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ORIGINAL ARTICLE

p38 Expression and Modulation of STAT3 Signaling in Oral Cancer

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Abstract

p38 protein belongs to Mitogen-activated protein kinases family that link extracellular stimuli with intracellular responses participating in numerous of fundamental cell processes. Persistent activation of STAT3 has been associated with cell proliferation, differentiation and apoptosis in oral squamous cell carcinoma (OSCC). This study examines the effects of p38 modulation on STAT3 signaling and cellular activities in OSCC cells and investigates possible correlation of p38 expression with tumor degree of differentiation. Phospho-p38 immunostaining was performed in 60 OSCC including well, moderately and poorly differentiated tumors. Semiquantitative analysis was used, by calculating intensity, percentage and combined scores. Protein expression levels of STAT3 (total, tyrosine and serine phosphorylated), p38 and cyclin D1 were assessed in two OSCC cell lines. p38 inhibition was achieved by pharmacological agent(SB2023580). Cell proliferation and viability rates were also evaluated. Phospho-p38 immunoexpression was intense in almost all tumor specimens, nevertheless did not correlate with tumor differentiation. Inhibition of p38 with SB203580 did not appear to affect tyrosine or serine phosphorylated STAT3 as well as cyclin D1 levels in both cell lines. Moreover, p38 inhibition resulted in mild dose-dependent decreases in cell growth and viability in both cell lines. p38 is highly expressed in OSCC but does not seem to mediate the oncogenic STAT3 pathway. However, changes found in proliferation and viability may suggest that p38 functions as potent regulator of HNSCC. Understanding the complexity of p38 signaling and cross-talk between other major molecules, may guide the development of novel pharmacologic therapies for cancer treatment and prevention.

Keywords p38 · STAT3 · Oral cancer · Tumor grade

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, related with a high rate of morbidity and mortality [1]. HNSCC presents approximately half million new incidences annually and despite progress in clinical management, 5-year survival rate remains at a low of 50% [2]. Several alterations in signaling molecular pathways including EGFR, Ras, NF-κB, STAT, Wnt/b-catenin, TGF-b, and PI3K-AKT-mTOR are considered significant HNSCC promoting factors [3]. However, the molecular basis of the disease in not fully elucidated, hence understanding the complexity role of these pathways will open new frontiers in therapeutic approaches.

Signal transducers and activators of transcription (STATs) are transcription factor proteins that have been widely involved in oncogenesis [3, 4]. Over the past decade, compelling evidence suggested that persistent STAT activation by cytokines and growth factors [5, 6] is present in many types of cancer [7–10]. In recent years, several studies revealed that constitutive activation of STAT3, a major member of STAT family, strongly associated with HNSCC development and progression [11, 12]. Indeed, cell cycle deregulation, enhanced proliferation and prevention of apoptosis, as well as adverse clinical parameters in HNSCC correlate with aberrant STAT3 activation [7, 11, 12]. Particularly, previous studies considered constitutive STAT3 activation as a response to aberrant signaling of upstream pathways, notably TGF-α/EGFR, in HNSCC [8, 11–13]. Other lines of evidence demonstrated that STAT3 can be also activated through various pathways such as 7-nicotinic receptor, interleukin (IL-6,

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IL-10, IL22) receptors, and erythropoietin receptor pathways in several malignancies including HNSCC [14–16].

Mitogen-activated protein kinases (MAPK) are a family of evolutionarily conserved kinase modules that link extracellular stimuli with intracellular responses by serine and/or threonine phosphorylation of specific downstream target molecules [17]. MAPKs comprise of three major groups, including extracellular signal-regulated kinases (ERKs), p38 MAPKs, and c-Jun NH2-terminal kinases (JNKs) [18]. Aberrant MAPK signaling has been proposed in several types of cancer, including HNSCC [17–19] where MAPKs are considered downstream targets of potential oncogenic molecules such as epidermal growth factor receptor (EGFR), Raf kinase and ROS (Reactive oxygen species) [20–22]. p38 proteins are also members of the MAPK family that are activated by inflammatory cytokines and a variety of environmental stresses [23]. The role of p38 in HNSCC is not completely understood. While some authors concluded that p38 promotes proliferation, survival and induces angiogenesis and lymphangiogenesis [24, 25], others suggested that p38 decreases cell proliferation [26] and has antimigrating effects on oral cancer cells [27]. Hence, it is believed that p38 MAPK plays a dual role in cancer and its regulation depends both on the type of stimulus and cells [28]. Interestingly, previous studies attempted to examine the existence of a potential crosstalk between activation of specific members of the MAPK family and STAT3 signaling [2, 29–31]. For example, JNK was suggested to negatively regulate oncogenic STAT3 constitutive signaling in OSCC cells [2] while Erk1/2 inhibition resulted in decreases in p-ser STAT3 and cyclin D1 and increases in p-tyr STAT3 in OSCC cells [29]. Moreover, Xue et al. described that ERK1/2 and p38 signaling pathways inhibited STAT3 activities in human lung adenocarcinoma [32]. However, the role of MAPKs and particularly p38 in cancer and STAT3 regulation is still obscure, indicating the need to further investigate the molecular mechanisms underlying the potential of this association.

