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RESEARCH ARTICLE

# Cytokeratin 5/6 Phenotypic Expression in Urothelial Bladder Carcinoma: Relation to Clinicopathological Parameters, FAP and CD147 Immunohistochemical Expression

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## ABSTRACT

**Background**: Urothelial bladder carcinoma is a heterogenous disease with variable prognosis. The basal cytokeratin (CK5/6) is associated with basal molecular subtype of urothelial carcinoma and is speculated to be associated with aggressive tumor behavior. **Aim**: This study aimed to evaluate immunohistochemical expression of CK5/6, fibroblast activation protein (FAP) and CD147 in urothelial bladder carcinoma in relation to clinicopathological parameters, and to determine clinicopathological characteristics of CK5/6 positive urothelial carcinoma. **Material and Methods:** The current work included 60 cases of urothelial bladder carcinoma, which were subjected to immunohistochemical staining using CK5/6, FAP and CD147. The association between these markers' expression and clinicopathological parameters was assessed. **Results:** Positive expression for CK5/6 was detected in 48.3% of cases. CK5/6 immunoreactivity was significantly related to tumor grade (p-value = 0.002*), muscle invasion (p-value = 0.021*), squamous differentiation (p-value = 0.020*), FAP expression (p-value<0.001*), and CD147 expression (p-value=0.001*). No significant association was detected as regard patients' age, sex, or lymphovascular invasion. In turn, FAP expression in tumor microenvironment was significantly related to tumor grade and lymphovascular invasion, but not to muscle invasion or squamous differentiation. In addition, the expression of CD147 showed significant association with tumor grade and muscle invasion. **Conclusions:** Cytokeratin 5/6 expression in urothelial bladder carcinoma is associated with features of aggressive tumor behaviour (in terms of high tumor grade, advanced stage, and squamous differentiation), FAP and CD147 immunoreactivity. Such markers can be considered as progression markers for urothelial carcinoma.

**Keywords:** CK5/6, CD147, FAP, Tumor microenvironment, Urothelial bladder carcinoma

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## INTRODUCTION

Bladder cancer ranks as the fourth most common diagnosed cancer in males with a rising incidence every year. It represents a leading cause of cancer related morbidity and mortality (Siegel et al., 2019). Urothelial bladder carcinoma, *the most common histological subtype*, is a heterogeneous disease with divergent outcome and response to therapy (Als et al., 2007). Despite its good prognosis, it has been reported that about 10-20% of the superficial non-muscle invasive cases will progress into more aggressive deeply invasive disease and the reported recurrence rate of bladder carcinoma is about 40-70%. The prognosis of the deep muscle invasive cases is worse, with about 50% survival rate and high rate of metastasis even after radical cystectomy (Kang et al., 2017). Hence, studying novel biomarkers that help to stratify urothelial carcinoma cases and serve as potential therapeutic targets is mandatory (Wittschieber et al., 2011).

Recently, molecular and genomic analyses further classify urothelial carcinoma into two main molecular subtypes: *basal and luminal subtypes,* utilizing surrogate immunohistochemical markers, akin to

molecular classification of breast carcinoma (Choi$_a$ et al., 2014 and Wang et al., 2019). Molecular classification provides more association with biological tumor behavior and may provide basis for individualized treatment (Zhu et al., 2020). GATA 3 and CK5/6 are amongst the primary markers commonly used to reflect luminal and basal subtypes of bladder carcinoma, respectively, with more than 90% accuracy (Dadhania et al., 2016). However, data on the prognostic significance of these biomarkers are controversy. Bladder carcinomas with basal phenotype are aggressive tumors with poor prognosis and high rate of chemotherapy resistance (Yuk et al., 2019). Even before the era of molecular classification, the expression of CK5/6 in invasive urothelial carcinoma has been reported to be associated with poor outcome (Gaisa et al., 2011).

The profound role of tumor microenvironment on cancer progression has attained much attention. Several studies have emphasized the potential role of tumor-stromal interaction on neoplastic progression, spread and metastasis (Kang et al., 2017).

Cancer associated fibroblasts (CAFs) represent a major component of tumor microenvironment. They are activated fibroblasts and myofibroblasts which are immunoreactive to fibroblast activated protein (FAP), smooth muscle actin (SMA) and platelet derived growth factor receptors (PDGFR) (Xing et al., 2010). They can affect tumor progression through production of many cytokines, growth factors and proangiogenic factors that favor tumor growth and promote metastasis. Moreover, CAFs are involved in epithelial mesenchymal transition (EMT), a process which facilitate metastasis of malignant tumors (Schulte et al., 2012). Fibroblast activation protein (FAP) is a transmembrane peptidase which is overexpressed in activated fibroblasts surrounding wide range of epithelial malignancies, with no expression in normal resting fibroblasts or in benign and premalignant lesions (Liu et al., 2012).

