightly regulated by proteolytic cleavage [22, 23]. uPA is secreted from cells in a virtually inactive single chain, pro-uPA, which upon proteolytic cleavage is converted into its 2-chain enzymatically active form [24].

A specific glycolipid-anchord receptor, uPAR, localizes both pro-uPA and uPA and plasminogen is strongly enhanced. This likely enables tumor cells to migrate through tissue barriers [24, 25].

Some tumors, including breast carcinoma, and malignant melanoma, pancreatic and liver cancer, have been shown to up-regulate uPA and uPAR when exposed to hypoxia in vitro [26, 27]. The hypoxia-induced up-regulation of uPAR shown to enhance concentration of uPAR on the cell surface, elevated levels of cell-associated uPA, and increased invasiveness [27]. Modifying the signaling through uPAR by the hypoxia-responsive expression of Low density lipoprotein receptor-related protein/alpha 2 (LRP/a2) macroglobulin receptor can alter the invasive activity [28, 29], whereas the blocking uPAR signaling with an engineered construct can suppress tumorgenicity in vivo [28].

Beside uPA and uPAR, plasminogen activator inhibitors appear to play an important role in the biology of the uPA system [30]. Inhibitory regulatory steps are required to prevent the reaction from getting out of control, most notably by inactivation of uPA by inhibitors [31]. These inhibitors include four proteins known by their inhibitory activity toward uPA: plasminogen activator inhibitors (PAIs)-1, -2 and -3 and a protein called nexin (PN1), inhibit uPA after endocytosis of uPA/uPAR complexes [12, 32-33].

Most relevant in the metastatic process is PAI-1, which exists in three different forms: nonactive-latent, cleaved, and the active form [34]. The active form of PAI-1, forms 1:1 complexes, specifically with the active (two chain) forms of plasminogen activators [35].

Materials and Methods:

Cell Lines and Culture Conditions

All experiments were performed using established cell lines of human oral tongue squamous carcinoma cells (SCCs: SCC-4,-9, and Cal 33) obtained from American Type Culture Collection (ATCC; Manassas, VA). For these studies, cells were cultured in a 1:1 mixture of Ham's F12 and Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS), 100 units of penicillin, 100 Ag/ml streptomycin, and 0.4 Ag/ml hydrocortisone (Sigma, St. Louis, MO) at 37°C in a 5% CO_2 air atmosphere (normoxia). Cells were subcultured by disaggregation with trypsin (0.1%)–EDTA (0.01%) in phosphate-buffered saline (PBS) at pH 7.5. For control cells, three cell lines of primary normal human oral keratinocytes (NHOK) were isolated from oral mucosa (Gingiva, Tongue, and Palate). Tissues were kindly provided by the Oral and Maxillofacial Surgery Department, as part of therapeutic procedures in accordance with Institutional Review Board approved protocol. Normal human keratinocytes were cultured in keratinocyte growth medium (KGM) containing a low level (0.15 mM) of Ca_2^+ and supplementary growth factor bullet kit (Clonetics, San Diego, CA) (Kang et al., 2000) [36]. In some experiments, serum-free medium was added to the cells for up to 24 h to produce conditioned medium. Cells were grown at

37°C under normoxic condition (20% O_2, 5% CO_2) and environmental hypoxic conditions (2%) were achieved in an airtight humidified chamber and continuously flushed with a gas mixture containing 5% CO_2 and 95% N_2. Maintenance of the desired O_2 concentration was constantly monitored during incubation using a microprocessor-based oxygen controller (Coy Laboratory Products, Inc.).