The aim of the present investigation was to evaluate the frequency and significance of p38 expression and activation, in oral squamous cell carcinoma (OSCCs) tissue specimens of various degrees of differentiation. Furthermore, we sought to assess the effects of p38 modulation on STAT3 expression and activation (through tyrosine and serine phosphorylation), as well as on cell proliferation and viability in OSCC cell lines.

Materials and Methods

Materials

The study material comprised sixty OSCC cases, obtained from established tissue repository of the Department of Oral Pathology and under the auspices of tissue bank protocol approved by the National and Kapodistrian University of Athens, Greece, Institutional Review Board. The tumors were classified according to Anneroth's grading system into well (WD), moderate (MD) and poorly differentiated (PD) (3 groups of 20).

Immunohistochemical Staining Paraffin-embedded tissue sections of tumor samples were deparaffinized, immersed in ethanol 100% and 95%, and heated for antigen retrieval in 0.01 M citrate buffer (C2488, Sigma-Aldrich) for 25 min in a pressure cooker inside a microwave oven. After dehydration in hydrogen peroxide, the sections were incubated with primary antibodies at room temperature for 1 h. The applied antibody was monoclonal phosphor-p38 (p-p38) (1:200) (Santa Cruz Bio.inc). To validate the staining in HNSCC samples, positive controls of Endometrial cancer tissue sections known to express phosphor-p38 were used.

Standard streptavidin–biotin–peroxidase complex method was employed to bind to the primary antibody along with multilink concentrated biotinylated anti-IgG as secondary antibody (1:2000, rabbit anti-Human IgG, ThermoFisher Scientific). Reaction products were visualized by counterstaining with the 3,3 V-diaminobenzidine reagent set (Kirkegaard and Perry Laboratories, Gaithersburg, MD). Sections were counterstained with hematoxylin. As a negative control, sections were treated with PBS, with the omission of the primary antibody. Additionally, tumors were stained with Harris' hematoxylin (Harleco, Kansas City, MO) and eosin (Sigma Chemical Co.) for microscopic evaluation. Immunostains were reviewed by 3 independent evaluators (NN, GR, IG).

Positive Criterion for Immunohistochemical Staining

The immunopositive staining was evaluated in 5 randomly selected areas of the tissue section and specific staining in cancer cells was defined as positive staining. Sections were scored as positive if cancer cells showed immunopositivity in the nucleus when observed by all 3 evaluators, independently, who were blinded to grading of the tissue samples, while scoring the immunoreactivity. The tissue sections were scored based on the percentage of immunostained cells as: 0% to 10% =0; 10% to 30% =1; 30% to 50% =2; 50% to 70% = 3; and 70% to 100% = 4. Sections were also scored on the basis of staining intensity as negative =0; mild = 1; moderate = 2; intense = 3. To validate staining intensity in HNSCC samples, positive controls of previously studied tissues known to express p-p38 were used and their intensity was classified as moderate. Lower intensity (light brown) compared to the brown staining of the positive control was classified as weak, while higher intensity (dark brown) compared to the positive control was classified as strong. Finally, a total score was obtained by adding the score of percentage positivity and intensity.

Ten non-malignant tissues (with histologically confirmed normal oral epithelium) were also evaluated for p-p38 proteins

expression (control). The source of normal tissues was from adjacent normal epithelium of routinely surgically excised traumatic fibromas of patients with no smoking or alcohol consumption habits.

Statistical Analysis The baseline characteristics of patients were summarized as mean and standard deviation (SD) for continuous or ordinal data and as absolute (n) and relative (%) frequency for categorical variables.