Extracellular matrix metalloproteinase inducer (EMMPIN), also known as CD147, is a transmembrane glycoprotein of the immunoglobulin family. Tissue expression of CD147 increases in remodeling processes including inflammation, repair, tumor development and progression (Xue et al., 2011). CD147 has been reported to be overexpressed in a range of malignant tumors. It has been reported to be involved in anaerobic glycolysis of malignant neoplasm favoring tumor cell proliferation, tumor invasion and metastasis (Peng et al., 2020). Moreover, CD147 may be a promising therapeutic target in many malignant tumors including bladder cancer (Xue et al., 2011).

The current work aimed to evaluate the immunohistochemical expression of the basal cytokeratin (CK5/6), FAP and CD147 in urothelial bladder carcinoma and to assess their relationship with the clinicopathological parameters. In addition, we aimed to determine clinicopathological characteristics of CK5/6 positive urothelial carcinoma.

## MATERIALS AND METHODS

The current retrospective study included 60 cases of urothelial bladder carcinoma, retrieved as 35 transurethral resection of bladder tumor (TURBT) and 25 cystectomy specimens, from the archives of the Pathology Department, Faculty of Medicine, Tanta University, during the period from October 2018 to October 2020. Cases with scanty neoplastic tissue and TURBT cases not including muscularis propria were excluded from the study. All cases were subjected to H&E staining. The grade of differentiation and depth of invasion (pT category) were evaluated according to WHO grading system and the recommendations of American joint committee of cancer staging 2017, respectively. The tumor stage was further divided into non-muscle invasive (stages pTa, and pT1) and muscle invasive (pT2-4). Approval from the research ethics committee (REC) was obtained before conducting the study.

Immunohistochemical staining for the included cases was done using the following antibodies: anti-CK5/6 antibody, a mouse monoclonal antibody (1:100 dilution; D5/16 B4; Dako, Glostrup, Denmark), anti-CD147 antibody, a mouse monoclonal antibody (1:200 dilution; 8D6 sc-21746; Santa Cruz Biotechnology, Inc, USA), and anti-FAP antibody, a mouse

monoclonal antibody (1:70 dilution; clone 427819; Santa Cruz Biotechnology, Inc, USA).

Sections (5 um thick), on positively charged slides, were left to dry for 30 min at 37 °C. Deparaffinization and antigen retrieval were performed in a Dako PT Link unit. Both high and low pH EnVision TM FLEX Target Retrieval Solutions were used at 97 °C for 20 min. Dako automated immune-stainer (Link 48) was used for immunostaining. The slides were incubated with the primary antibodies for 20–30 min, following treatment with a peroxidase-blocking reagent for 5 min; with subsequent addition of horseradish peroxidase (HRP) reagent for 20 min and diaminobenzidine (DAB) chromogen solution for 10 min. Hematoxylin was applied for counterstaining.

**Assessment of CK5/6 immunohistochemistry:** CK5/6 was assessed as cytoplasmic and/or membranous staining in the neoplastic cells. The immunoreactivity was semiquantitatively classified, according to the percentage of stained tumor cells, into negative or minimal (less than 10%), partial staining (10–80%) and diffuse staining (more than 80% staining). For statistical purpose, cases with partial and diffuse staining were considered as positive group (Wang et al., 2019).

**Assessment of FAP immunohistochemistry:** FAP immunoreactivity was assessed as positive cytoplasmic staining in the stromal fibroblasts adjacent to neoplastic nests. Epithelial and macrophages staining was ignored. The staining was semiquantitatively scored by multiplying the percentage of stained stromal fibroblasts and the intensity of staining. The intensity was classified into mild, moderate, strong on a scale from (1 to 3). The percentage of staining was classified into; 0 (less than 25% staining), 1 (26–50% staining), 2 (51–75% staining), 3 (more than 75% staining). The final score was further classified into negative/ low expression score (0-3) and high expression score (4-9) (Cao *et al.,* 2018 and Mezheyeuski et al., 2020).

**Assessment of CD147 immunohistochemistry:** CD147 immunoreactivity was assessed as membranous staining in the neoplastic cells and semiquantitatively scored by multiplying the percentage of stained cells and the intensity of staining. The percentage of staining was scaled from 0–4 as following; 0 (negative staining), 1 (less than 25% staining), 2 (26–50% staining), 3 (51–75% staining), 4 (more than 75%). The intensity signal was scaled from 1 to 3:1 (mild signal), 2 (moderate signal), 3 (strong signal). The final score was divided into: negative/ low score (0–4) and high score (6–12) (Peng et al., 2020).

