Co-expression of BubR1 and UCHL1 in salivary gland tumors

Mohammed K. Mahmood[1], Balkees T. Garib[2], Marwa A. Hamied[2]*

Abstract

Objective: Salivary gland tumors (SGTs) are one of the most heterogeneous and challenging neoplasms affects human. Several biomarkers have been used to study proliferation, angiogenesis, prognosis, metastasis and recurrence of SGTs. The aim of this study was to evaluate, compare and correlate the co-expression of Budding Uninhibited by Benz imidazole Related 1 (BubR1) and Ubiquitin C-terminal hydrolase-L1 (UCHL1) immunomarkers in SGTs.

Methods: The immunohistochemical expression of BubR1 and UCHL1 were performed with formalin fixed paraffin embedded tissue sections of 35 retrieved blokes of SGTs. The expression, pattern of reactivity, intensity and subcellular localization of these markers are studied. T-test was used to find statistical difference in expression immunomarkers.

Results: All of the cases were positive for both BubR1 and UCHL1. The intensity of reaction differed between the tumor types. A significant difference was seen in the expression of BubR1 in benign versus malignant tumors (P=.002) and pleomorphic adenoma versus mucoepidermoid carcinoma (P=.001). While statistically significant difference was not seen in the expression of UCHL1 between the tumors mentioned above (P=.81 and P=.83, respectively). Finally, there was a significant difference between the expressions of BubR1 and UCHL1 in SGTs (P=.001), indicating a higher expression of UCHL1 in SGTs.

Conclusions: UCHL1 has a higher percentage and intensity of reactivity in SGTs as compared to BubR1, While BubR1 is a better immunomarker for distinguishing between benign and malignant tumors.

Keywords: *BubR1, UCHL1, Immunohistochemistry, Salivary gland tumors.*

Submitted: March 14, 2018, Accepted: May 18, 2018

Cite this article as: Mahmood MK, Garib BT, Hamied MA. Co-expression of BubR1 and UCHL1 in salivary gland tumors. Sulaimani Dent J. 2018;5(1):1-15.

DOI: https://doi.org/10.17656/sdj.10071

1. *Said Sadiq Hospital, Sulaimani, Iraq.*
2. *Department of Oral Diagnosis, College of Dentistry, University of Sulaimani, Sulaimani, Iraq.*

** Corresponding author: marwa.hamied@univsul.edu.iq*

Introduction

Salivary gland tumors are uncommon. They comprise approximately 1% of all neoplasms of the whole body. Malignant SGTs accounts for 0.3% of human malignancies and 3% to 6% of all head and neck cancers. The WHO classification system in 2005 recognizes 24 malignant and ten benign salivary epithelial neoplasms[1]. A pathological diagnosis of common types of SGTs is not difficult in typical cases[2]. However, salivary neoplasms often exhibit more than one growth pattern; significant morphologic variability may exist within a single tumor and between different tumors[3]. The overlap in the histopathological features of various types of SGTs often causes difficulties in obtaining a final and accurate diagnosis and may present a considerable diagnostic challenge. Therefore, immunohistochemistry (IHC) can be of great help[4]. immunohistochemistry has also provided insight into tumor histopathogenesis and has contributed to more accurate determination of patient prognosis[5].

Benz imidazole Related 1 (mitotic-checkpoint protein) directs proper attachment of microtubules to kinetochores (a complex of proteins associated with the centromere of a chromosome during cell division, to which the microtubules of the spindles attach) and links regulation of chromosome-spindle attachment to mitotic checkpoint signaling. Thus, disruption of BubR1 activity results in loss of checkpoint control, chromosomal instability caused by premature anaphase and the early onset of tumorigenesis[6]. Regarding the expression of BubR1 in SGTs, little is known. However, a study shows that the expression levels of Bub1 mRNA and its protein were higher in malignant SGTs than in benign SGTs and normal salivary gland tissue[7]. Another study concluded that Bub1 appeared to play a limited role in predicting prognosis in salivary ductal carcinomas[8].

Modification of proteins by ubiquitination is a fundamental mechanism in the regulation of numerous cellular activities such as DNA repair, cell cycle regulation, antigen presentation, cell-cell communication, cell differentiation and apoptosis. Deubiquitination is the opposite of this process, carried out by deubiquitinating enzymes (DUBs), which are important for regulating different cellular processes. UCHL1 belongs to the family of DUBs[9]. Regarding the SGTs, there is only one published study in English literature in which UCHL1 is considered as an important tumor suppressor gene in SGTs that may contribute to their carcinogenesis[10]. Therefore, this study was conducted to detect changes in the IHC expression of BubR1 and UCHL1 among different SGTs.

Materials and methods

A retrospective cross-sectional study was conducted in Sulaimani University from April 2017 to March 2018. The study was approved by the Ethical Committee in the College of Dentistry. A total of 35 formalin fixed paraffin blocks of primary SGTs was collected from histopathological centers in Sulaimani city. Serial 5µm tissue sections were cut from each block, one section subjected to routine H&E staining, and the remaining sections used for IHC staining as described below[11].

Sections were deparaffinized in xylene and rehydrated through series of ethanol. Antigen was retrieved by boiling in citrate buffer (pH-6, 15mins). At room temperature, sections stayed for another 15mins, and then washed with phosphate buffered saline (PBS) twice (3 mins each). Sections were wiped with a gauze pad and a circle is drawn around the tissues by a pap pen. Endogenous peroxidase activity was blocked by hydrogen peroxidase (10 mins) then protein block was applied (10 mins). Sections were incubated with primary antibodies (rabbit monoclonal anti-BuBR1 and rabbit polyclonal anti-UCHL1, dilution 1:100, Abcam; UK) for 45mins and then washed four times with PBS. After that, they were incubated with complement (10 min) and washed by PBS (3 min). Mouse anti-rabbit HRP conjugate was applied for 15mins and then washed. Sections were stained by Diaminobenzidine (DAB) (5mins in the dark) and counter-stained with hematoxylin (20 secs). Then they were dehydrated, cleared and mounted with Distyrene-plasticizer-xylene (DPX) to be ready for microscopical examined. According to manufactural instructions, normal spleen tissue for BubR1 and normal tonsil tissue for UCHL1 were served as positive controls. The negative control includes a non-immune serum by omitting primary antibody and applying antibody diluents alone. The negative and positive control tissue specimens were run with each batch of stain. All incubations were done at 37°C. The sections were not allowed to dry during the staining procedure by placing the slides in a humidified chamber.

Assessment of immunoreactivity

The slides were examined under a light microscope by two observers. Five different high spot fields (X400) of each case were selected and their digital images were uploaded to an Image J software program for Windows. Immunostained cells were counted by using the grid system of the program. The immunohistochemical signal specification was

demonstrated by the presence of immunostaining in recommended positive controls and its absence in the negative control slides. Cells were considered immunoreactive if they show cytoplasmic and membranous expression and counted. The intensity of the stain was evaluated using a semi-quantitative immuno-reactive score (IRS), which took into account the intensity of the color reaction and the percentage of positive cells. BubR1scores followed Maciejczyk et al. [12] method, the percentage of positive cells /1000 counted cells was scored as follows; 0= no positive cells, 1=<10% positive cells, 2=10-50% positive cells, 3=51-80% positive cells, and 4=>80% positive cells. For UCHL1, scoring was carried according to Hamied et al. [11] method. The percentage of positive cells /1000 counted cells was scored as follows; 0= < 5% positive cells, 1= 6-25% positive cells, 2= 26-50% positive cells, and 3= > 51% positive cells. Immunoreactivity was graded according to intensity for both BubR1 and UCHL1 as 0= no stain, 1= weak; 2= moderate; and 3= strong. The pattern of expression was evaluated at low power magnification (X100), diffuse expression and focal immune-reactive area.