The two tailed Fisher's exact test was performed in order to evaluate possible differences in the frequency distribution of clinical and pathologic features of patients between men and women, as well as in the frequency distribution of cases with positive and negative IHC staining between the three groups of tumor differentiation. Comparisons concerning the age of patients were based on Student's t-test, while ordinal data were compared with the use of the Kruskal-Wallis one way analysis of variance by ranks. Spearman's rank correlation coefficient rho *(r)* was calculated for the evaluation of possible correlations between the ranks of the various p-p38 IHC scores under study with the progression of tumor differentiation.

Cell Lines and Cell Culture Experiments were performed using established cell lines of human OSCC (SCC9 and SCC25), which were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in a 1:1 mixture of Ham's F-12 (Sigma-Aldrich, St. Louis, MO, USA cat# 51651C) and Dulbecco's modified Eagle's (Sigma-Aldrich, St. Louis, MO, USA, cat#D5796) medium containing 10% fetal bovine serum (GIBCO-BRL, Eggenstein, Germany, cat#10437–077), 100 U penicillin and 400 ng/ml hydrocortisone (Sigma-Aldrich, St. Louis, MO, USA) at 37 °C in a 5% CO_2 air atmosphere. Cells were subcultured by disaggregation with trypsin (0.1%) and ethylenediaminetetraacetic acid (0.01%)(GIBCO-BRL, Eggenstein, Germany, cat# 25300120) in phosphate-buffered saline (PBS) (Sigma-Aldrich, St. Louis, MO, USA cat# 1314-87-0) at pH 7.5.

Selective Inhibition of p38 Cells were plated in 6-well plates at a density of 5 × 104 cells/well and were allowed to grow to 80% confluency. Then, cells were either treated with vehicle alone [dimethyl sulfoxide (DMSO) at a maximum concentration of 0.1%] or with the selective p38 inhibitor SB203580 (Calbiochem; EMD Millipore, Billerica, MA, USA) at concentrations of 10–20 μM for 24 h.

Western Blot Analysis Western blot analysis was performed as we previously described [2]. Cells were lysed and sonicated in the lysis buffer. Equal amounts of protein were subjected to SDS-PAGE. After protein separation by electrophoresis, samples were transferred to polyvinylidene difluoride films (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Blotted films were placed in blocking solution for 1 h at room temperature. Subsequently, they were probed with indicated primary antibodies overnight at 4 °C: monoclonal STAT3 (1/250) (mouse #9139), polyclonal phospho-STAT3 (Tyr705) (1:250)(rabbit #9131), polyclonal phospho-STAT3 (Ser727) (1:200) (rabbit #9134), total polyclonal p38 (1/300) (rabbit # 9212S), monoclonal p-p38 (1/200) (rabbit # 4631) and polyclonal cyclin-D1(1/250) (rabbit #2922). All antibodies were purchased from Cell Signalling (Cell Signaling, Beverly, MA, USA). The film was washed thoroughly, incubated with goat polyclonal anti-rabbit IgG horse radish peroxidase secondary antibody (1:3.000; Santa Cruz Biotechnology, Santa Cruz, CA, USA, # sc-2301) or anti-mouse IgG antibody (dilution, 1:3.000; Santa Cruz Biotechnology, CA, USA,# sc-2031) with shaking at room temperature for 1 h at 25 °C; β-actin was used as control (Santa Cruz Biotechnology, Santa Cruz, CA, USA, # sc-47,778). Proteins were visualized using an enhanced chemiluminescence system and band intensity was quantified using Image J software1.48 (https://imagej.nih.gov/ij/).

Cell Proliferation and Viability Cells were counted with a Neubauer hemocytometer under an inverted Zeiss *Axioplan* microscope (*Zeiss*, *Carl Zeiss Ltd*, Jena, Germany). Cell viability upon treatment was determined by the Trypan blue dye (Bio-Rad Laboratories, Inc., Hercules, CA, USA #1450021) exclusion test. All assays were performed in quadruplicate and the results are reported as the mean ± standard deviation.

Statistical analyses Results of protein expression levels, cell viability and cell number of treated cells were compared with the results of untreated (control) cells respectively. Paired groups were compared with the Student's t test and level was set at 5% ($p < 0.05$).

Statistical analyses were performed using the SPSS® software application (version 21.0; IBM® SPSS Statistics, Chicago, IL, U.S.A.) with $P < 0.05$ as the threshold of significance.