**Statistical analysis**

The collected data were statistically analyzed using the SPSS software statistical computer package (version 23). Kolmogorov- Smirnov test was used to verify the normality of distribution of variables. Data were expressed in terms of frequencies (number of cases) and percentages for categorical variables and mean±standard deviation (SD) for continuous variables. For comparing categorical data, Chi-square ($\chi 2$) test was used as a test of significance. Fisher's exact test or Monte Carlo test were used when appropriate. P values of $<0.05$ were considered statistically significant.

## RESULTS

### Clinicopathologic characteristics

Sixty cases of bladder urothelial carcinoma were included in the current study. Thirty-three cases were high grade carcinomas; representing (55%) of cases, 28 cases (46.7%) showed muscle invasion (pT2-4), while lymphovascular invasion was detected in 27 (45%) of cases. Squamous differentiation was detected in 50% of the studied cases. Positive expression of CK5/6 (either partial or diffuse positivity) was detected in 48.3% of cases. High FAP and CD147 expression was detected in 63.3% and 53.3% of cases, respectively. The clinicopathological features of the studied urothelial carcinoma cases were represented in Table 1.

### Immunohistochemical results

Statistically significant relation could be detected between CK5/6 expression and histological tumor grade (p-value = 0.002*) as 66.7% of the high grade tumors showed positive CK5/6 immunoreactivity, while 25.9% of the low grade tumors were CK5/6 positive. The pattern of CK5/6 expression varied between low and high-grade tumors. Staining confined to basal cell layer was frequent in low grade tumors, while high grade tumors showed more diffuse

expression. The expression of CK5/6 was also significantly related to muscle invasion (p-value=0.021*) and squamous differentiation (p-value=0.020*), as 64.3% of the muscle invasive cases and 63.3% of the cases exhibiting squamous differentiation showed positive CK5/6 immunoreactivity (Figure 1). In addition, CK5/6 expression was significantly related to FAP and CD147 immunohistochemical expression (p-value=0.001*). No significant relation was detected as regard patients' age (p-value=0.604), sex (p-value=0.809), or lymphovascular invasion (p-value=0.311). The immunohistochemical expression of CK5/6 in relation to clinicopathological features was illustrated in Table 2.

As regard to FAP expression, statistically significant relation was detected between FAP expression and tumor grade (p-value = 0.001*) as 81.8% of the high grade tumors, while 40.7% of low grade tumors exhibited high FAP expression in peritumoral fibroblasts (Figure 2). Moreover, a significant relation was detected between FAP expression and lymphovascular invasion (p-value = 0.036*).

**Table 1.** Distribution of the studied cases according to clinicopathologic parameters (n = 60)

| Item | No. (%) |
|---|---|
| **Sex** | |
| Male | 32 (53.3%) |
| Female | 28 (46.7%) |
| **Age (years)** | |
| Mean ± SD. | 51.2 ± 10.7 |
| Median (Min. – Max.) | 53 (33 – 78) |
| **Grade** | |
| Low | 27 (45%) |
| High | 33 (55%) |
| **Muscle invasion** | |
| Non-muscle invasive (pTa, pT1) | 32 (53.3%) |
| Muscle invasive (pT2-4) | 28 (46.7%) |
| **Lymphovascular invasion** | |
| Yes | 27 (45%) |
| No | 33 (55%) |
| **Squamous differentiation** | |
| Yes | 30 (50%) |
| No | 30 (50%) |
| **CD147 expression** | |
| Negative /Low | 28 (46.7%) |
| High | 32 (53.3%) |
| **FAP expression** | |
| Negative/ Low | 22 (36.7%) |
| High | 38 (63.3%) |
| **CK5/6 expression** | |
| Negative / minimal (<10%) | 31 (51.7%) |
| positive (≥ 10%) | 29 (48.3%) |

Yet, no significant relation was detected between FAP expression and muscle invasion (p- value = 0.224), or squamous differentiation (p-value = 0.284). The immunohistochemical expression of FAP in relation to clinicopathological features was illustrated in Table 3.

CD147 immunohistochemical expression was also significantly related to tumor grade (p-value < 0.001*), as 87.9 % of the high-grade and 11.1% of low-grade cases showed high CD147 immunoreactivity (Figure 2). Also, significant relation was detected as regard muscle invasion (p-value<0.001*), and FAP expression (P-value=0.002*). No significant relation was detected between CD147 expression and age, sex, lymphovascular invasion or squamous differentiation. The association between CD147 immunohistochemical expression and clinicopathological parameters was illustrated in Table 4.