Statistical analysis

Data were analyzed by using SPSS 20.0 software for Windows and applying t-test. For all analyses, $P<0.05$ was considered as significant.

Results

The studied sample included ten types of SGTs (3 benign and seven malignant tumors, Table 1). The normal spleen cells showed cytoplasmic and nuclear BubR1 expression (Figure 1 A and B), and the UCHL1 expression was cytoplasmic and membranous in tonsillar lymphoid cells (Figure 1. C and D).

Concerning the benign SGTs, results showed that all pleomorphic adenoma (PA) cases (n=16) were positively reactive to BubR1 (100%). The expression was focal within the growth. The intracellular localization of the expression was only cytoplasmic (Figure 2 A) in 9 cases, the remaining 7 cases showed both cytoplasmic reaction and some membranous expression (Figure 2 B). The ductal (luminal) cells were intensely positive in all cases. However, few cases also showed some faintly positive abluminal cells. The stroma of all PA cases was negative. Warthin tumor (WT) showed positive expression of the oncocytic cells. The expression was diffuse and dense membranous in the luminal (columnar) cells making the intercellular bridges distinct (Figure 2 C). Basal cell adenoma (BCA) showed a diffuse and moderate cytoplasmic and membranous reaction of the basaloid islands. The inner cells stained dense, while the outer cells were faint. The modified myoepithelial cells among the basaloid nest and cords were generally negative (Figure 2 D).

On the other hand, sections of malignant SGTs showed that Mucoepidermoid carcinoma (MUC) had diffuse BubR1 expression. The reactivity was mainly in the cytoplasm. The epidermoid and intermediate cells intensely stained, while the mucous and clear cells were negative (Figure 3 A and B). High-grade tumors showed a higher reactivity and strong intensity. Furthermore, adenocystic carcinoma (AdCC) also showed a diffuse immune reaction. The expression was cytoplasmic, dense, and focal within the islands of the cribriform pattern (the remaining cells showed faint or negative expression since they had scant dendritic cytoplasm) (Figure 3 C). While the solid type showed diffuse cytoplasmic expression with higher intensity and reactivity (Figure 3 D). The two studied Acinic cell carcinoma (ACC) cases showed a moderate cytoplasmic and membranous staining in few cells with limited strong nuclear staining (Figure 3 E). The expression was diffuse and moderate immunoreactivity in one case and focal in the other case (Figure 3 F). The expression in the ductal cells was mainly cytoplasmic and scant membranous. Acinic cells were immuno-negative.

Myoepithelial carcinoma (MEC) showed a diffuse or focal moderate cytoplasmic BubR1 reaction (Figure 4.A). Nuclei were either negative or showed focal/diffuse nuclear reaction. Mitotic cells were positive in the cytoplasm but negative in the dividing nucleus (Figure 4 B). Epithelial-myoepithelial carcinoma (EMEC) sections showed a diffuse cytoplasmic expression. Pleomorphism, hyperchromatism and mitotic activity was seen (Figure 4 C and D). Polymorphous low-grade adenocarcinoma (PLGA) showed a diffuse strong cytoplasmic and membranous immunoreactivity (Figure 4 E). Carcinoma ex-pleomorphic adenoma (Ca ex-PA) showed a diffuse and strong expression in the cancerous epithelial cells. The expression was cytoplasmic and membranous (Figure 4 F).

Both WT and PLGA showed the highest percentage of positive cells for BubR1 (98%), followed by Ca ex-PA (94.5%), AdCC (85.5%) and MUC (77.1%).

Table 1: Summary of histopathological diagnosis of SGTs studied sample.

Diagnosis	No.	Percentage
Pleomorphic adenoma (PA)	16	45.7
Mucoepidermoid carcinoma (MUC)	6	17.1
Adenoid cystic carcinoma (AdCC)	3	8.5
Myoepithelial carcinoma (MEC)	2	5.7
Acinic cell carcinoma (ACC)	2	5.7
Warthin tumor (WT)	2	5.7
Epithelial-myoepithelial carcinoma (EMEC)	1	2.8
Polymorphous low-grade adenocarcinoma (PLGA)	1	2.8
Basal cell adenoma (BCA)	1	2.8
Carcinoma ex-pleomorphic adenoma (Ca ex-PA)	1	2.8
Total	35	100

Table 2: Scores and description of positive BubR1 expression in SGTs.

SGT	no.	Mean +ve cells	Score			Subcellular localization	Type of cells
			Reaction	Intensity	IRS		
PA	16	38.4	2	2	0	Mainly cytoplasmic, Some membranous	+ve in ductal luminal, few abluminal, some epithelial cells.
WT	2	98	4	3	1	Membranous	+ve in oncocytic, -ve in lymphocytes
BCA	1	70	3	2	1	Mixed*	+ve in basaloid, -ve in modified myoepithelial
MUC	6	77.1	3	3	1	Mainly cytoplasmic	+ve in epidermoid and intermediate, -ve in mucous and clear cells
AdCC	3	85.5	4	3	1	Cytoplasmic	+ve in basaloid cells
ACC	2	50	2	2	0	Mixed, Some membranous	-ve in acinar cells
MEC	2	68.5	3	2	1	Mixed	+ve in epitheliod and myoepithelioid
EMEC	1	70	3	2	1	Cytoplasmic	+ve in basaloid, -ve modified myoepithelial
PLGA	1	98	4	3	1	Cytoplasmic, membranous	+ve in the round and polygonal
Ca ex-PA	1	94.5	4	3	1	Cytoplasmic, membranous	+ve in epithelial and myoepithelial

*Mixed= Cytoplasmic and nuclear.

Table 3: Scores and description of positive UCHL1 expression in SGTs.

Tumor	Case (n)	Mean +ve cells	Score		Subcellular localization	Type of cells
			Reaction	Intensity		
PA	16	89.7	3	3	Mixed, some membranous	+ve in ductal luminal, epithelial and myoepithelial cells
WT	2	98	3	3	Mixed*	+ve in oncocytic and lymphocytes
BCA	1	83.5	3	2	Mixed, some membranous	+ve in basaloid and modified myoepithelial
MUC	6	89.7	3	3	Cytoplasmic	+ve in epidermoid and intermediate, -ve mucous and clear cells
AdCC	3	94.2	3	3	Mixed, some membranous	+ve in epithelial, myoepithelial, basaloid and ductal cells.
ACC	2	62.5	2	2	Mixed	+ve acinar cells
MEC	2	92.5	3	3	Mixed	+ve in epitheliod and myoepithelioid cells
EMEC	1	91.5	3	2	Cytoplasmic	+ve in basaloid, -ve modified myoepithelial
PLGA	1	91.5	3	3	Mainly cytoplasmic, some membranous	+ve in the round and polygonal cells
Ca ex-PA	1	92.6	3	3	Cytoplasmic	+ve in epithelial and myoepithelial cells

*Mixed= Cytoplasmic and nuclear.

PA showed the lowest percentage of positive cells (38.4%), with a reaction ranging from (2-83%). The intensity of reactivity was strong in WT, MUC, AdCC, PLGA and Ca ex-PA, while it was moderate in PA, BCA, ACC, MEC, and EMEC. The immunoreactive score (IRS) was 1 for all, except PA and ACC which were scored as 0 (Table 2).

