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UCHL1expression in OSCC In Relation To the Invasive Front Grading System

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Abstract:
Background : *Ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) is an abundant neuronal deubiquitinating enzyme that has been recent implicated in the pathogenesis and progression of several human cancers.*
Aims of the study: To evaluate the immunohistochemical pattern of expressions, scoring and intracellular localization of UCHL1 in relation to the invasive Bryne's grading system.
Materials and methods: *a total of thirty formalin fixed paraffin embedded blocks of oral squamous cell carcinoma were included in this study. Routine stained sections were histologically graded by Bryne's systems. Sections stained with anti-UCHL1 were evaluated for subcellular localization, pattern of expression, and stain intensity. Kruskal Wallis tests and Somers'd correlation coefficient were applied for analysis. P<0.05 was considered statistically significant.*
Results: *UCHL1 showed 86.6% total positivity with 73.3% cytoplasmic expression, with significant high positivity (score 2 and 3) and diffuse strong homogeneous pattern and strong staining intensity. There was no significant differences regarding intracellular localization, percentage of positivity, pattern distribution and staining intensity among different histopathological grades of Bryne's system.*
Conclusion*: OSCC had a high percentage of UCHL1 positive expression in homogenous distribution pattern and strong intensity. However, basal cells loss this expression. There is small percentage of negative case and membranous expression. The expression of UCHL1 do not related to histological invasive grading.*
Keywords: *Bryne's grading system, oral squamous cell carcinoma, UCHL1.*

I. Introduction

The process of tumorigenesis requires multistep initiation of cellular and molecular pathways leading to a series of mutations resulting in the acquisition of replication and growth factor independence, resistance to growth-inhibitory signals, tissue invasion, and metastasis [1].

The normal cellular homeostasis is tightly regulated by protein degradation process (ubiquitination) both through proteasomal targeting and by direct sorting to the lysosome [2]. This process is reversible through deubiquitinases. The ubiquitin C-terminal hydrolases L1 (UCHL1) is a deubiquitinase enzymegoverning the subcellular trafficking of proteins [3]. It influences many ubiquitination-dependent cellular processes, including proteasomal degradation, DNA damage repair, trafficking, cell signaling, endocytosis, and lysosomal degradation [4]. The precise mechanism of UCHL1mediated tumorigenesis is not fully understood. Different studies have identified its role either as an oncogene[5]or a tumor suppressor gene [6]. It may be implicated in the regulation of cell cycle progression, cell survival, cell motility, and invasion [4].

The mechanism of cancer cell invasion is not well known but cell migration is clearly an important factor [7].Histomorphological identification of un-cohesiveness and infiltrative pattern of growth that mostly found in poorly differentiated tumorsare related to a poorer outcome; that associated with recurrence, lymph node involvement and worst disease-free survival [8]. Thus invasive front grading system proposed by Bryne's is well accepted as a prognostic tool in oral cancers [9].

Many studies were conducted to find the expression of UCHL1 in different cancers. It was overexpressed in high metastatic colorectal and prostate cancer cells with enhancing cell migration and invasion [10, 11], while it wasdown regulatedin lung carcinoma with reducing cancer cells migration [12].To date, little is known about its role in OSCC. There is only one published paper detected DNA promoter hypermethylation of UCHL1 in esophageal SCC [13]. Therefore, this study was conducted to evaluate IHC expression and intracellular localization of UCHL1 in oral squamous cell carcinoma (OSCC) in regarding to Bryne's grading systems.

II. Materials And Methods

A retrospective study conducted in oral pathology Dept. School of Dentistry / University of Sulaimani, during the period 1st April to 30th September 2014. The study was approved by the ethical committee in the Faculty of Medical Sciences.The sampleincluded 30 FFPE blocks of OSCC that contain invasive front areas.Demographic and clinical data were registered from archive case sheets and any recurrent OSCC or patient received radiotherapy or chemotherapy was excluded.