## DISCUSSION

Management of bladder carcinoma has been largely dependent on histopathological features of tumor for several decades. However, tumors with similar pathological features may have different biological behaviors and different response to therapy. Initiation and progression of urothelial bladder carcinoma is now considered to involve alterations in a range of molecular pathways. Molecular subtyping of urothelial carcinoma may permit risk stratification and development of more individualized therapy (Zhu et al., 2020).

In the current study, CK5/6 immunohistochemical expression was detected in 48.3% of cases. Across previous studies, the frequency of CK5/6 expression in urothelial carcinoma ranged from 19% to 57%. The difference between various reports may be attributed to inclusion/exclusion of urothelial carcinomas exhibiting specific cytological differentiation*(squamous)* differentiation in different studies. The lowest frequency of CK5/6 expression (19.7%) was reported by Hashmi et al., (2018) who excluded urothelial carcinoma with squamous differentiation from their study.

**Table 2.** Relation between CK5/6 expression and different parameters (n=60)

| | CK5/6 expression | | Test of Sig. | P-value |
|---|---|---|---|---|
| | Negative / minimal (n = 31) | Positive (n = 29) | | |
| **Sex** | | | | |
| Male | 17 (53.1%) | 15 (46.9%) | $\chi^2$= 0.058 | 0.809 |
| Female | 14 (50%) | 14 (50%) | | |
| **Age (years)** | | | t= 0.522 | 0.604 |
| Mean ± SD. | 50.5 ± 9.5 | 51.9 ± 11.9 | | |
| **Grade** | | | | |
| Low | 20 (74.1%) | 7 (25.9%) | $\chi^2$= 9.870* | 0.002* |
| High | 11 (33.3%) | 22 (66.7%) | | |
| **Muscle invasion** | | | | |
| Non-muscle invasive (pTa, pT1) | 21 (65.6%) | 11 (34.4%) | $\chi^2$= 5.350* | 0.021* |
| muscle invasive (pT2-4) | 10 (35.7%) | 18 (64.3%) | | |
| **Lymphovascular invasion** | | | | |
| Yes | 12 (44.4%) | 15 (55.6%) | $\chi^2$= 1.025 | 0.311 |
| No | 19 (57.6%) | 14 (42.4%) | | |
| **Squamous differentiation** | | | | |
| Yes | 11 (36.7%) | 19 (63.3%) | $\chi^2$= 5.406* | 0.020* |
| No | 20 (66.7%) | 10 (33.3%) | | |
| **CD147 expression** | | | | |
| Low | 21 (75%) | 7 (25%) | $\chi^2$= 11.446* | 0.001* |
| High | 10 (31.2 %) | 22 (68.8%) | | |
| **FAP expression** | | | | |
| Low | 18 (81.8%) | 4 (18.2%) | $\chi^2$= 12.646* | <0.001* |
| High | 13 (34.3%) | 25 (65.7%) | | |

$\chi$2: Chi square test, t: Student t-test p: p value for comparing between CK5/6 and different parameters *: Statistically significant at p ≤ 0.05

**Table 3.** Relation between FAP immunohistochemical expression and different parameters (n=60)

| | FAP expression | | Test of Sig. | P-value |
|---|---|---|---|---|
| | Negative /Low (n = 22) | High (n = 38) | | |
| **Sex** | | | | |
| Male | 12 (37.5%) | 20 (62.5%) | $\chi^2$= 0.021 | 0.886 |
| Female | 10 (35.7%) | 18 (64.3%) | | |
| **Age (years)** | | | t= 0.357 | 0.722 |
| Mean ± SD. | 50.5 ± 9 | 51.5 ± 11.6 | | |
| **Grade** | | | | |
| Low | 16 (59.3%) | 11 (40.7%) | $\chi^2$= 10.790* | 0.001* |
| High | 6 (18.2%) | 27 (81.8%) | | |
| **Muscle invasion** | | | | |
| Non-muscle invasive (pTa, pT1) | 14 (43.8%) | 18 (56.2%) | $\chi^2$= 1.482 | 0.224 |
| Muscle invasive (pT2-4) | 8 (28.6%) | 20 (71.4%) | | |
| **Lymphovascular invasion** | | | | |
| Yes | 6 (22.2%) | 21 (77.8%) | $\chi^2$= 4.411* | 0.036* |
| No | 16 (48.5%) | 17 (51.5%) | | |
| **Squamous differentiation** | | | | |
| Yes | 9 (30%) | 21 (70%) | $\chi^2$= 1.148 | 0.284 |
| No | 13 (43.3%) | 17 (56.7%) | | |
| **CD147 expression** | | | | |
| Low | 16 (57.1%) | 12 (42.9%) | $\chi^2$= 9.479* | 0.002* |
| High | 6 (18.7%) | 26 (81.3%) | | |