Regarding the second studied marker (UCHL1), again all PA cases showed immunoreactivity. The reactivity was mainly cytoplasmic; however, few cells had membranous and nuclear expression. The epithelial cells showed a stronger intensity as compared with the other types of cells. Generally, the myoepithelial cells reacted positively, while mucous and clear cells were negative. Ductal cells, both luminal and abluminal cells were positive. The majority of the stromal cells were also positive (Figure 5 A). The two studied cases of WT showed diffuse and strong staining against UCHL1. In one case, both the oncocytes and lymphocytes showed a concomitant cytoplasmic and nuclear staining (Figure 5 B), while the second case showed only cytoplasmic expression in both kinds of the cells (Figure 5 C). BCA showed a diffuse staining and moderate intensity. The expression was mainly cytoplasmic, although membranous and nuclear expressions were also seen (Figure 5 D). The cells in the center stained darker than the outer cells.

Mucoepidermoid carcinoma cases showed diffuse and strong reactivity against UCHL1. The epithelial, myoepithelial and intermediate cells demonstrated mainly cytoplasmic staining, while the mucous cells remained negative (Figure 6 A). AdCC cases showed a diffuse and strong cytoplasmic, nuclear and membranous expression. The intensity of the solid variant was stronger than the cribriform pattern (Figure 6 B). Except for few myoepithelial cells, the other types of cells stained positively, including epithelial, modified myoepithelial and ductal cells (Figure 6 C and D). One of the studied ACC cases showed a diffuse and strong nuclear and cytoplasmic expression (Figure 6.E), while the second case showed only a focal reaction (Figure 6 F).

Table 5: Comparison between BubR1 and UCHL1 expression in benign and malignant SGTs.

Expression	Type	n	Mean of +ve cells	SD	A p value of each marker	Comparison of both marker P value
BubR1	Benign	19	46.41	28.47	.002	0.001
	Malignant	16	76.15	23.66		
	Total	35	60.00	30.03		
UCHL1	Benign	19	90.06	20.46	.81	
	Malignant	16	88.53	17.14		
	Total	35	18.76	3.17		
P <.05 significant difference, p <.001 highly significant difference.						

Myoepithelial carcinoma cases showed diffuse and strong, cytoplasmic and nuclear UCHL1 expression. All variants of the myoepithelial cells were positive, including small round (Figure 7 A), spindle-shaped, large oval cells (Figure 7.B). EMEC showed a diffuse and moderate cytoplasmic expression. Both epithelial and myoepithelial cells reacted positively (Figure 7 C), while the clear cells remained unstained (Figure 7 D). The studied case of PLGA showed a diffuse and strong cytoplasmic reaction in the uniform round cells of PLGA (Figure 7 E). The studied case of Ca ex-PA showed a diffuse and strong cytoplasmic reactivity to UCHL1. The epithelial cells from the cancerous part of the tumor showed a higher intensity than the benign part of PA (Figure 7 F). The modified myoepithelial cells of the stroma also demonstrated a faint reactivity (Figure 7 G).

Warthin tumor gave the highest percentage of counted positive cells for UCHL1 (98%), followed by AdCC (94.2%), Ca ex-PA (92.6%), MEC (92.5), PLGA (91%), EMEC (91%), MUC (89.7%), PA (89.7%), BCA (83.5) and ACC (62.5%). The intensity of the reactivity was strong in PA, WT, MUC, AdCC, MEC, PLGA and Ca ex-PA, while it was moderate in BCA, ACC and EMEC (Table 3).

Comparing the expressions of BubR1 and UCHL1 between benign and malignant tumors, indicated only a significant difference in BubR1 expression (P=.002).

This difference was in particular between PA and MUC (P=.001, Table 4); malignant SGTs showed diffuse strong expression. Finally, SGTs showed higher UCHL1 expression than BubR1 (Table 5).

Discussion

Salivary gland tumors represent a diverse group of tumor types with a wide range of biological behaviors and histopathologic characteristics, which complicates their diagnosis and management. This study focused on the co-expression of BubR1 and UCHL1 in SGTs. Generally, both of the markers showed various subcellular localizations such as diffuse cytoplasmic, granular cytoplasmic, diffuse nuclear, partial nuclear and concomitant mixed expressions. The cause of these variations may be related to differences in tumor composition, tumor size, histopathological grade of the tumor, the age of the patient, sampling techniques and presence or absence of co-existing tumors in the same area.

The spindle assembly checkpoint ensures accurate separation of chromosomes. The checkpoint is mediated by a signal transduction system composed of Mad and Bub proteins. The human Bub1 gene is a protein kinase which localizes to kinetochores very early in prophase and plays a surveillance role in preventing the missegregation of chromosomes[7].

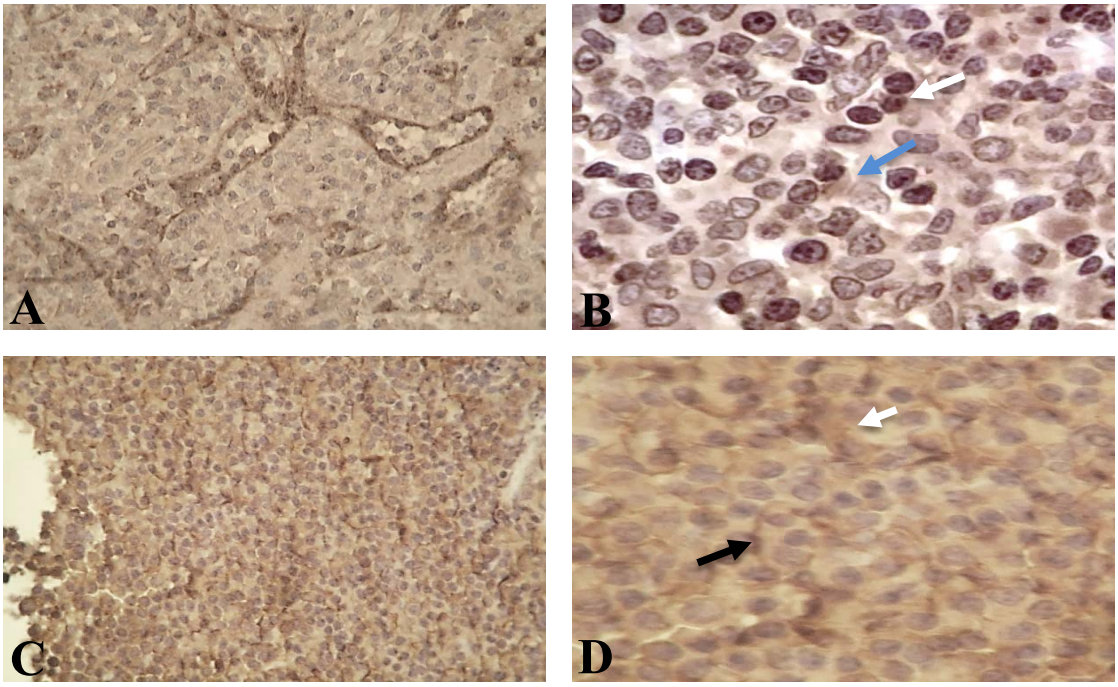

Figure 1: Positive controls: (A, B) Positive cytoplasmic (blue arrow) and nuclear (white arrow) expression of BubR1 in normal spleen cells, (C, D) Cytoplasmic (white arrow) and membranous (black arrow) expression of UCHL1 in tonsillar lymphoid cells. (IHC, A, C 100X; B, D 400X).