RNA Extraction and Northern Blot Analysis

mRNA was isolated from cells previously exposed to normoxic or hypoxic conditions for 24 h, and extracted according to the method described by (Chomczynski and Saachi, 1987) [37]. Briefly, 1 x 10^6 cells were mixed with 0.5 ml RNA extraction solution (RES) containing phenol–guanidine–isothiocyanate, and RNA was separated from DNA and proteins by adding 200 Al chloroform and centrifuged at 12,000 x g for 30 min at 4ºC; the aqueous phase was recovered and RNA was precipitated with equal volume of isopropanol and incubated for 2 h at 4ºC. Total RNA was precipitated with ethanol and quantitated by ultraviolet (260nm) spectrophotometry (BioSpec 1601, Shimadzu Scientific Instruments, Columbia, MD). Fifteen micrograms of total RNA was separated on a 1.4% agarose–2% formaldehyde gels and transferred by capillary blotting to nitrocellulose membrane (Hybond-N, Amersham Pharmacia Biotechnology, Piscataway, NJ). The blots were then prehybridized, hybridized, and washed under appropriate conditions for cDNA probes. After overnight hybridization at 42ºC with the labeled cDNA probe with specific activity of > 1 x 10^5 cpm/ml, membranes were washed and exposed to an intensifying screen in cassette (Kodak, Rochester, NY) for 24 h and analyzed with a phosphorimaging scanner (ImageQuant, Molecular Dynamics, Sunnyvale, CA). Images were quantified using ImageQuant analysis software (Molecular Dynamics); a 28S ribosomal RNA was used for normalization.

Probe synthesis

cDNA fragment complementary to human uPA specific primers based on the published human cDNA sequences were utilized. For amplification of the human uPA gene, oligonucleotide primers were, sense 5´-GGCAGCAATGAACTTCATCAAGTTCC-3´ and antisense 5´-TATTTCACAGTGCTGCCCTCCG-3´, this primer was synthesized from mRNA and amplified using reverse transcription polymerase chain reaction (RT-PCR) according to manufacturer's protocol (Life Technologies). Both fragments were subcloned into pGEM-T Easy Vector System (Promega, Madison, WI), labeled with α32P-dCTP using the Rediprime™ II Random Prime DNA labeling System (Amersham Biosciences, Little Chalfont Buckinghamshire, UK), and purified through Sephadex G-50 Quick Spin columns (TE; Roche Diagnostics Corp., Indianapolis, IN).

Protein Extraction and Western Blot Analysis

To determine protein expression of uPA, cells were grown to 80% confluence on 100 x 20 mm polystyrene tissue culture plates (Fisher, Irvine, CA). Fresh media were added and cells were incubated in normoxic and hypoxic conditions at 37ºC for 24 h. At time of harvest, cell count was approximately 1.0 x 10^6 cells / plate. Cells were collected by scraping and lysed in 100 ml lysis buffer (50 mM Tris–HCl, 5mMEDTA, 150mMNaCl, 0.5% Nonidet P-40, 1mM phenylmethylsulfonyl fluoride, pH 8.0). Samples were centrifuged at 17,000 x g for 15 min to recover cellular lysates. Protein content was determined using the BCA assay kit (Pierce Endogen, Rockford, IL). All samples were normalized according to protein concentration, and 100 µg of proteins/sample was resolved on 10% SDS-PAGE gels and electroblotted onto nitrocellulose membranes (Hybond ECL, Amersham Pharmacia, Piscataway, NJ) using wet transfer blotting system (Bio-Rad, Hercules, CA). Trans-blots were washed twice with Tris-buffered saline (TBS: 120 mM NaCl, 50 mM Tris–HCl, pH. 7.4) containing 0.1% Tween 20 and three times with TBS buffer without Tween 20. To prevent nonspecific binding, blots were blocked in TBS/5% skim milk for 3 h. Anti-human uPA antibody (1:1000) (American Diagnostica, Greenwich, CT) were added to the blots in 0.1% Tween 20/TBS/0.25% skim milk/0.1% BSA and incubated overnight at 4ºC. Subsequently, membranes were washed extensively and incubated with goat anti-mouse IgG conjugated with peroxidase (1:5000; Pierce) for 1 h at room temperature, followed by chemiluminescent detection (SuperSignal Pico, Pierce). Film exposures were performed on Kodak X-OMAT-AR autoradiographic film (Fisher Scientific). In addition, levels of secretory uPA was determined from conditioned media of cultured cells (normalized to cell count) on 10% SDS-PAGE gels, followed by electroblotting and chemiluminescent detection.