$\chi^2$: Chi square test, t: Student t-test, p: p value for comparing between FAP and different parameters, *: Statistically significant at p ≤ 0.05

**Table 4.** Relation between CD147 immunohistochemical expression and different parameters

| | CD147 expression | | Test of Sig. | P-value |
|---|---|---|---|---|
| | Negative/Low (n = 28) | High (n = 32) | | |
| **Sex** | | | | |
| Male | 16 (50%) | 16 (50%) | $\chi^2$= 0.306 | 0.580 |
| Female | 12 (42.9%) | 16 (57.1%) | | |
| **Age (years)** | | | t= 0.043 | 0.966 |
| Mean ± SD. | 51.2 ± 9.2 | 51.1 ± 11.9 | | |
| **Grade** | | | | |
| Low | 24 (88.9%) | 3 (11.1%) | $\chi^2$= 32.20* | <0.001* |
| High | 4 (12.1%) | 29 (87.9 %) | | |
| **Muscle invasion** | | | | |
| Non-muscle invasive (pTa, pT1) | 22 (68.8%) | 10 (31.2%) | $\chi^2$= 13.44* | <0.001* |
| Muscle invasive (pT2-4) | 6 (21.4%) | 22 (78.6%) | | |
| **Lymphovascular invasion** | | | | |
| Yes | 11 (40.7%) | 16 (59.3%) | $\chi^2$= 0.693 | 0.405 |
| No | 17 (51.5%) | 16 (48.5%) | | |
| **Squamous differentiation** | | | | |
| Yes | 12 (40%) | 18 (60%) | $\chi^2$= 1.071 | 0.301 |
| No | 16 (53.3%) | 14(46.7%) | | |
| **FAP expression** | | | | |
| Low | 16 (72.7%) | 6 (27.3%) | $\chi^2$= 9.479* | 0.002* |
| High | 12 (31.6%) | 26 (68.4%) | | |

$\chi^2$: Chi square test, t: Student t-test, p: p value for comparing between CD147 and different parameters, *: Statistically significant at p ≤ 0.05.

In the current study, CK5/6 expression was associated with high nuclear grade and increase depth of invasion. Our results were consistent with data observed by Hashmi et al., (2018). Jangir et al., (2019), also found that CK5/6 expression was associated with increase tumor stage, but the association didn't reach statistical significance. Cytokeratin5/6 is frequently expressed in basal subtype of urothelial carcinoma. Basal tumors are associated with aggressive behavior, which may be due to the expression of stem cell markers as CD44 and markers of EMT that facilitate tumor cell invasion and metastasis (Choi$_a$ et al., 2014 and Calvate et al., 2019).

On the other hand, Rodriguez Pena et al., (2019) reported significant association between CK5/6 expression and low nuclear grade in a cohort of non-muscle invasive bladder carcinoma. The discrepancy may be due different study population and different evaluation method used to assess CK5/6 immunoreactivity. Moreover, the expression pattern of CK 5/6 varies between low- and high-grade urothelial carcinoma. Expression confined to the basal layer is more detected in low grade papillary urothelial tumors, while diffuse expression is more common in high grade tumors.

In the current work, positive expression of CK5/6 was significantly related to squamous differentiation in the studied urothelial carcinoma cases. Similar observation was detected by Geisa et al., 2011 and Jangir et al., (2019). Moreover, Choi$_a$ et al., (2014) reported that the aggressive basal subtype of urothelial carcinoma in their studied cohort was enriched with squamous and sarcomatoid features. CK5/6 is considered as marker of basal and squamous differentiation in normal epithelium as well as in tumors (Reis-filho et al., 2003). Urothelial carcinoma with squamous differentiation has been associated with aggressive behavior and advanced stage at presentation (Liu et al., 2017). The interplay between tumor cells and the surrounding tumor microenvironment is supposed to activate stromal fibroblasts with conversion into CAFs through paracrine signals. In turn, CAFs provide collagen niche for aggressive tumor cells supporting their growth, migration and chemoresistance (Kang et al., 2017).

In the current work, high expression of FAP was detected in peritumoral fibroblasts in 63.3% of the studied cases. In agreement with Mezheyeuski et al., (2020), FAP expression in current study was significantly associated with high tumor grade and lymphovascular invasion.