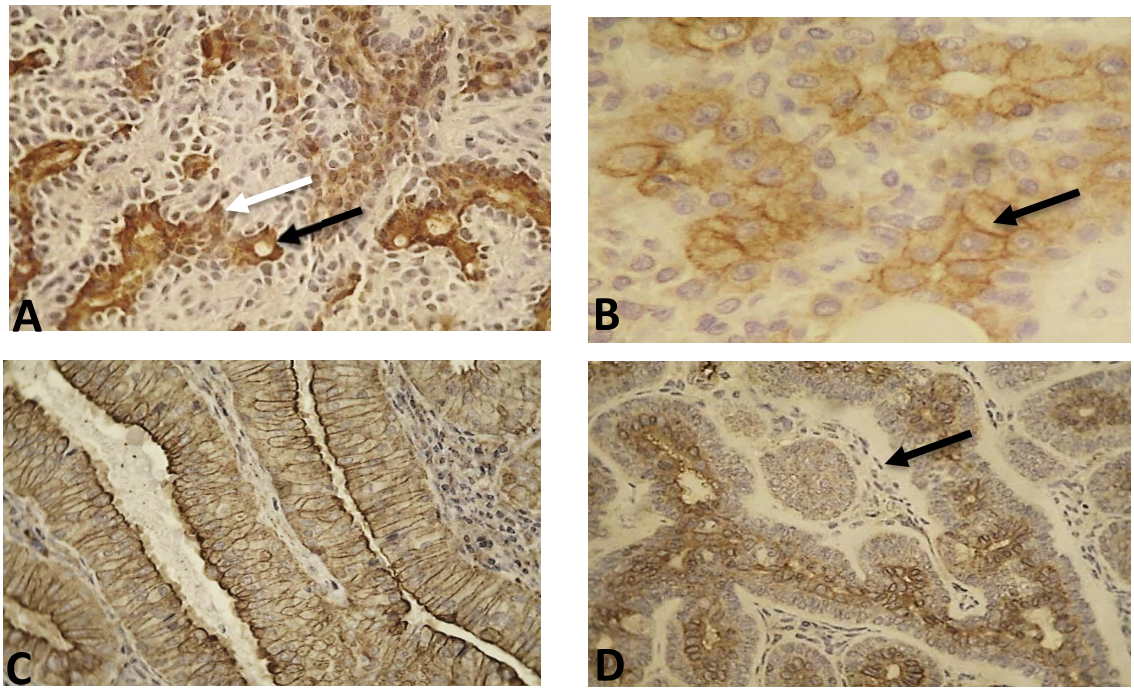

Figure 2: BubR1 expression in benign SGTs, (A) PA: Intense cytoplasmic expression in the ductal luminal cells (black arrow) and few abluminal cells (white arrow). The stromal cells are negative (IHC 100X), (B) PA: Membranous expression (arrow) (IHC 400X), (C) WT: Dense membranous expression in the oncocytic cells (IHC 100X), (D) BCA: Cytoplasmic and membranous expression of inner basaloid cells, modified myoepithelial cells were negative (arrow) (IHC 100X).

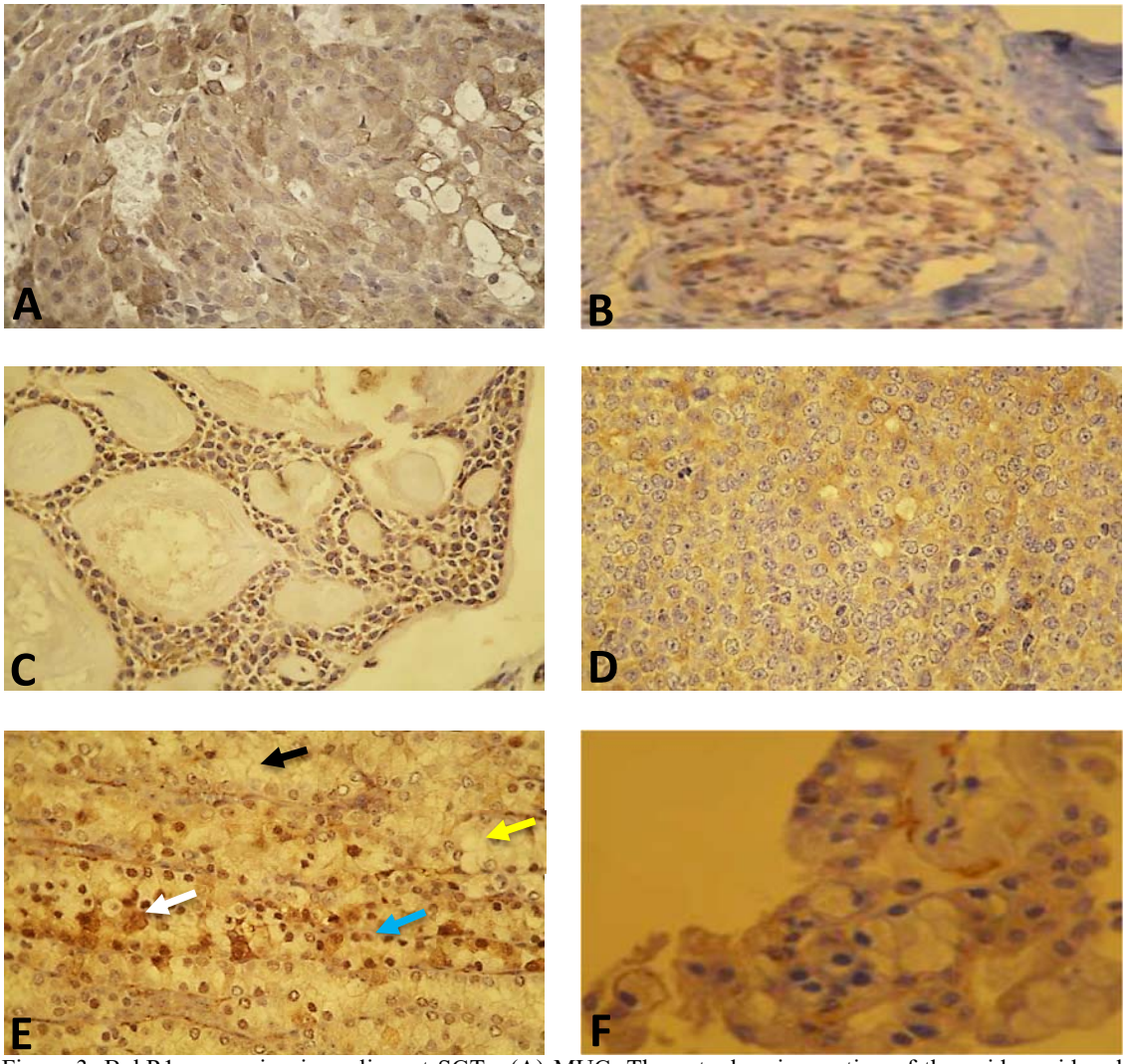

Figure 3: BubR1 expression in malignant SGTs, (A) MUC: The cytoplasmic reaction of the epidermoid and intermediate-grade tumor with negative mucous and clear cells (right) (IHC 400X), (B) MUC: Low-grade tumor mainly composed of negative mucous and clear cells (IHC, 400X), (C) AdCC: Cytoplasmic expression focal within the islands of the cribriform pattern (IHC 100X), (D) AdCC: Diffuse cytoplasmic expression in the solid pattern (IHC 400X), (E) ACC: Cytoplasmic (white arrow), membranous (black arrow) and nuclear expression (blue arrow). The acinic cell is negative (yellow arrow) (IHC 100X), (F) ACC: Few cells with faint staining (IHC, 400X).