Two 5µm tissue sections were cut from each block. One of them wasmounted on ordinaryslide for routine H&E staining and graded by Bryne's systems [14]. The other section was mounted on positively charged slides for IHC staining as describe previously [15]. In brief, sectionsdeparaffinized in xylene and rehydrated through series of ethanol. Antigen was retrieved by boiling in citrate buffer (pH-6, 15min). At room temperature,sections washed with PBS twice (3 min each). Endogenous peroxidase activity was blocked by hydrogen peroxidase (10 min) then protein block was applied (10 min). Sections incubated with primary antibody (rabbit polyclonalanti-UCHL1, dilution 1:200,abcam) for 45 min and washed 4 times with PBS. After that they incubated with complement (10 min) and washed by PBS (3min).Goat anti-rabbit HRP conjugate applied for 15 min and then washed. Sections stained by DAB (5 min in the dark) and counter-stained withhematoxylin (20 sec).Then they dehydrated, cleared and mounted with DPX to be ready for microscopical examined. The nerve tissue served as positive internal controls [16], while the negative control include a non-immune serum by omitting primary antibody and applying antibody diluents alone.

For immune-histological evaluation, five hot spot fields at high power (X400) were selected and their digital images were uploaded to the computer to be analyzed by Image J software after plugins grid for counting immune-positive stained cells. Intracellular localization of UCHL1 was assessed either to be membranous or cytoplasmic. The percentage of positive cells /1000 counted cells was scored according to Diomedi-Camassei et al (2) as follows ,0= < 5% of cell positive,1= 6–25% of cells positive, 2= 26–50% of cells positive, 3= > 51% of cells positive. Immunoreactivity was graded according to intensity as: 1= weak; 2= moderate; and 3= strong [17]. Statistical analysis was done by using SPSS 20.0 software and data analyzed by Kruskal Wallis test andSomers'd correlation coefficient. Probabilities of less than 0.05 accepted as significant.

III. Results

The expression of UCHL1 in normal oral epithelia is cytoplasmic in the basal, parabasal and polyhedral cell layers, as well as in the nucleus of fewparabasal and polyhedral cells (Fig -1A). Sections containing perinural invasion showed positive strong cytoplasmic expression in the neural cells (Fig -1-B).

UCHL1 positive expression was detected in 26 OSCC cases (86.6%) as either membranous (23.3%) or cytoplasmic (73.3%)in various cells within the same lesion (Table-1). The basal cell layer in OSCC nests was negative or had faint cytoplasmic expression, while polyhedral layer had prominent strong cytoplasmic with few faint discontinuous membranous expression. Cord and sheets of OSCC showed cytoplasmic stain. Regarding the percentage of cell positivity, pattern, and stain intensity, 36.7% of the cases was in score 2 and 30%was in score 3. Oral SCC expressed strong homogenous distribution(36.7%) with strong stain intensity (36.7%). Only 4 cases were negative (Table-1).

UCHL1 expression percentage mean ranks were analyzed (by Kruskal-Wallis test) in relation to score positivity, pattern of expression and intensity. Considering the membranous expression percentage alone, it had a higher mean rank in;both score 0 and 3, both reduce and strong diffuse homogeneous pattern, as well asboth weak and strong stain intensity,with no significant difference ($P>0.05$). On the other hand, cytoplasmic expression percentagemean rank alone was significantly high in score 3, in diffuse strong homogeneous pattern, and in strong intensity ($p<0.05$). Finally, if both expressions evaluated collectively, the positive cells percentage was highly significantly different. The higher mean rank was in the diffuse strong homogeneous pattern and in strong intensity ($p<0.001$), Table 2.

The UCHL1 cytoplasmic expression percentage mean rank in well differentiated (WD) OSCC wasless (13.5) than moderate differentiated (MD) OSCC (19.04)but higher than poor differentiated (PD) SCC (11.5). There was no significant difference of intracellular localizationamong different histopathological grades ($p>0.05$) (Table-3). Furthermore, WDSCC has negative or faint UCHL1 expression in basal and parabasal layers, and strong or faint membranous or cytoplasmic stain in polyhedral cell layers. In MDOSCC, UCHL1 expression was cytoplasmic with moderate stain intensity. Lastly, the expression was negative or weak membranous or cytoplasmic in PDOSCC. (Fig-1 C,D,E). Although, score 2 withthe strong diffuse homogeneous pattern and strong intensity were most frequent in WDSCCs (6 cases), yet significant difference among grads was not found. According to Somers'd strength of association, the relation between the grades on one hand, and the score, pattern of expression and intensity of the stain on the other hand were reverse and low ($d< -0.3$) (Table -4).