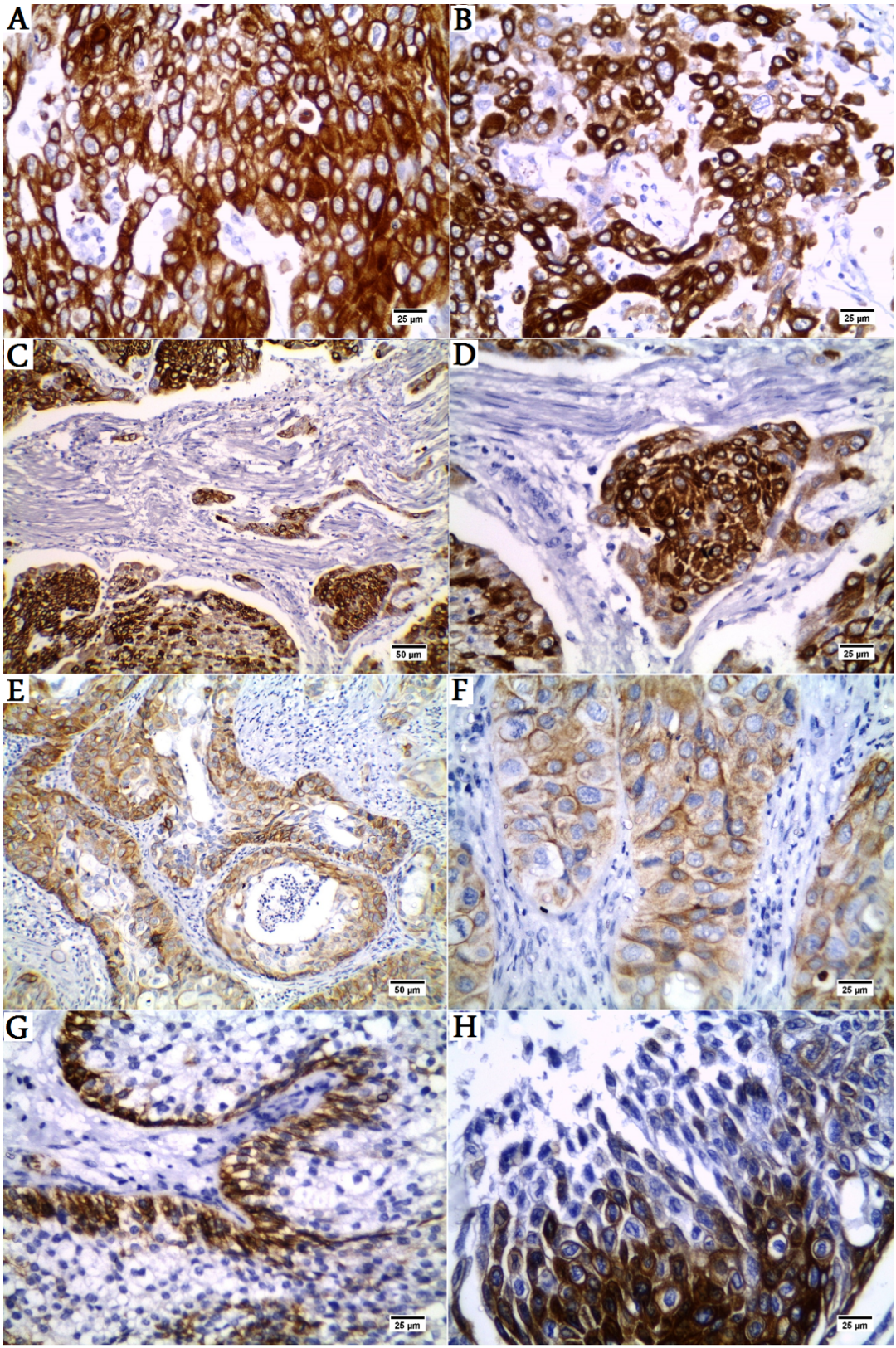


**Figure 1. Immunohistochemical expression of CK5/6 in urothelial bladder carcinoma**. (A) Diffuse expression (more than 80% stained neoplastic cells) in high grade urothelial carcinoma (x400). (B) Diffuse expression in high grade urothelial carcinoma exhibiting cellular decohesion (x400). (C) Diffuse expression in muscle invasive urothelial carcinoma (x200), (D) higher magnification of the previous image (x400). (E) Diffuse expression in urothelial carcinoma with squamous differentiation (x200). (F) Higher magnification of the previous case (x400). (G) Minimal CK5/6 expression (less than 10% stained neoplastic cells) confined to the basal layer of low grade papillary urothelial carcinoma (x 400). (H) High grade urothelial carcinoma with CK5/6 expression extending beyond the basal layer to the more superficial neoplastic cells (x400).