Zymography of uPA (Fibrin overlay)

uPA activity was assessed by fibrin overlay method as described above; however, with the omission of human urokinase to the indicator gel.

SDS-fibrin Zymography gel

Another zymographic method for the detection of uPA activity was by using a modified methods

described by Choi et. al. 2000 [38], where plasminogen was either copolymerized within a fibrin containing SDS-gel or pre-incubated with samples and electrophoresed. The enzyme-substrate reaction between plasmin and fibrin would result in clear bands against a stained gel. Briefly, plasminogen (0.1 NIH unit/ml, Sigma Chemical) was added to a 10% polyacrylamide. 10% SDS gel containing: 0.012 g/ml of bovine fibrinogen and 1 NIH unit/ml of thrombin (Sigma Chemical). Samples were electrophoresed according to the method of Laemmli, 1970 [39]. Following separation, the gel was washed with Triton-X-100 for 30 min, and then incubated in reaction buffer (30 mM Tris, pH 7.4, 200 mM NaCl, and 0.02% NaN_3) at 37ºC for 12-36 hrs. followed by electroblotting and chemiluminescent detection.

Immunocytochemistry

For detection of uPA expression, NHOK and tongue cancer cell lines (Cal33, SCC-9, and SCC-4) were seeded on Labtek chamber slide (Fisher Scientific, Irvine, CA), cells were grown under normoxic and hypoxic conditions for 24 h. Cells were fixed with 2% paraformaldehyde in Triton-X, washed with sterile PBS, and 10% goat serum was added for 30 min at RT to decrease nonspecific binding. Slides were then incubated overnight with mouse monoclonal IgG uPA (1:50; Chemicon International Inc., Temecula, CA, USA) at 4ºC. Subsequently, cells were exposed for 1 h at room temperature to anti-mouse secondary antibody (DAKO Corporation, Carpinteria, CA, USA) at a dilution of 1:200, followed by incubation with ABC detection kit according to the manufacturer's protocol (Vector Laboratories, Burlingame, CA) for 30 min at RT. Each step was followed by brief wash with sterile PBS. The reaction product was visualized with 3,3Vdiaminobenzidine tetrahydrochloride (DAB) chromogen (Sigma), followed by counter stain with hematoxylin, for optimal visualization and evaluation of positive reaction. Determination of the staining reaction was carried out quantitatively using Image Analysis System (KS300 Optoronics Zeiss) according to established protocol. For each case, five fields were analyzed for immune-positivity and their means recorded.

Statistical analysis

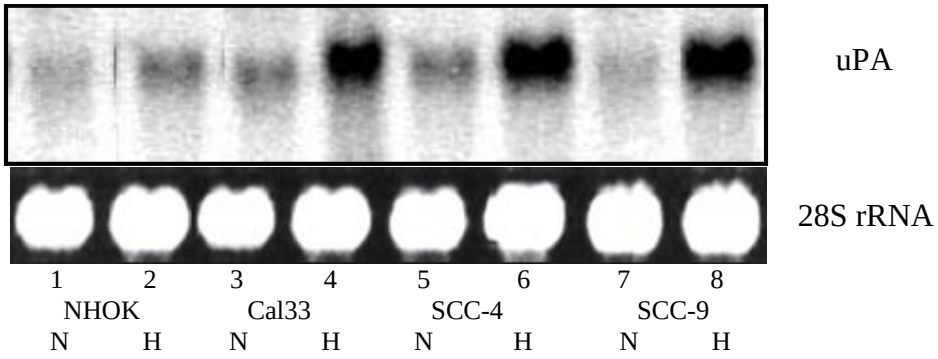
Experiments were repeated three times. uPA expression in oral cancer cell lines and NHOK were compared; results were expressed as means ± SEM of the three different experiments. Statistical significance was determined by Student's t test. A P value of < 0.05 was considered to be statistically significant.