Results

Study Sample Patients' Demographics

The frequency distribution of selected characteristics of the patients whose biopsy specimens comprised the study sample are presented in Table 1, according to gender. The sample consisted of 60 cases, namely 33 men (55.0%) and 27 women (45.0%). The mean age was 61.2 years (±7.8 years; range, 42–73 years) for men and 63.4 years (±9.3 years; range, 40–83 years) for women. Mean ages between these two groups

Table 1 Frequency distributions of selected demographic variables according to gender

Characteristics	Men	Women	*P*	Total
n	33 (55.0%)	27 (45.0%)		60 (100%)
Age:				
Mean (±S.D.)	61.2 (±7.8)	63.4 (±9.3)	0.323*	62.2 (±8.5)
Range	42–73	40–83		40–83
Tumor site:				
Tongue	21 (35.0%)	19 (31.7%)	0.677**	40 (66.7%)
Floor of mouth	7 (11.7%)	6 (10.0%)		13 (21.7%)
Alveolar crest	5 (8.3%)	2 (3.3%)		7 (11.6%)
Positive history of:				
Smoking	25 (41.7%)	23 (38.3%)	0.519**	48 (80.0%)
Alcohol intake	15 (25.0%)	8 (13.3%)	0.189**	23 (38.3%)

S.D., Standard Deviation

* Student's t-test

** Two tailed Fisher's exact test

were comparable ($P = 0.323$). A non-statistically significant distribution according to tumor site and the history of smoking and alcohol intake was also observed between men and women, as can be deduced from Table 1.

Immunohistochemical (IHC) Expression Patterns and their Correlation with Tumor Grade

IHC staining was positive in 84.2% (16/19) of WD, in 85.0% (17/20) of MD and in 94.4% (17/18) of PD cases. WD, MD as well as PD cases demonstrated minor differences in the mean scores in all three studied variables, as depicted in Fig. 2. p-p38 is highly expressed in OSCC, however two Tailed Fisher's Exact Test revealed that p-p38 immunoexpression did not appear to correlate with tumor degree of differentiation (Table 2). Kruskal-Wallis one way analysis pointed out that the distribution of p-p38 intensity, percentage and combined scores are the same across categories of differentiation (Fig. 3) while Spearman's Correlation Coefficients analysis found no statistically significant correlation between p-p38 IH levels (intensity, percentage, combined) and progression of tumor grade (Table 3) .

Effects of p38 Selective Inhibition in OSCC Cell Lines Selective inhibition of p38 activity in OSCC cells treated with SB203580 resulted in a slight decrease in cell viability and a higher but not statistical significant decrease in absolute number of living cells in both cell lines.

Western blot analysis showed that endogenous levels of total p38 were almost steady when 10μM and 20μM of SB203580 inhibitor were used. As expected, decreased levels of p-p38 were also observed in a dose-dependent manner. The relative STAT3 phosphorylation levels remained almost unchanged for both pSTAT3 Tyr705 and Ser727. Moreover, cyclinD1 levels did not appear a significant change.

Discussion

Several lines of evidence highlighted the involvement of p38 in many types of cancer such as prostate [33–35], breast [36] and bladder cancer, [37, 38]. Indeed, activation of p38 has been correlated to the epithelial-mesenchymal transition (EMT) of cells in the primary tumor and enhanced the ability of tumor cells to invade and migrate to the surrounding tissues in gastric adenocarcinoma and pancreatic cancer [39, 40]. In contrast, p38 MAPK inhibition was associated with the resistance to anoikis in canine kidney epithelial cells [41], while p38 activation was responsible for pharmacological-induced apoptosis in human lung cancer cells [42], p53 activation and p53-induced apoptosis and cell cycle arrest in mice embryo fibroblasts [43].

In the present study, high p-p38 immunoexpression levels appeared to the majority of OSCC tissue specimens, however p-p38 immunoexpression did not correlate with differentiation