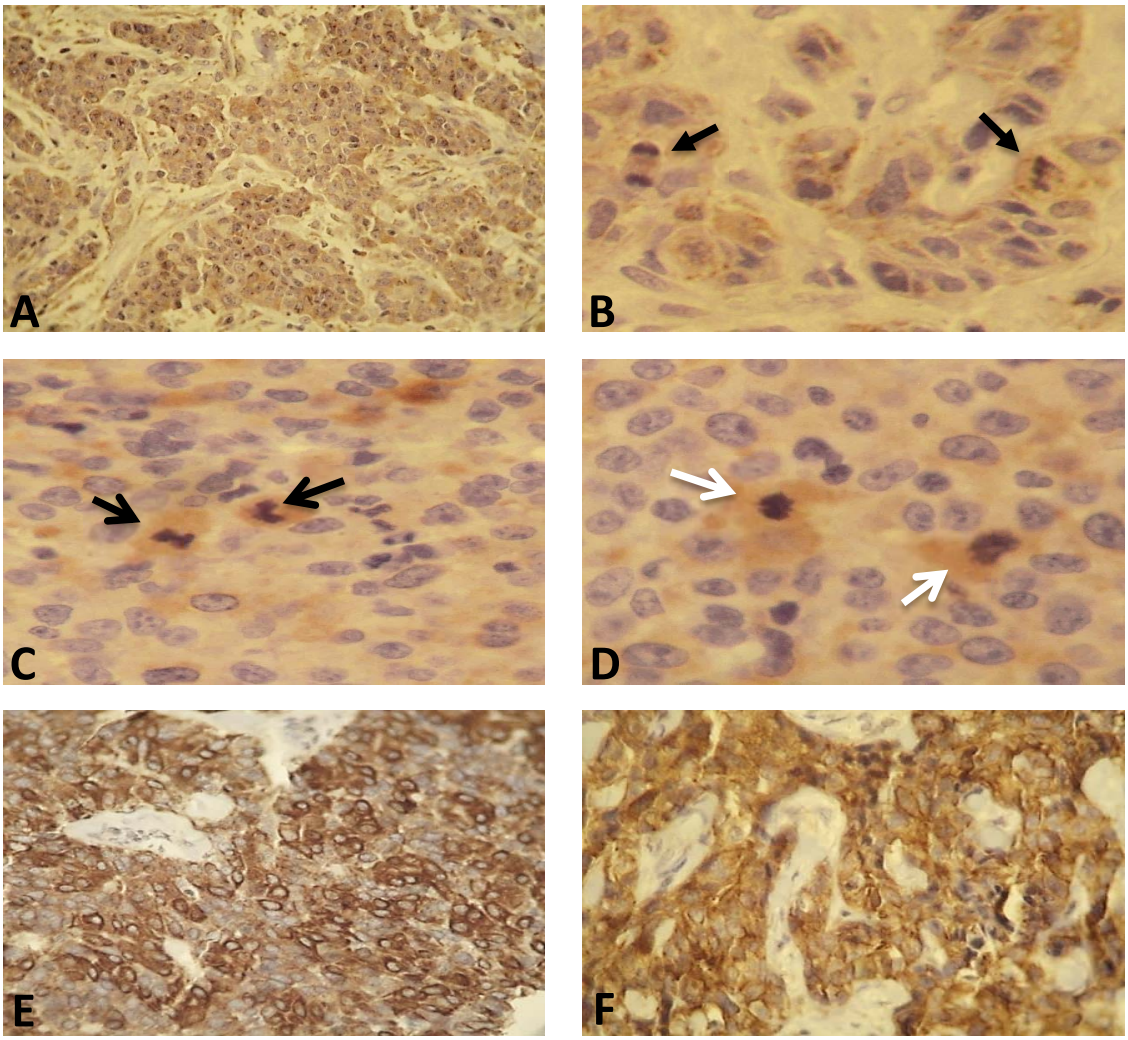

Figure 4: BubR1 expression in malignant SGTs, (A) MEC: Immunoreactivity of epithelioid and myoepithelioid cells (IHC, 100X), (B) MEC: Positivity in the cytoplasm and negativity in the nucleus of mitotic cells (IHC, 400X), (C and D) EMEC: Cytoplasmic expression of mitotic cells (black arrows), Cytoplasmic expression of hyperchromatic cells (white arrows) (IHC, 400X), (E) PLGA: Cytoplasmic and membranous expression (IHC, 400X), (F) Ca ex-PA: Diffuse cytoplasmic and membranous expression in the epithelial cells (IHC, 400X).

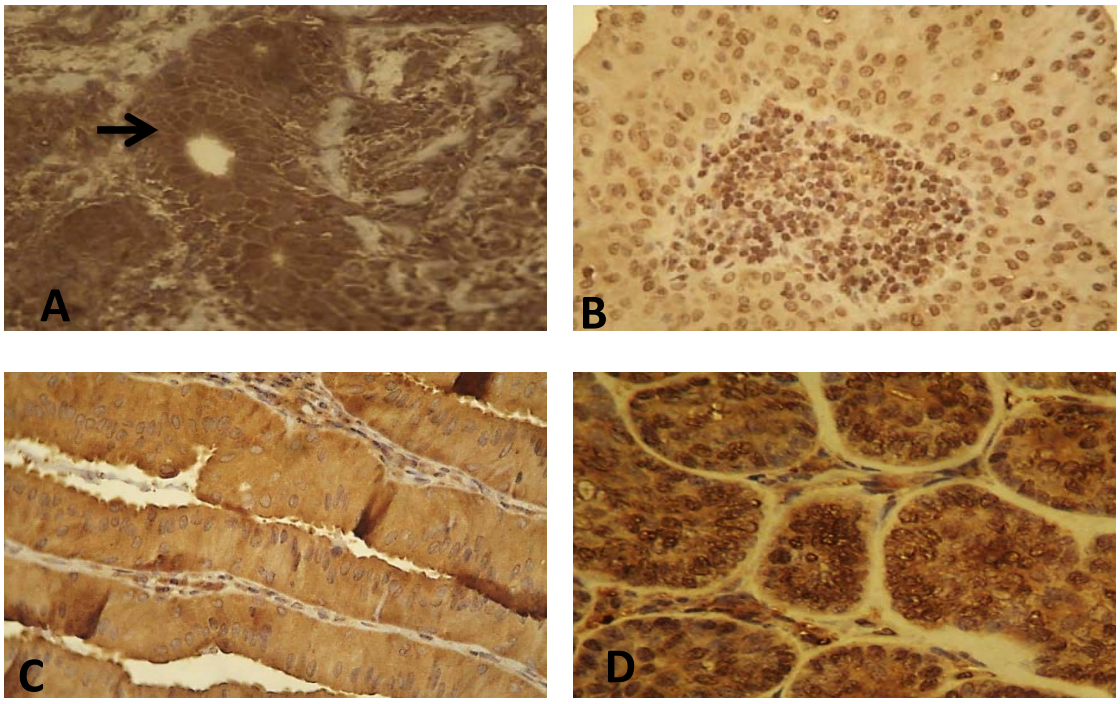

Figure 5: UCHL1 expression in benign SGTs, (A) PA: Expression in the luminal and abluminal cells of ducts. Some cells show nuclear staining also (arrow) (IHC, 400X), (B) WT: Both the oncocytes and the lymphocytes show both cytoplasmic and nuclear staining (IHC, 400X), (C) WT: The oncocytes and the lymphocytes show cytoplasmic expression with a retained blue color of their nuclei (IHC, 100X), (D) BCA: Cytoplasmic and membranous expression (IHC, 400X).