Results:

Upregulation of uPA mRNA Expression by hypoxia:

All data are representative of three independent experiments for NHOK and OTSCC. To examine the effect of hypoxia on uPA gene expression in normal human oral keratinocytes [NHOK] and oral human tongue cancer cell lines [cal33, SCC-4 and SCC-9], cells were incubated in both normoxia and hypoxia conditions for 24 h. uPA expression was detected in normal human oral keratinocytes (NHOK) as well as tongue cancer cell lines (SCCs; cal33, SCC-4 and SCC-9). Tongue cancer cell lines showed higher levels of uPA expression under normoxia condition particularly, cal33 and SCC-4 cell lines, while under hypoxia condition all three oral cancer cell lines showed higher expression of uPA than NHOK. Hypoxia upregulated uPA expression in all tongue cancer cell lines ($P = 0.00012$) (Fig. 1A, B) while upregulation in normal oral mucosa was not statistically significant ($P > 0.05$).

Fig.1:
[A]



[B]

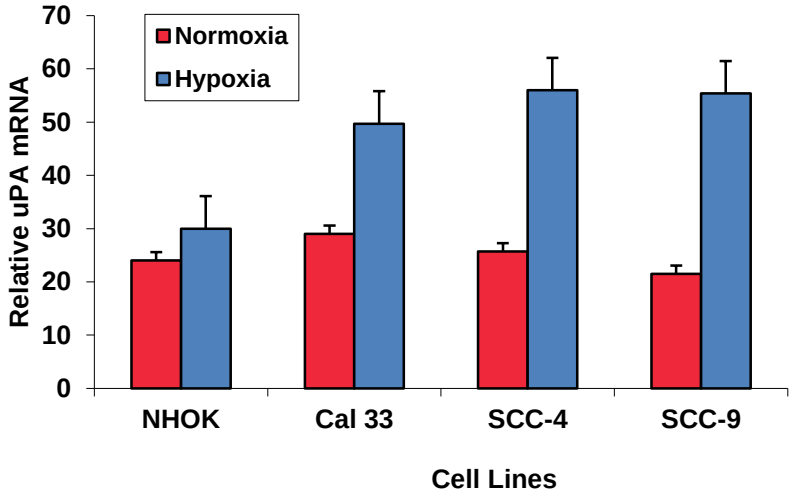


Fig. 1 [A] Northern blot analysis of uPA mRNA. N = normoxia; H =hypoxia. Lane 1, NOHK (N); lane 2, NHOK (H); lane 3, Cal33 (N); lane 4, Cal 33 (H); lane 5, SCC-4 (N); lane 6, SCC-4 (H); lane 7, SCC-9 (N); lane 8, SCC-9 (H). [B] Densitometric analysis of Northern blot. The results are representative of three independent experiments. uPA mRNA levels expressed relative to 28S ribosomal RNA.

Upregulation of uPA protein expression by hypoxia:

Western blot analysis was performed to determine if hypoxia modulates protein expression of the metastatic factor uPA. Western blotting with monoclonal anti-uPA demonstrated uPA expression in NHOK as well as oral tongue squamous cell carcinoma cell lines. uPA protein expression was detected at 48 kDa (Fig. 2 A). uPA basal level in tongue SCC cell lines than that in NHOK particularly SCC-4 and SCC-9 (P= 0.029)(Fig. 2 B). Hypoxia upregulated uPA expression in OSCC compared to NHOK (P= 0.005).