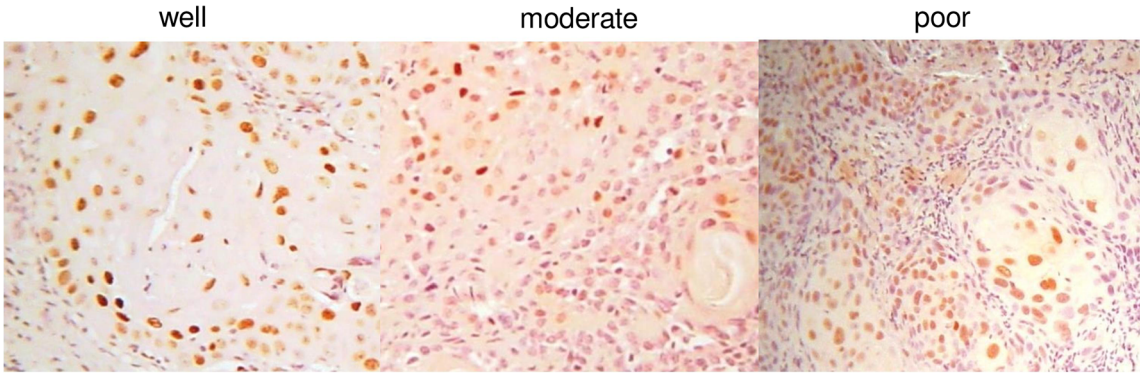

Fig. 1 Indicative staining for p-p38 protein including well, moderate and poor differentiated cases. Immunohistocemical staining of p-p38 in OSCC (magnification X200). Representative photomicrographs for each molecule in well, moderately and poorly differentiated tumors are depicted. Overall, p-p38 immunoexpression did not show noticeable differences among cases of variable differentiation

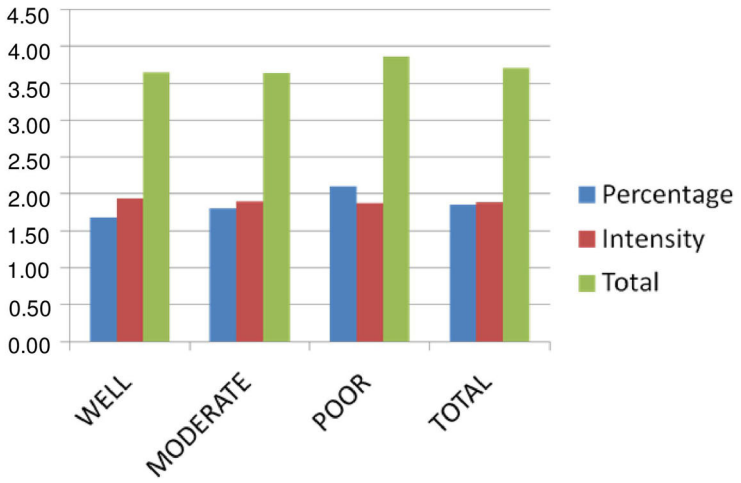

Fig. 2 IHC staining was positive in 84.2% (16/19) of WD, in 85.0% (17/20) of MD and in 94.4% (17/18) of PD cases. WD, MD as well as PD cases demonstrated minor differences in the mean scores in all three studied variables

p-p38	% Positive Cases	Percentage score	Intensity score	Total score
WELL	16/19 (84.2%)	1.68	1.94	3.65
MODERATE	17/20 (85%)	1.80	1.90	3.63
POOR	17/18 (94.4%)	2.10	1.88	3.86
TOTAL	50/57 (87.7%)	1.85	1.89	3.71

or the progression of tumor grade. Nevertheless, high rates of expression may imply a role of p38 in HNSCC tumorigenesis. Similarly, previous immunohistochemical studies in series of human HNSCC tissues demonstrated significant elevated levels of p-p38 in 79% of studied cases, whereas relative p-ERK1/2 and p-JNK elevated levels were present in just 33% and 16% of the cases, respectively [25]. In addition, both p38 and p-p38 expression levels were considered independent prognostic risk factors in a tissue microarray assay of patients with Diffused Large B Cell Lymphoma [44], while a predictive model of high ATF6α but low p-p38 expression was proposed as potential biomarker of recurrence risk in resected biliopancreatic adenocarcinoma tissues [45]. Considering the aforementioned controversial evidence, we could hypothesize that the significance of p38 as a prognostic biomarker in cancer may vary according to the type and status of the studied cells.

Table 2 Two Tailed Fisher's Exact test: p-p38 immunoexpression did not appear to correlate with tumor degree of differentiation

IHC p-p38	Grade of Differentiation								
	Well ($n = 19$)		Moderate ($n = 20$)		P (Well vs Moderate)	POOR ($n = 18$)		P (Well vs Poor)	P (Moderate vs Poor)
	n	%	n	%		n	%		
Intensity									
0	3	15,8%	3	15,0%	0.999	1	5,6%	0.476	0.651
1	4	21,1%	5	25,0%		7	38,9%		
2	3	15,8%	3	15,0%		4	22,2%		
3	9	47,4%	9	45,0%		6	33,3%		
Percentage									
0	3	15,8%	3	15,0%	0.969	1	5,6%	0.699	0.874
1	5	26,3%	5	25,0%		4	22,2%		
2	6	31,6%	5	25,0%		5	27,8%		
3	5	26,3%	7	35,0%		8	44,4%		
Combined score									
0–2	6	31,6%	5	25,0%	0.714	5	27,8%	0.999	0.921
3–4	4	21,1%	7	35,0%		5	27,8%		
5–6	9	47,4%	8	40,0%		8	44,4%		