IV. Discussion

Carcinoma is a heterogeneous growth with a wide variety of characteristics and an emphasis placed on using the most reliable malignancy grading system which is restricted to the deep invasive front area of the growth [14and 18]. Such system provides definitive histological scoring assessment related to cell biological behavior. In this study, for the first time, the morphological invasive features of OSCC were evaluated in relation to the expression of specific molecules related to invasion (UCHL1).

Despite the controversy regarding the exact function of UCHL1 in oncogenesis, previous studies suggest that UCHL1 is an important regulator of tumor formation and maturation, and it might play different roles depending on the tissue type. Herein, normaloral epithelium showed cytoplasmic UCHL1 expression in the basal and polyhedral cells.In OSCC, the basal cells tend to be negative (loss expression) and 23% of malignant cells showed membranous expression, beside 73.3% retained the normal cytoplasmic localization. This shifting and changing is directly related to B-catenin expression (unpublished data). Thus it may act as an oncogenes as has been suggested in colorectal cancer via activation of the β-catenin/TCF pathway [5]. On the other hand, the lowerUCHL1 expression reported in other types of cancers [13, 19, and 20] was attributed to promoter hypermethylation.In the present study, OSCC had high percentage of UCHL1 expression. It characterized by high scored cytoplasmic expression with strong homogenous distribution and strong stain intensity. This possibly indicates various potentials of the growth at different enzyme levels. This reflects thealteration in the ubiquitin-proteasome system related to epithelial cellular activity, butit did not related to the invasion. However, clinical follow up of cases is recommended to clarify its role in migration and metastasis.

It has been shown that the percentage of mean rank membranous UCHL1 expression did not provide meaningful results. In fact it minimizes the significant findings when considered in the total expression. Therefore, this abnormal membranous localization needs to be evaluated in different manner. Besides that, oral stratified squamous epithelial has well organized cells' layers related to stage of maturation. This cells architecture is observed in potentially malignant oral lesions and well differentiated carcinoma, but is lost in poor differentiation carcinomas. Lacking of scoring system that evaluates molecules expression in relation to theepithelial cells architecture may obscure part of the fact.

Thiswork provides preliminary results that limited by the small number of poor differentiated case, and because of lacking previous study to compare the results, it is difficult to draw conclusions about its respective role in OSCC tumorgenesis.

V. Conclusion

OSCC had a high percentage of UCHL1 positive expression in homogenous distribution pattern and strong intensity. However, basal cells loss this expression. There is small percentage of negative case and membranous expression. The expression of UCHL1 do notrelated to histological invasive grading.

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Table 1: The frequency distribution and percentage of UCHL1 expression in OSCCs

		No.	%
Expression	Membranous	7	23.5
	Cytoplasmic	22	73.3
	Total positive	26	86.6
Score	0	4	13.3
	1	2	6.7
	2	11	36.7
	3	9	30.0
Pattern	Absent	4	13.3
	Focal hetero	7	23.3
	Reduced homo	8	26.7
	Strong homo	11	36.7
Intensity	Negative	4	13.3
	Weak	11	36.7
	Moderate	4	13.3
	Strong	11	36.7

Table 2 : UCHL1 expressions % mean ranks in relation with total score, pattern of expression and intensity of the stain of OSCCs

		Expression % mean ranks					
Expression		Membranous	P value	Cytoplasmic	P value	Total	P value
Score	0	15.7	0.22	4	0.002		
	1	10		7.5			
	2	11.3		13.41			
	3	15.8		19.17			
Pattern	Absent	12	0.78	4.5	0.001	2.5	0.000
	Focal heterogeneous	15.8		11		9.9	
	Diffuse reduced homogeneous	16		16.06		16.25	
	Diffuse strong homogeneous	16.18		21.91		23.23	
Intensity	Negative	12	0.83	4.5	0.000	2.5	0.000
	Weak	16		12		11.7	
	Moderate	15.75		18.5		17.63	
	Strong	16.18		21.9		23.23	