Fig.2:
[A]

[B]

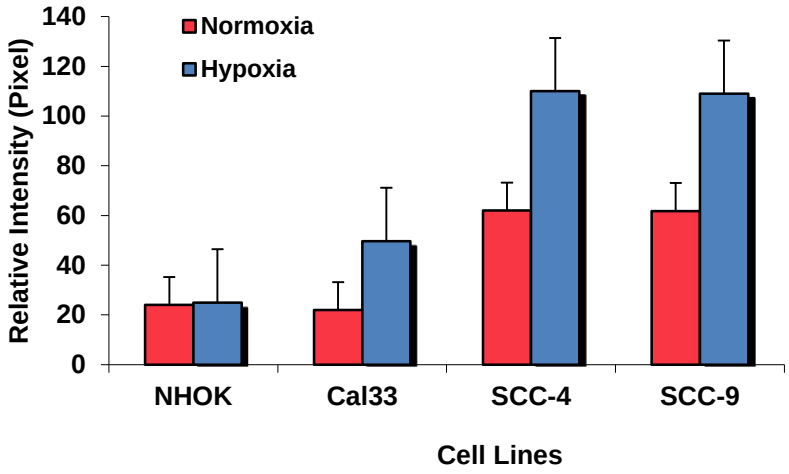
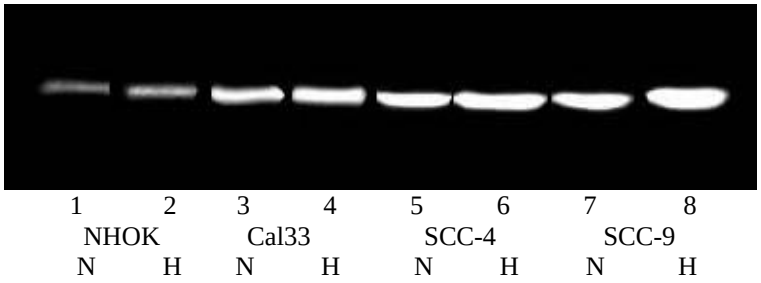


Fig. 2: (A) Total intracellular protein were extracted; equal amounts were loaded in each lane. Western blot showing uPA accumulation of intracellular extract. (t = 24 normoxia and hypoxia conditions). N = normoxia; H= hypoxia. Lane 1, NOHK (N); lane 2, NHOK (H); Lane 3, Cal33 (N); lane 4, Cal 33 (H); lane 5, SCC-4 (N); lane 6, SCC-4 (H); lane 7, SCC-9 (N); lane 8, SCC-9 (H). (B) Quantifying analysis of uPA protein level by Eagle-Eye 2 Image Analyzer and software (Stratagene, San Diego, CA). Samples were normalized to equal cell count of approximately 100,000 cells. (N: normoxia and H: hypoxia).

Detection of Active component of uPA Expressed Protein:

In order to determine the activity of secreted uPA fibrin overlay zymography was performed. The basal level of uPA activity was higher in all three tongue squamous cell carcinoma cell lines (OTSCCs) compared to that of normal human oral keratinocytes (NHOK) under both normoxic and hypoxic conditions)(Fig. 3 A). Under normoxic conditions, the mean uPA activity in NHOK was 21.13 ± 4.75 (mean ± SD) and OSCC 35.586 ± 4.78 (mean ± SD) (Fig. 3 B). The difference in uPA activity between NHOK and tongue SCCs was statistically significant (P= 0.001). Hypoxia upregulated uPA activity in both NHOK and SCC. NHOK uPA activity was 30 ± 1.75 (mean ± SD) while tongue SCCs showed 74.33 ± 24.8 (mean ± SD) and the difference was statistically significant (P= 0.008).