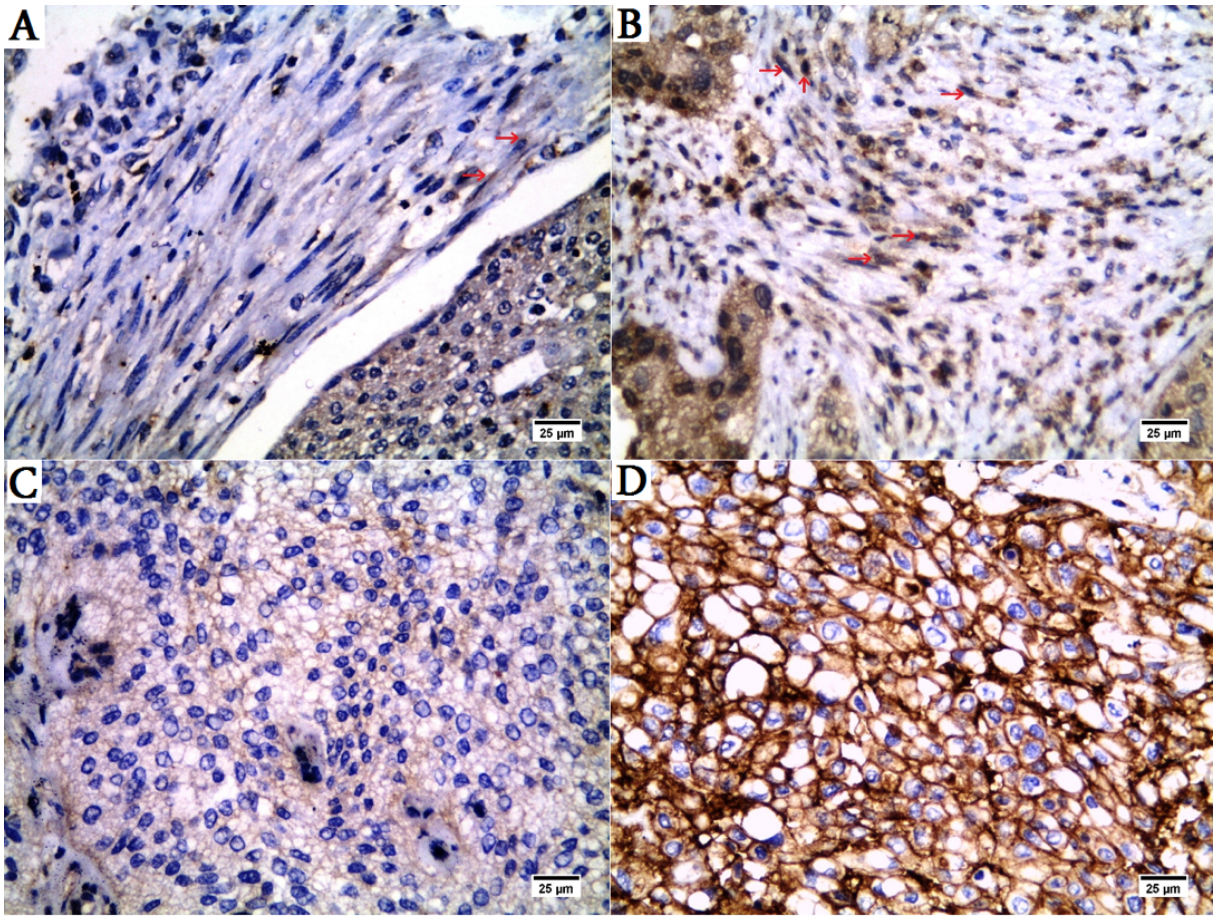


**Figure 2. Immunohistochemical expressions of FAP and CD147 in urothelial bladder carcinoma**. (A) Negative FAP expression in stromal fibroblasts surrounding low grade urothelial carcinoma (score 0) ( few weekly stained stromal cells *marked by red arrows* x400). (B) High score FAP expression highlights numerous stromal fibroblasts surrounding high grade urothelial carcinoma (score 6) [intensity 3X percentage 2] (*marked by red arrows* x400). (C) Low score CD147 expression in low grade urothelial carcinoma (score 3) [intensity 1X percentage 3] (x400). (D) High score CD147 expression in high grade urothelial carcinoma (score 12) [intensity 3X percentage 4] (x400).

Expression of FAP in stromal fibroblasts stimulates tumor cell proliferation, cellular dedifferentiation, angiogenesis, remodeling of extracellular matrix, and loss of cellular adhesion. FAP can degrade extracellular matrix either directly by its proteolytic effect or indirectly through remodeling function of other stromal proteases (Liu et al., 2012). Moreover, CAFs have been correlated with the expression of EMT markers. The phenomenon of epithelial mesenchymal transition is associated with invasiveness and migration of tumor cells (Potenta et al., 2008).