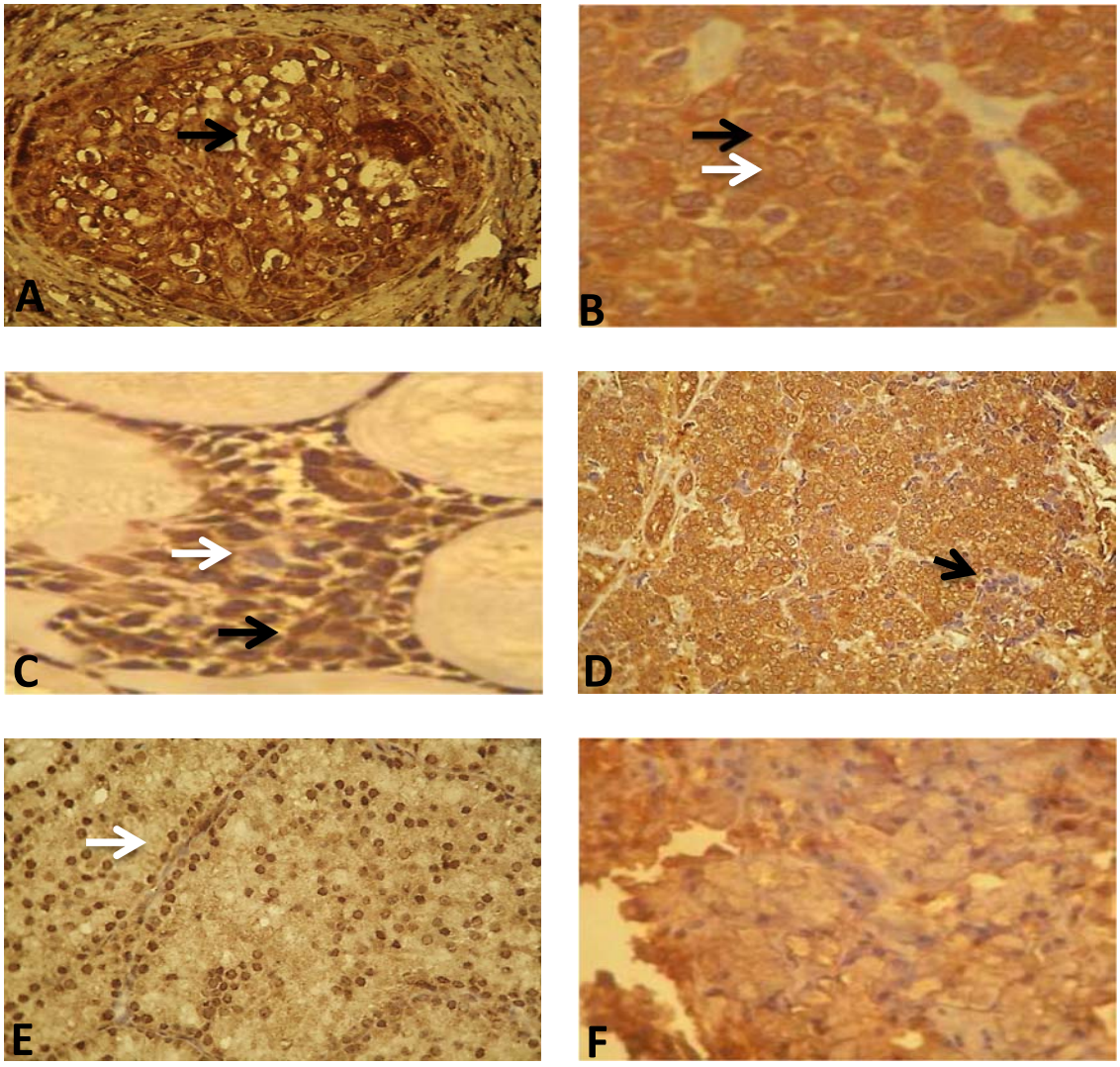

Figure 6: UCHL1 expression in malignant SGTs, (A) MUC: Immunoreactivity of pleomorphic epidermoid and intermediate cells mixed with non-staining mucous cells (arrow) (IHC, 400X), (B) AdCC: strong intensity in the solid variant, dark nuclear expression in a mitotic cell (black arrow), membranous and cytoplasmic expression (white arrow) (IHC, 400X), (C) AdCC: Cribriform variant, nuclear and cytoplasmic expression (black arrow), negative blue nuclei (white arrow) (IHC, 400X), (D) AdCC: Cytoplasmic and nuclear expression of round epithelial cells. The spindle-shaped myoepithelial cells with blue nuclei remain non-stained (arrow) (IHC, 100X), (E) ACC: Nuclear expression (arrow) (IHC, 400X), (F) ACC: Focal cytoplasmic reactivity (IHC, 400X).