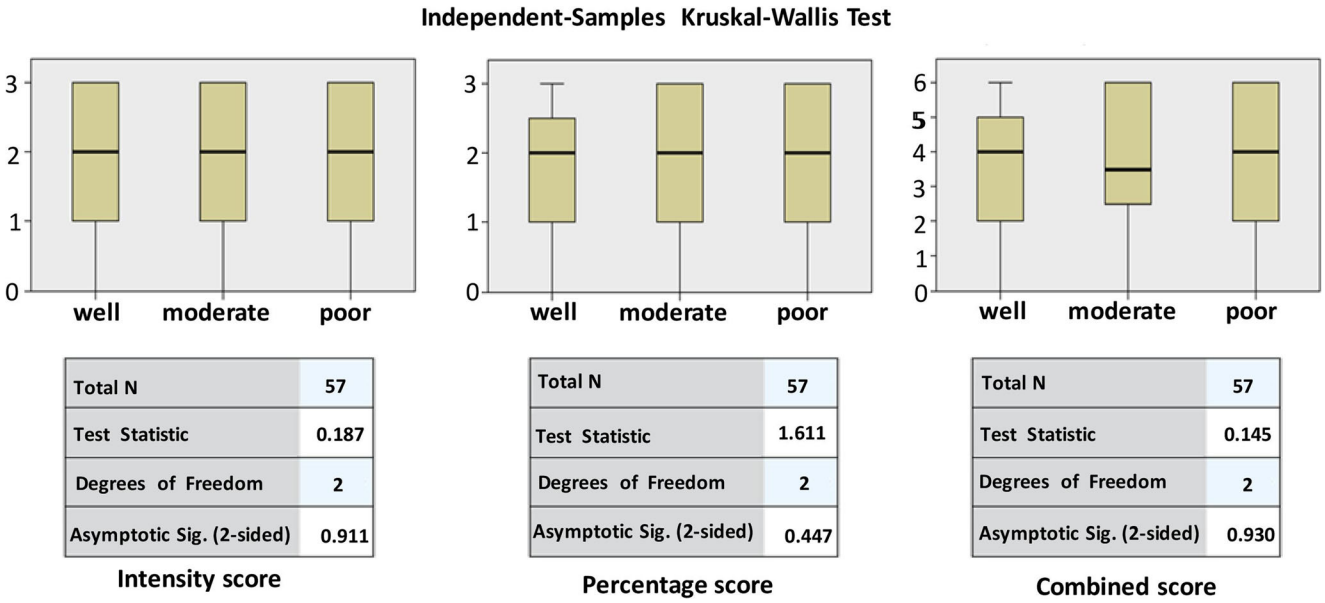

Fig. 3 Independent-Samples Kruskal-Wallis Test showed that the distribution of p-p38 intensity, percentage and combined scores are the same across categories of differentiation

Furthermore, we sought to examine the effects of p38 chemical inhibition on cell proliferation and viability of OSCC cells. According to our results, inhibition of p38 activity had almost no effect in cell viability and showed a slight decrease in absolute number of living cells in both cell lines. Consistent with our in vitro data, previous studies indicated that pharmacological inhibition of p38 did not affect autophagy regulatory proteins and autophagosome formation in HNSCC [46], [*6-(N,N-Dimethylamino)-2-(naphthalene-1-yl)-4-quinazolinone*] DPQZ-induced cell death in HNSCC [47] and mevastatin-induced cell growth inhibition and apoptosis of salivary adenoid cystic carcinoma cells [48]. Furthermore, in a MAPK assay, Shen et al. reported that ERKs and JNKs, but not p38, were responsible for the cyclin D1 decrease in human embryonic lung fibroblasts [49]. However, a deep insight on the role of p38 in HNSCC reveals controversial reviews between research groups, giving motivation for intensive investigation. Indeed, Juntila et al. [24] reported that p38 promoted proliferation and survival of HNSCC cells while Leelahavanichkul et al. [25] suggested that p38α functioned as a positive regulator of HNSCC, controlling cancer cell proliferation as well as tumor-induced angiogenesis and lymphangiogenesis. In addition, AKT, p38 and Src Family Kinases (SFK) inhibitors were proposed to reduce survival of HNSCC, in combination with radiotherapy [50]. Likewise, NF-κB, p38, and JNK inhibitors suppressed IL-6 expression and enhanced sensitivity of HNSCC to erlotinib [51].