Fig.3:
[A]



[B]

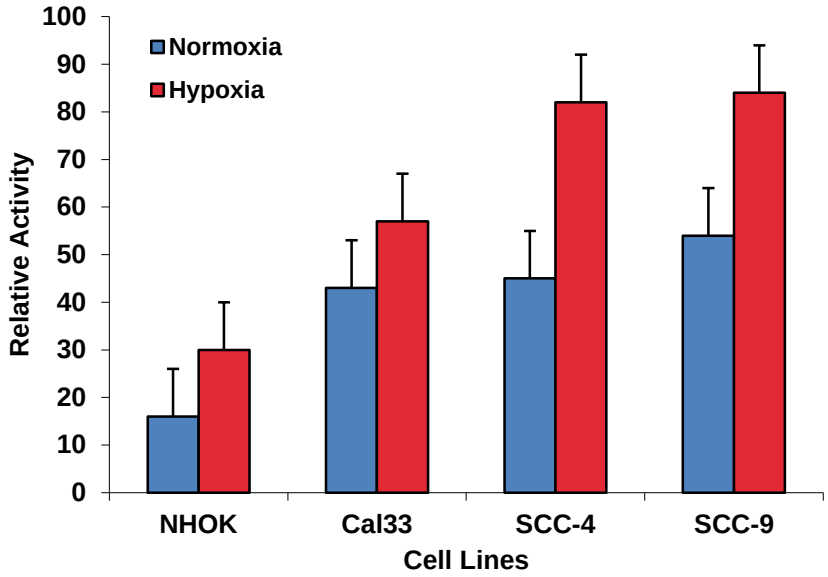


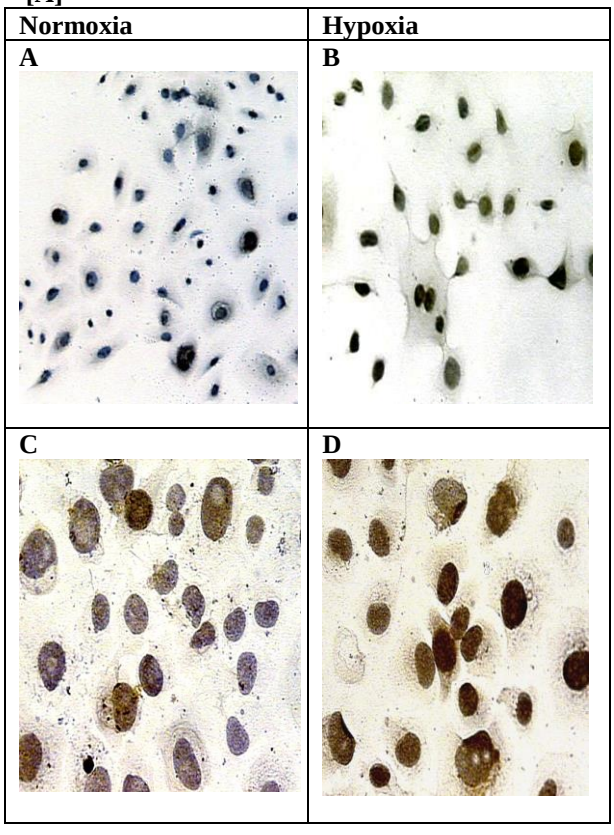
Fig. 3: (A) Total intracellular protein were extracted; equal amounts were loaded in each lane. Zymography of showing uPA activity (t = 24 normoxia and hypoxia conditions), N = normoxia; H= hypoxia. Lane 1, NOHK (N); lane 2, NHOK (H); Lane 3, Cal33 (N); lane 4, Cal 33 (H); lane 5, SCC-4 (N); lane 6, SCC-4 (H); lane 7, SCC-9 (N); lane 8, SCC-9 (H). (B) Quantifying analysis of active uPA protein level by Eagle-Eye 2 Image Analyzer and software (Stratagene, San Diego, CA). Samples were normalized to equal cell count of approximately 100,000 cells. (N: normoxia and H: hypoxia).