Table-3. The percentage distribution of UCHL1 mean rank expression and its intracellular localization with relation to Bryne's grading systems

Expression	Mean rank percentage			Kruskal-Wallis
	Well9	Moderate13	Poor4	P value
Total positive	15.91	17.58	10.25	0.24
Membranous	16.64	15.12	14.25	0.73
Cytoplasmic	13.5	19.04	11.5	0.13

Table-4 : The score and pattern of UCHL1expression with stain intensity in relation to Bryne's grading system

Expression		Well	Moderate	Poor	P value	Somers'd
Score	0	0	2	2	0.097	-0.211
	1	0	2	0		
	2	6	4	1		
	3	3	5	1		
Pattern	Absent	2	0	2	0.207	-0.215
	Focal heterogeneous	1	5	1		
	Diffuse reduced homogeneous	2	4	2		
	Diffuse strong homogeneous	6	4	1		
Intensity	Negative	2	0	2	0.118	-0.262
	Weak	2	6	3		
	Moderate	1	3	0		
	Strong	6	4	1		

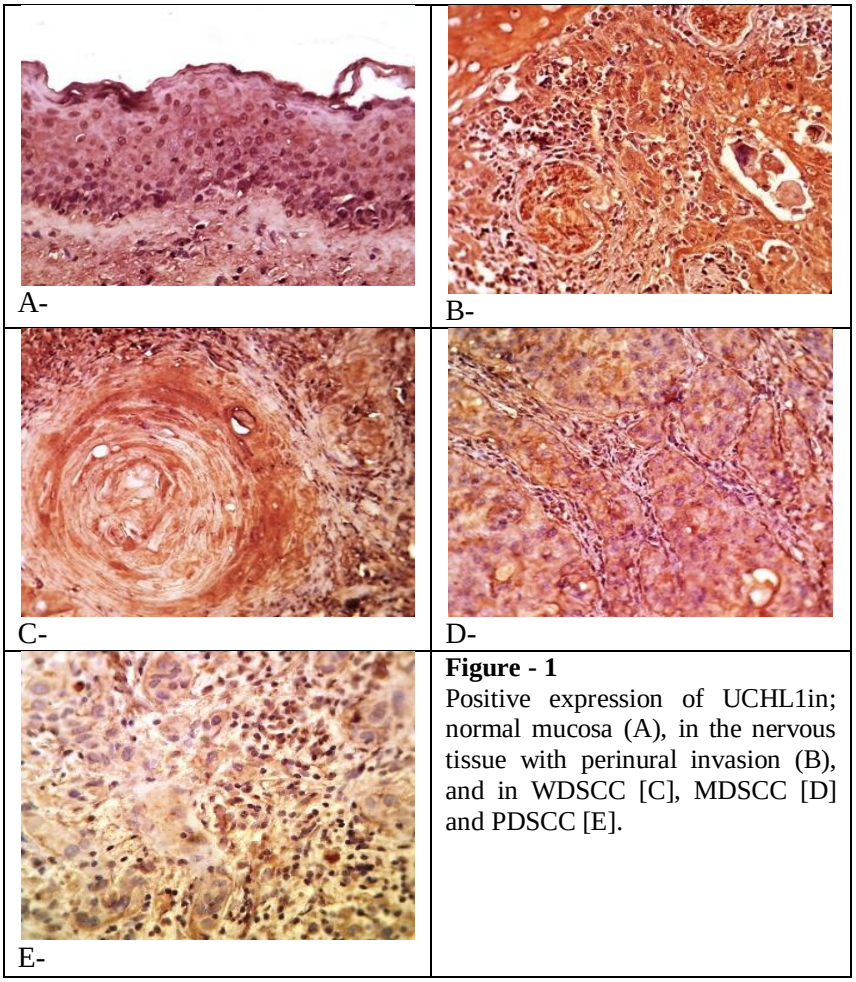

Figure - 1
Positive expression of UCHL1in; normal mucosa (A), in the nervous tissue with perinural invasion (B), and in WDSCC [C], MDSCC [D] and PDSCC [E].