In contrast, Riebe et al. [26] suggested that activation of p38 decreased cell proliferation and Yen et al. [27] concluded that cardiotoxin III posed antiproliferative effects on oral cancer cells through p38-MAPK signaling. Moreover, IFNg treatment of HNSCC cells induced apoptosis through mitochondrial and endoplasmic reticulum (ER) stress-associated pathways including p38 [1], while TNF treatment enhanced p38 phosphorylation and increased chemosensitivity [52]. Other studies, introducing chemotherapeutic agents, demonstrated that sequential treatment with bortezomib and celecoxib favored apoptosis through p-p38-mediated cell cycle arrest associated with ER stress respone in HNSCC cells [53]. Similarly, celecoxib antitumor effect on HNSCC cells depended on upregulation of ERK and/or p38 signaling pathways [54].

As regards to the role of p38 in HNSCC metastasis, it was suggested that chemical inhibition or silencing of p38 resulted to a less invasive phenotype [55, 56]. In addition, individual or combined treatment with Epigallocatechin gallate (EGCG) and gefitinib followed by downregulation of ERK, JNK, p38 and AKT, reduced metastatic potential of HNSCC cells [57] while irradiation enhanced AKT, p38 MAPK and ERK expression resulting in elevated tumor cell migration [58]. In

Table 3 Speaman's Correlation Coefficients; Results indicating no statistically significant correlation between p-p38 IH levels (intensity, percentage, combined) and progression of tumor grade

		Differentiation
p-p38 intensity score	Correlation Coefficient	-,057
	Sig. (2-tailed)	,676
	N	57
p-p38 percentage score	Correlation Coefficient	,165
	Sig. (2-tailed)	,221
	N	57
p-p38 combined score	Correlation Coefficient	,051
	Sig. (2-tailed)	,708
	N	57

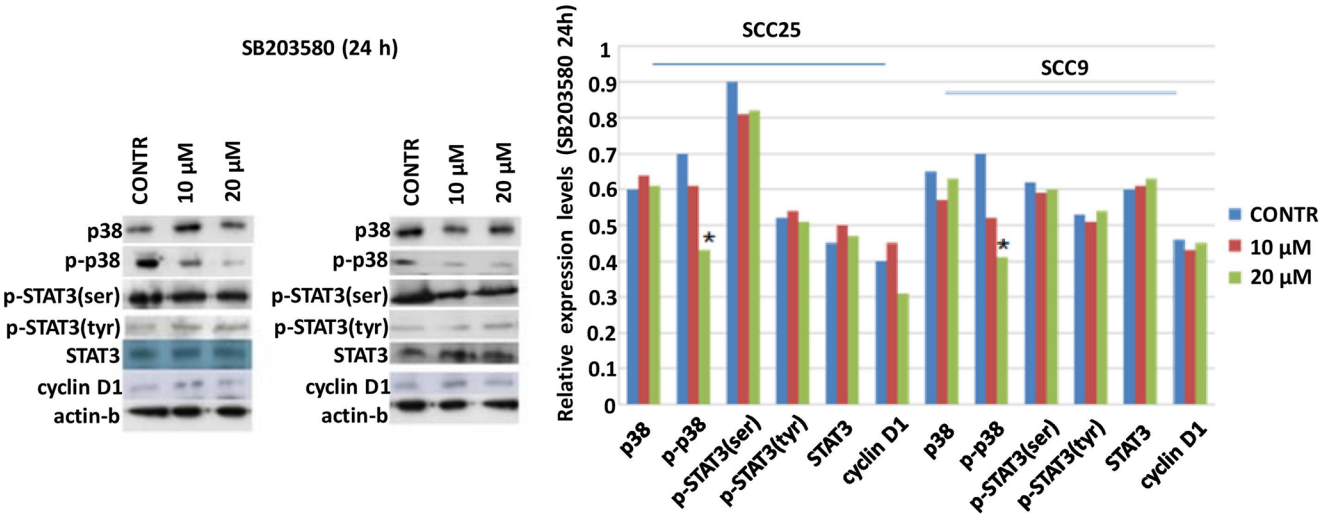

Fig. 4 Western blot analyses and relative quantification of WB protein levels (compared with β-actin protein) in the two studied cell lines. *Statistical significant difference ($P < 0.05$) compared with control. Western blot analysis showed that endogenous levels of p38 were almost steady when 10μM and 20μM of SB203580 inhibitor were used. SB203580 induced a dose-dependent decrease in p-p38 levels. The relative STAT3 phosphorylation (ser and tyr) levels remained almost unchanged for both pSTAT3 Tyr705 and Ser727. Moreover, cyclin D1 levels did not show significant changes

contrast, Yen et al. [27] reported that cardiotoxin III increased JNK and p38 phosphorylation but decreased MMP-2 and MMP-9 expression as well as migration of oral cancer cells.