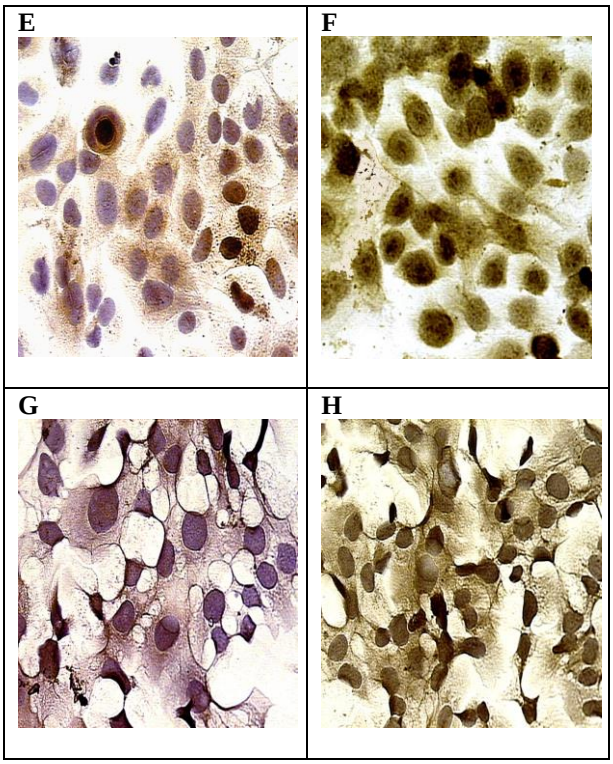
Immunocytochemical Analysis of uPA:

uPA expression was confirmed by the presence of cytoplasmic brown-stained cells in the lab-tek chamber slides (Fig. 4A). Quantitative analysis using an Image Analysis System showed that in normoxic conditions, NHOK cells expressed uPA at a mean level of 13 ± 0.8 (mean ± SD), while tongue SCCs showed higher expression 21.3 ± 4.38 (mean ± SD) (Fig. 4B). There was a statistically significant difference in uPA expression between tongue SCCs and NHOK at (P = 0.08). Hypoxia upregulated uPA expression in OTSCCs 41.13 ± 4.75 (mean ± SD) and to a lesser extent NHOK 22.76 ± 1.35 at (P = 0.0015).

Fig. 4:

[A]





[B]

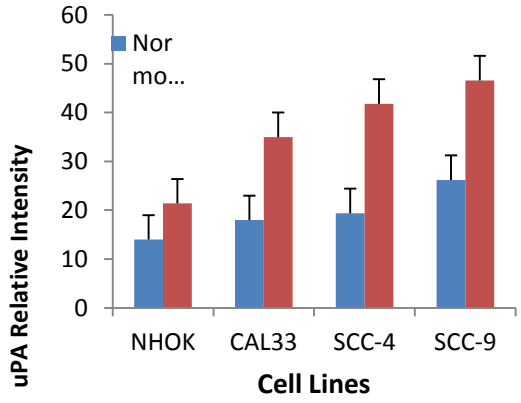


Fig. 4: [A] Detection of uPA in normal mucosa and oral tongue squamous cell carcinoma by immunocytochemistry. (A, B) NHOK; (C, D) Cal33; (E, F) SCC-4; (G, H) SCC-9. Panels A, C, E, and G represent normoxic conditions and panels B, D, F, and H represent hypoxic conditions (magnification 200x). [B] Quantifying analysis of nuclear uPA protein staining by Image Analysis System (KS300 Optoronics Zeiss) according to established protocol. Samples were normalized to equal cell count of approximately 100,000 cells. (N: normoxia and H: hypoxia).

Discussion:

The main cause of death from cancer is local cell invasion and formation of distant metastatic lesions. Cancer metastasis is equivalent to invasive cell migration; a process in which extracellular matrix (ECM) represents an obstacle for cell migration. In order to degrade the ECM, malignant cells produce proteolytic enzymes required for disruption of basement membrane and interstitial tissues and penetration of cell layers. Two families of enzymes though to participate in the invasive and metastatic process of tumor cells are plasminogen activators (urinary type uPA and tissue type tPA) and matrix metalloproteinases [1, 4-6]. The urokinase plasminogen activator (uPA) system has been implicated in the progression, metastasis and angiogenesis of numerous solid tumors [1]. Moreover, expression of uPA is correlated with poor prognosis [11]. Hypoxic conditions in tumors result in the release of cytokines promoting vascularization and enhancing tumor growth and metastasis [1]. Hypoxia stimulates expression of hypoxia inducible factor 1-α (HIF-1-α), a transcriptional activator mediating changes in gene expression in a group of genes in response to changes in cellular oxygen concentration [5]. In our previous research we approved expression of HIF-1-α in human tongue oral squamous cell carcinoma cell lines, hypoxia upregulated the expression of VEGF and HIF-1 α in oral cancer cells and also found HIF-1-α expression correlated with VEGF expression which is an important angiogenic gene in the same cell lines [11].