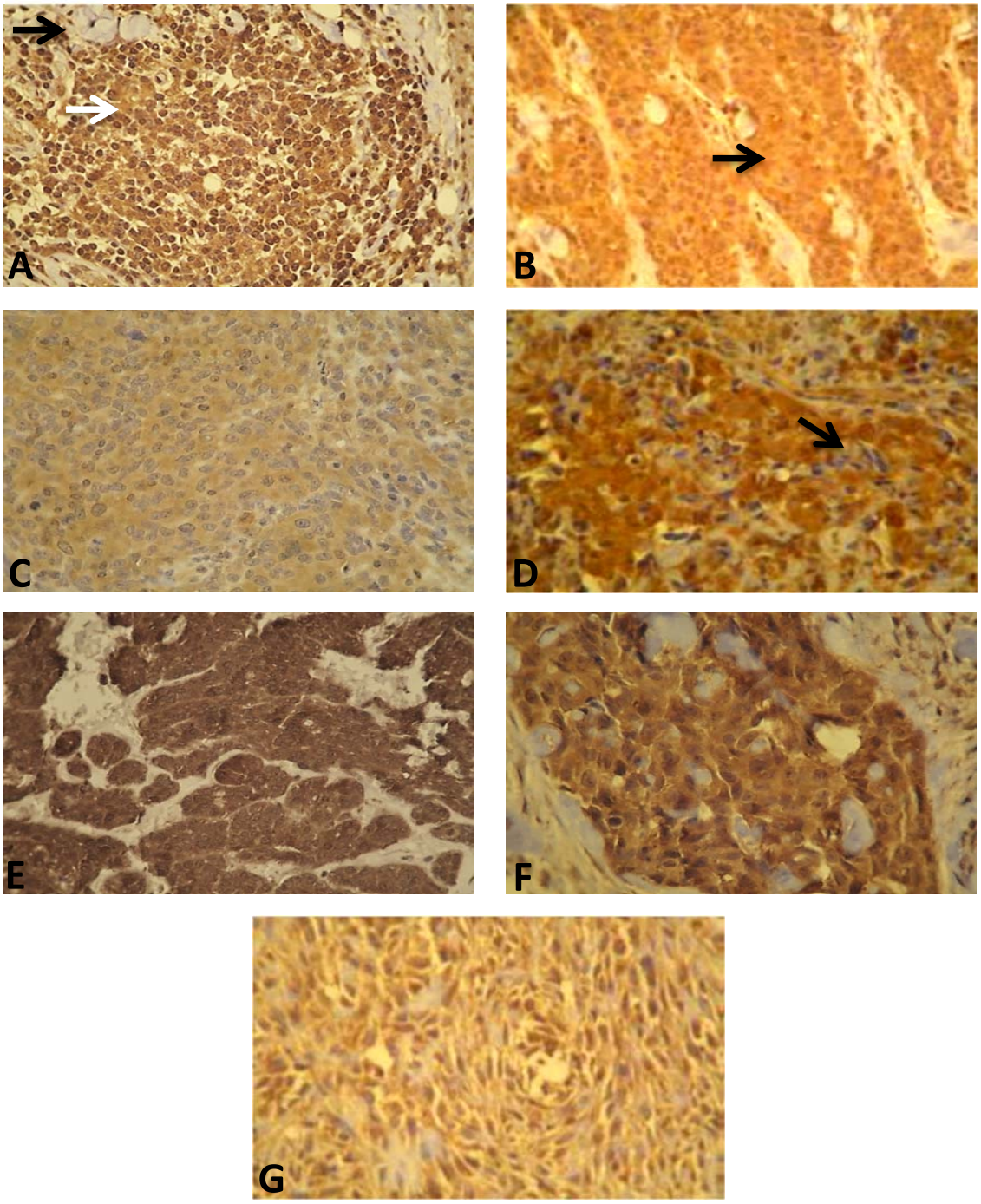

Figure 7: UCHL1 expression in malignant SGTs, (A) MEC: Cytoplasmic and nuclear staining of round and small cells (white arrow). Clear cells are negative (black arrow) (IHC, 400X), (B) MEC: Expression in large oval cells (arrow) (IHC, 400X), (C) EMEC: Cytoplasmic expression in large polyhedral epithelial cells (IHC, 400X), (D) EMEC: spindle-shaped myoepithelial cells remained not stained cells (arrow) (IHC, 400X), (E) PLGA: Cytoplasmic expression in uniform round cells (IHC, 400X), (F) Ca ex-PA: Strong expression from the cancerous part of the tumor. (IHC, 400X), (G) Ca ex-PA: Faint expression of the benign portion of the tumor (IHC, 400X).

Regarding the immunohistochemical expression of BubR1 in SGTs, to our knowledge, there are only two published studies in the English literature; one of them dealt with the correlation of human BubR1 expression with tumor proliferating activity in SGTs[7]. While the other one studied the expression of mitotic checkpoint proteins BuB1B and MA2L1 in salivary ductal carcinomas[8]. However, both of these papers did not mention the immunohistochemical expression of BubR1 in different types of SGTs.

In this study, the immunohistochemical expression of BubR1 in all studied cases was positive. It was mainly cytoplasmic and focal membranous with nuclear staining was also seen. Interestingly, the expression of BubR1 was not noted predominantly in the nuclei of tumor cells, but throughout the cytoplasm in cells of SGTs. This result is in accordance with the previous study for BubR1 expression in salivary ductal carcinomas[8]. However, it differed from a study that noted nuclear and cytoplasmic localization of BubR1 in different cancers, including pancreatic adenocarcinoma, SCC of the skin and colon cancers. This latter study recorded that BubR1 stained the cytoplasm of all normal tissues' cells, but the subcellular localization of BubR1 shifted from cytoplasmic to the nucleus in malignant ones[13]. This study agrees with this finding, in the way that we observed more nuclear staining in malignant tumors as compared to benign tumors.

Ductal luminal cells were the most reacting cells, followed by epithelial cells (in the form of nests, cords, and sheets) and some abluminal cells. The wide range of reactivity of PA (2%-83%) may reflect the pleomorphic and heterogenic nature of PA. Histologically, nine PA cases were cell-poor, therefore, they had a low percentage and intensity of reactivity as compared to the cell-rich cases. On the other hand, in the BCA which had a highly cellular solid growth and it is still a benign tumor, BubR1 reactivity is confined to the center of the islands or sheets. Despite the benign nature of WT, it had the highest percentage of reactivity (98%) such as PLGA; this may be related to high uptake ability of the oncocytic cells of WT. ACC contained uniform well-differentiated cells, resembling normal acinic cells, nevertheless, it differed from PA by showing nuclear BubR1 expression. Despite its malignant nature, the ACC had a relatively low percentage of reactivity (50%) and an IRS score of 0. This result may be due to the vast difference between the expressions of the two studied cases since one of them scored (95%), while the other was (5%). The possible cause for this may be related to the tumor composition; the more differentiated tumor with acinic cells the lesser reaction and the more solid growth, the stronger reaction.

It is worth to mention that the difference in BubR1 expression between the two MEC studied could be attributed to the difference in the proportion or the epithelium such as myoepithelial cells, the existence of atypia and mitosis within the stained sections. In the studied case of Ca ex-PA, the reactivity and intensity were higher in the malignant portion of the tumor compared with the benign portion, pointing again to the relation of BubR1 expression and mitotic activity. A higher percentage of reactivity may be related to a more aggressive tumor. Thus, malignant SGTs showed diffuse strong expression and altered intracellular localization. They can show more membranous and nuclear strong expression beside the cytoplasmic one. It should be noted that functional BubR1 is key importance for dividing cells but not for resting cells. Thus, no activity of BubR1 should be noted in non-dividing cells. The activity of BubR1 correlates mainly with an aggressive form of the tumor. High expression reflects the high proliferative ability of neoplastic cells[14]. The significant difference between expressions of BubR1 in benign and malignant tumors is in agreement with other papers that compared BubR1 expression in potentially malignant oral lesions and OSCC, in which the reactivity of OSCC was significantly higher than the potentially malignant oral lesions[15, 16]. Ubiquitination and deubiquitination are vital processes in protein metabolism carried out by addition or separation of ubiquitin monomers to the proteins. UCHL1 belongs to the family of UCH of the DUBs and its role in tumorigenesis has been investigated in many tumors[17]. Regarding the immunohistochemical expression of UCHL1 in SGTs, there is only one published paper in the English literature which studied quantitative methylation profiles for multiple tumor suppressor gene promoters in SGTs, including UCHL1, but the immunohistochemical reactivity of UCHL1 in various SGTs was not mentioned.

The high percentage of UCHL1 positive cytoplasmic expression is in agreement with its expression in breast cancer[18] and neurodegenerative diseases[19]. While its focal nuclear and membranous expressions were also reported by Bheda et al. (2010)[20] and Caballero et al. (2002)(21). Ubiquitin C-terminal hydrolase-L1 expression in PA included luminal, abluminal and even the stromal cells, thus differing from the BubR1 expression which was mainly confined to the luminal cells. The UCHL1 and BubR1expressions in the BCA were of the similar pattern (darker stained in inner than the outer cells), except for the fact that UCHL1 total positivity was stronger. WT had the highest percentage

of reactivity, pointing out again to the possible high uptake of oncocytic cells. Interestingly, one of the WT cases had a concomitant cytoplasmic and nuclear expression, while the other one only had a cytoplasmic reactivity.