Another interesting finding of the present research was that treatment of OSCC cells with pharmacological inhibitor of p38 (SB203580), down-modulated the phosphorylation of p38 but led to no significant changes in tyr705 or ser727 STAT3 expression. In agreement with our results, Platanias et al. [59] reported that p38 played an important role in Type I IFN-dependent transcriptional regulation, without affecting activation of STAT pathway. Also, Tanabe et al. [60] demonstrated that 8-Bromo cAMP treatment of glial cells induced IL1β-dependent STAT3 phosphorylation but did not affect IκB, p38 or JNK phosphorylation. However, Riebe et al. [61] described that down-regulation of p38 expression levels by siRNA-targeting strongly reduced phosphorylation of STAT3 tyr705 without affecting phosphorylation of STAT3 ser727 in HNSCC cell lines. In contrast, activation of ERK and p38-dependent pathways inhibited STAT3 activity in human lung adenocarcinoma cells [32], while p38 was suggested to inhibit IL-6-JAK-STAT3 pathway in inflammatory and other cell types [30]. Based on these findings, we could speculate that p38 function and regulatory effect on STAT3 signaling significantly varies, probably depends on the activated pathway and seems to be stimuli as well as cell type-specific [28, 62, 63].

In summary, our findings indicate that p38 does not appear to modulate oncogenic STAT3 pathway activation in OSCC cells. Even though changes found in proliferation and viability

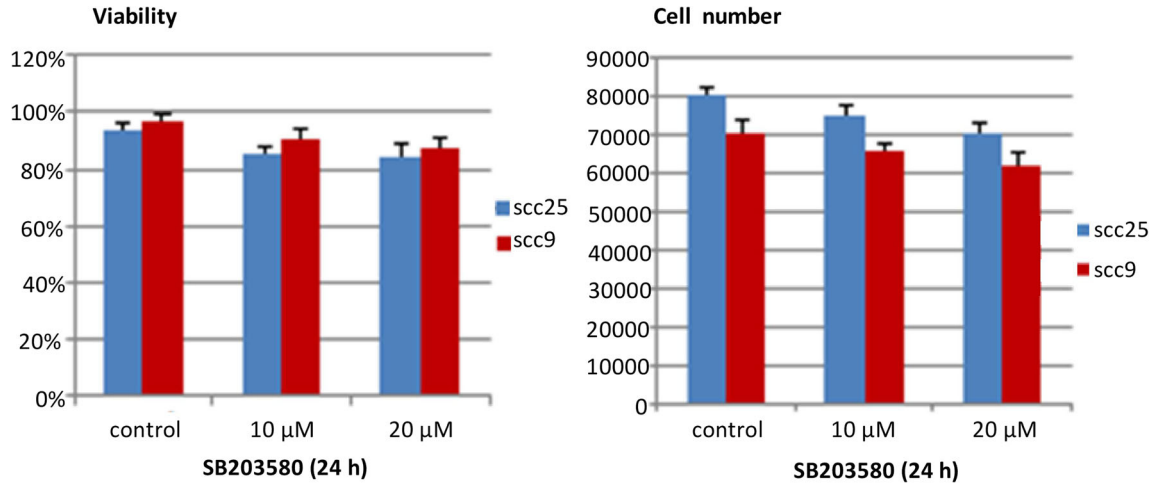

Fig. 5 Selective inhibition of p38 activity by SB203580 in OSCC cells resulted in a slight decrease in cell viability and a higher but not statistical significant decrease in absolute number of living cells in both cell lines

after p38 inhibition were modest, high expression observed in all OSCC tissues may suggest that p38 functions as a potent regulator of HNSCC. It is possible that the role of p38 in cancer and STAT3 regulation varies according to the type and status of the studied cells, supporting the need for further investigation. Understanding the complexity of MAPK pathway and the mechanism underlying cross-talk between other major molecules such as STAT3 will elucidate novel potential targets for molecular-based therapies.

Acknowledgments All authors listed above have contributed significantly to implementation, analysis, interpretation of the data and general presentation of the manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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