However, in the current work no significant relation was detected between FAP expression and muscle invasion. Also, Schulte et al., (2012) didn't detect significant relation between FAP expression in stromal fibroblasts and the invasiveness of the urothelial carcinoma, although the expression of other fibroblastic markers as SMA, S100B and PDGFB showed positive correlation with tumor invasive behavior. The expression of diverse biomarkers may reflect that CAFs are actually heterogenous population with different origins and functions during different stages of tumor development or probably different stages of maturation (Schulte et al., 2012). Hence, combination of multiple markers for detection of CAFs can be better correlated with prognosis than single marker.

Our results regarding relation between FAP expression and muscle invasion were inconsistent with data reported by Calvate et al., (2019) and Mezheyeuski et al., (2020). The controversy may be attributed to different study population and different evaluation method applied to assess FAP

immunoreactivity. Moreover, the heterogenous interaction between CAFs and tumor cells may explain the discrepancy in results (Yoshida, 2020). It has been reported that FAP may have both tumor promoting and suppressing functions, depending on interaction with different signaling molecules on tumor cells (Liu et al., 2012).

Furthermore, Shin et al., (2014) reported that stromal fibroblasts with activation of certain molecular pathways can exhibit tumor restraining rather than promoting function during transition from insitu into invasive bladder carcinoma.

It is suggested that FAP may be regarded as stromal therapeutic target, with less tendency to drug resistance, than frequently mutated genetically unstable tumor cells (Liu et al., 2012 and Kang et al., 2017). In vitro suppression of FAP in cancer cell lines was associated with decreased tumor progression (Teichgräber et al., 2015). FAP targeting prodrug has been utilized in breast and prostatic cancer with promising results (Brennen et al., 2014).

Regarding CD147 expression in urothelial carcinoma cases, high CD147 immunoreactivity was significantly related to high tumor grade and muscle invasion. These results were in line with data reported by Xue et al., (2011), Choi$_b$ et al., (2014) and Peng et al., (2020). CD147 is involved in extracellular matrix, basement membrane degradation and tumor metastasis through enhancement of matrix metalloproteinases (MMPs) production by tumor cells or stromal fibroblasts. CD147 is also a promotor of angiogenesis through stimulation of VEGF production by tumor cells (Tang et al., 2005). Moreover, Wittschieber et al., (2011) reported that CD147 can serve as progression marker for urothelial carcinoma as it may help to stratify patients with early-stage urothelial carcinoma who have tendency towards disease progression and further muscle invasion.

On the other hand, Afonso et al., (2011), didn't detect a significant correlation between CD147 expression in urothelial carcinoma cases and other pathological parameters including tumor grade and stage. However, they reported that CD147 expression added to the predictive value of advanced tumor stage on patient outcome. Based on its molecular structure, CD147 has been proposed to be a promising therapeutic target for urothelial carcinoma. Xue et al., (2011) reported decrease in MMP2, MMP9 and VEGEF with subsequent inhibition of migration and invasion after targeting CD147 in bladder cancer cell lines.

The current study was limited by the retrospective design, with lack of follow-up and survival analysis, and the relatively small sample size. However, the study detected relation between biomarkers involved in tumor aggressive behavior (FAP and CD147) and the basal marker CK5/6. Also, Calvate et al., (2019) detected a correlation between FAP and basal markers expression. However, the correlation didn't reach clinical significance. The progression of tumor is not only associated with acquiring aggressive characters in tumor cells themselves, but in the tumor microenvironment as well. CAFs promote cellular dedifferentiation and aggressive behavior acquisition (Kang et al., 2017). Hence, recent advances in molecular subtyping of bladder cancer have included heterogeneity in tumor microenvironment including extracellular matrix, immune cells and surrounding stromal cells (Zhu et al., 2020).

## CONCLUSION

Basal cytokeratin (CK5/6) expression in bladder urothelial carcinoma is associated with features of aggressive tumor behavior as high tumor grade, muscle invasion and squamous differentiation. Moreover, it showed significant relation to FAP and CD147 immunoreactivity. In turn, FAP and CD147 immunoreactivity were significantly related to features of aggressive tumor behavior. Hence, these markers can be considered as progression markers for urothelial carcinoma and potential targets for therapy but need further validation through large scale prospective studies. Moreover, molecular based studies especially on the aggressive basal phenotype with the development of subtype directed target therapy should be encouraged.

## CONFLICT OF INTEREST

All authors declare no conflicts of interest.

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## AUTHORS' CONTRIBUTION

Both authors contributed equally to the design of work, analysis, and interpretation of data, drafting and revision of the manuscript. The manuscript was finally approved by both authors.

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