The current study investigated uPA expression in human tongue oral squamous cell carcinoma cell lines (OSCC) compared to normal human oral keratinocytes (NHOK) and also effect of hypoxia on uPA expression at both transcription and translation levels on both conditions. The upA transcription was examined utilizing cellular mRNA by northern blot analysis denoted uPA expression in both normal mucosa and tongue squamous cell carcinoma with higher basal level in tongue squamous cell carcinoma. Hypoxia upregulated that expression in tongue cancer cells to great extent compared to normal mucosa. uPA protein expression was evaluated with western blot analysis and the active component of that protein was examined with zymography. The results showed the basal level of uPA is higher in tongue squamous cell carcinoma cell lines compared to normal human oral keratinocytes and hypoxia upregulated that expression to greater extent in tongue cancer cells. uPA expression was confirmed with immunocytochemistry on cultured cells under both conditions. Some clinical data has demonstrated the correlation between high levels of uPA, tPA, uPAR, PAI-1, PAI-2 and tumor

aggressiveness of head and neck cancer and also poor patient prognosis. In accordance with this study, (Hattori et al, 2000) [40] studied the expression and distribution of uPA, uPAR and PAI-2 in 56 patients with esophageal squamous cell carcinoma, and found that uPA and uPAR were expressed only at the tumor cells while PAI-2 at the fibroblasts surrounding them. He also found that uPA and uPAR positive tumors, the tendency for metastasis is high and patients have poor survival rate. In another clinical study, (Bugge et al, 2004) [41] , studied plasminogen system (uPA, tPA, PAI-1, uPA-PAI-1 complex and tPA-PAI-1 complex) by laser capture microdissection (LCM) in specimens from patients having squamous cell carcinoma of tongue, floor of mouth, larynx, and vocal cords. He found that uPA expression is highly increased in tumor tissue compared to adjacent non-malignant tissue. In a review of the role of uPA/uPAR in human cancer (Sarkar et al, 2008) [42] , including oral, esophageal and different cancers found that it is responsible for increased metastasis, poor prognosis, inhibition of apoptosis, increased angiogenesis, aggressiveness and increased mortality. He also suggested that uPA system might be a good target for gene therapy in cancer patients. In a study of plasminogen activator system in oral squamous cell carcinoma (OSCC), (Dickneson et al, 2007) [43] , they found relation between components of the plasminogen activator system, in particular uPA, uPAR, and PAI-1 in invasion, metastasis, prognosis, and survival and also advised for further investigations of plasminogen system of oral squamous cell carcinoma. (Shirasuna et al 2007) [44], in their clinical study immunohistochemically investigated whether expression levels of uPA, uPAR, and PAI-1 components of plasminogen system correlates with clinicopatholoogical features of OSSC. They found that the expression levels of uPA and uPAR were related to the pattern of invasion and metastasis. (Gutkind et al 2009) [45], evaluated genetic expression in oral squamous cell carcinoma in relation to risk factor utilizing quantitative PCR of tissue specimen of the lesion and found that uPA is one of the upregulated genes in comparison to normal oral mucosa tissue.

Conclusion:

From both present and other studies of urokinase plasminogen activator, uPA genetic expression is an important regulator for metastatic behavior and invasiveness of oral tongue squamous cell carcinoma (OTSCC), and hypoxia plays an essential role for its upregulation.

Acknowledgement:

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