The extranuclear expression may be related to a more clinically aggressive tumor. Expression of UCHL1 in malignant tumors, such as MUC, AdCC, ACC, MEC and EMEC showed a higher percentage of reactivity as compared to their counterpart BubR1 reactivity. Thus, indicating that the protein modification and deubiquitination by UCHL1 takes place in a larger number of cells including dividing and resting cell, differing from BubR1 which is predominantly expressed in dividing cells. The independent sample t-test is used to compare the expressions of UCHL1 in benign vs. malignant and PA vs. MUC as representatives of benign and malignant tumors respectively. But none of them were significant. These results differ from a paper regarding the pancreatic endocrine tumors, in which a significant difference in the expression between benign and malignant tumors was recorded[22].

Finally, there was a statistically significant difference between the expressions of BubR1 and UCHL1 in SGTs, indicating that UCHL1 is expressed higher than the BubR1. This is probably because of the predominant expression of BubR1 in mitotically active cells since it is a mitotic checkpoint protein. While the UCHL1 could be expressed in both mitotically active and passive labile cells. For future studies, a larger sample is suggested since our sample contained 35 cases which are relatively small. The usage of cell-specific antibodies is suggested to specify different compartments like epithelial, myoepithelial cells, etc. Further correlation with clinical data is recommended.

Conclusions

Both BubR1 and UCHL1 are expressed in different SGTs in different subcellular localizations including cytoplasm, membrane and nucleus. Except for WT, which has a high uptake and percentage of reactivity despite its benign nature, the IHC expression of BubR1 can differentiate between benign and malignant SGTs, including the differentiation between PA and its malignant counterpart. Moreover,, BubR1 may differentiate the more active PAs from the less active ones since the active cases are expected to react stronger. But, UCHL1 expression cannot differentiate between benign and malignant tumors. UCHL1 expression is higher in percentage and stronger in intensity as compared to BubR1 since the latter is predominantly expressed in mitotically active cells, while the former is expressed both in the mitotic and passive labile cells. As a result, UCHL1 is expressed higher in the SGTs, but the BubR1 is more useful in differentiation between benign and malignant tumors.

References

1. Speight PM and Barrett AW. Prognostic factors in malignant tumors of the salivary glands. Br J Oral Maxillofac Surg. 2009;47(8):587-93.

2. Nagao T, Sato E, Inoue R, Oshiro H, Takahashi RH, Nagai T, et al. Immunohistochemical analysis of salivary gland tumors: application for surgical pathology practice. Histochem Cytochem. 2012;45(5): 269-82.

3. Ito FA, Jorge J, Vargas PA and Lopes MA. Histopathological findings of pleomorphic adenomas of the salivary glands. Medi Oral Pathol 2009; 14: 57-61.

4. Shieh YS, Chang LC, Chiu KC, Wu CW, Lee HS. Cadherin and catenin expression in mucoepidermoid carcinoma: correlation with histopathologic grade, clinical stage, and patient outcome. J Oral Pathol Med. 2003;32(5):297-304.

5. Lee KS, Kwak Y, Nam KH, Kim D-W, Kang S-B, Choe G, et al. Favorable prognosis in colorectal cancer patients with co-expression of c-MYC and ß-catenin. BMC Cancer. 2016;16(1):730.

6. Park H-Y, Jeon Y-K, Shin H-J, Kim I-J, Kang H-C, Jeong S-J, et al. Differential promoter methylation may be a key molecular mechanism in regulating BubR1 expression in cancer cells. Exper Mol Med. 2007;39(2):195-204.

7. Shigeishi H, Yoneda S, Taki M, Nobumori T, Ohta K, Higashikawa K, et al. Correlation of human Bub1 expression with tumor proliferating activity in salivary gland tumors. Oncol Rep. 2006;15(4):933-38.

8. Ko YH, Roh JH, Son YI, Chung MK, Jang JY, Byun H, et al. Expression of mitotic checkpoint proteins BUB1B and MAD2L1 in salivary duct carcinomas. J Oral Pathol and Med. 2010;39(4):349-55.

9. Schröder, C, Milde-Langosch K, Gebauer F, Schmid K, Mueller V, Wirtz RM, et al. Prognostic relevance of ubiquitin C-terminal hydrolase L1 mRNA and protein expression in breast cancer patients. J Cancer Res Clin Oncol. 2013;139(10):1745-55.

10. Durret ML, Mydlarz WK, Shao C, Zahurak ML, Chuang AY, Hoque MO, et al. Quantitative

methylation profiles for multiple tumor suppressor gene promoters in salivary gland tumors. PLoS One 2010; 5(5): e10828.

11. Hamied MA, Garib BT, Mohammad DN. UCHL1 expression in OSCC in relation to the invasive front grading system. IOSR-JDMS. 2015;14(7):93-97.

12. Maciejczyk A, Szelachowska J, Czapiga B, Matkowski R, Hałoń A, Györffy B, and Surowiak P. Elevated BUBR1 expression is associated with poor survival in early breast cancer patients: 15-year follow-up analysis. J Histochem and Cytochem. 2013; 61(5):330-39.

13. Burum-Auensen E, Deangelis PM, Schjølberg AR, Røislien J, Andersen SN & Clausen O. Subcellular localization of the spindle proteins Aurora A, Mad2, and BUBR1 assessed by immunohistochemistry. J Histochem & Cytochem. 2007;55(5):477-86.

14. Lee Y, Choi E, Kim MA, Park P, Park N & Lee H. BubR1 as a prognostic marker for recurrence-free survival rates in epithelial ovarian cancers. Br J Cancer. 2009;101(3):504-10.

15. Hsieh P-C, Chen Y-K, Tsai K-B, Shieh T-Y, Chang Y-Y, Chang J-G, et al. Expression of BUBR1 in human oral potentially malignant disorders and squamous cell carcinoma. J Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;109(2):257-67.

16. Lira RC, Miranda FA, Guimarães M, Simões RT, Donadi EA, Soares CP, et al. BUBR1 expression in benign oral lesions and squamous cell carcinomas: correlation with human papillomavirus. Oncol Rep 2010;23(4):1027-36.

17. Hurst-Kennedy J, ChinL-S and LiL. Ubiquitin C-terminal hydrolase l1 in tumorigenesis. Biochem Res Int. 2012;2012: 123706.

18. Xiang T, Li L, Yin X, Yuan C, Tan C, Su X, et al. The ubiquitin peptidase UCHL1 induces G0/G1 cell cycle arrest and apoptosis through stabilizing p53 and is frequently silenced in breast cancer. PloS one 2012; 7(1):e29783.

19. Lowe J, McDermott H, Landon M, Mayer RJ, and Wilkinson KD. Ubiquitin carboxyl-terminal hydrolase (PGP9.5) is selectively present in ubiquitinated inclusion bodies characteristic of human neurodegenerative diseases. J Pathol. 1990;161(2): 153-60.

20. Bheda A, Gullapalli A, Caplow M, Pagano JS & Shackelford J. Ubiquitin editing enzyme UCHL1 and microtubule dynamics: implication in mitosis. Cell Cycle. 2010;9(5):980-94.

21. Caballero OL, Resto V, Patturajan M, Meerzaman D, Guo MZ, Engles J, et al. Interaction and colocalization of PGP9. 5 with JAB1 and p27Kip1. Oncogene. 2002;21(19):3003-10.

22. Tomita T. PGP 9.5 immunocytochemical staining for pancreatic endocrine tumors. Islets. 2013;5(3):122-128.