SOHAG MEDICAL JOURNAL
Vol. 21 No.1 Jan 2017

IMMUNOHISTOCHEMICAL EXPRESSION OF P63 IN UROTHELIAL CARCINOMA

***Ola M. Nageeb *Afaf T. Elnashar, *Noha ED Hassab El-Naby, **Atef G.Abd El Wahab**
*Pathology and **Urology Departments, Sohag University Hospital

Abstract

Introduction: Urothelial carcinoma is the most common histologic type of urinary bladder cancer in Egypt. Detection of high-grade urothelial carcinoma is important for modification of therapy and improving the prognosis. P**63** is expressed at high levels in the basal layers of different epithelial tissues, including the urothelium. **Aim of the work**: to study the diagnostic and prognostic value of p**63** expression in urothelial carcinoma (UC). **Materials and Methods**: Fifty cases of bladder urothelial carcinoma were involved in the study; **12** cases of non-invasive UC, (**3** HG & **9** LG), and **38** cases of invasive UC, (**30** HG & **8** LG). Sixteen cases of UC showed Bilharziasis. All the specimens were stained with p**63** using immunohistochemical technique. **Results**: P**63** was expressed in **28**/**38** cases **(73.7%)** of invasive UC and in all cases of non-invasive UC, and also in **16**/**17 (94%)** low-Grade and in **24**/**33 (72.7%)** of high-grade UC, with a statistically significant relation between p**63** expression and both invasion **(p < 0.001)** and the tumor grade (p **<0.034). Conclusion**: **P63** can be used as a diagnostic and a prognostic factor for high grade invasive UC.

Key words: P**63**, urothelial carcinoma (UC), High Grade (HG), Low Grade (LG).

Introduction

Bladder cancer is the most common cancer among Egyptian men (**1).**Urothelial carcinoma (UC) is the most common histologic type of urinary bladder cancer, constituting more than **90%** of bladder cancer cases in United States (**2**). In Egypt, there was significant rise of UC from **16%** to **65.8%,** becoming at present the most common tumor type, with a significant decrease in squamous cell carcinoma (SCC) from **75.9%** to **28.4% (3).** This may be due to increased exposure to etiological factors as smoking and pesticides **(4).** Despite advances in surgical techniques as well as in intravesical and systemic therapy; up to **30%** of patients with non-invasive and **50%** of patients with invasive urothelial carcinoma experience disease progression, recurrence, and eventual death **(5)**. With regard to clinical management, urothelial neoplasms are divided into two major phenotypic variants; low-grade and high-grade invasive carcinoma. The former has and eventually provided better prognosis **(6**). **P63** is a transcription factor belonging to the **p53** family. It is a nuclear protein encoded by a gene on chromosome **3q27-29 (7 & 8). P63** is a marker of basal epithelial cells and is required for normal development of several epithelial tissues including bladder and prostatic glands **(9).** The human TP**63** gene-localized on chromosome **3**-consists of **15** exons and contains two promoters. **(10).** Different studies support the hypothesis that **p63** can function as a tumour suppressor, especially TAp**63** isoform. For example TAp**63** overexpression is responsible for the

activation of **p53** responsive genes leading to cell cycle arrest and apoptosis. **(11).** On the other hand, ΔNp**63** inhibits death receptors-mediated apoptosis and chemotherapy-induced mitochondrial apoptosis pathways and thus functions as an oncogene **(12).** Many studies have investigated the role of **p63** in urothelial neoplasms. The first group of investigators proposed that over expression of p63 mRNA relates to carcinogenesis and tumour progression **(13)**. In contrast, other studies revealed that high-grade invasive urothelial carcinomas frequently diminish **p63** expression, whereas low-grade tumours highly preserve the normal **p63** expression (**14).** Impaired **p63** expression is thought to be a prognostic marker along with the well-established prognostic factors, such as TNM stage, indicating that impaired **p63** characterizes biological aggressiveness of urothelial neoplasms **(15)**. Recently **p63** has been shown to be a marker of tumours of urothelial origin **(16).**

The aim of the work: to study the expression of **p63** in UC and to detect the value of using **p63** expression as a prognostic marker.

Materials and methods

The specimens of the study were obtained randomly from urothelial carcinoma (UC) cases admitted to The Urology Department and referred to the Pathology Laboratory at Sohag University Hospital, in the period between May **2014** and June **2015.** The total number of studied specimens was **50** cases divided into **17** cases low-grade UC**, 33** cases high-grade UC, **12** noninvasive UC and **38** invasive UC. Clinical data were obtained from the referral clinical reports. These data included: age, sex, clinical presentations, laboratory tests and the method of obtaining the specimen. The biopsies were obtained by either transurethral resection (TUR) or radical cystectomy. Each case in the study was stained by routine H&E stained slides to evaluate the diagnosis, grading according to World Health Organization classification of tumors **2004 (17)** and staging according to American Joint Committee on Cancer **2010** (**18)**. For immunohistochemical staining, Five micron-thick sections from the formalin-fixed paraffin-embedded blocks of all specimens were cut on chrome alum–gelatin adhesive coated slides and immunostained using peroxidase-labeled streptavidin-biotin immuno-enzymatic antigen-detection kit to detect **p63** expression. Mouse monoclonal **p63** antibody (clone **4A4**), catalog # (CM**163**A, B, C, H. BIOCARE MEDICAI corporation) was used. All the specimens were stained with the antibody. Tissue sections were deparaffinized in **2** changes of xylene, rehydrated through descending grades of alcohols and washed in distilled water. Endogenous peroxidase activity was blocked with hydrogen peroxide, and then washed in **20%** diluted phosphate buffered saline (PBS). Slides were immersed in antigen retrieval solution (**10** mmol sodium citrate buffer solution, pH **6.0**) and put in the oven at **100**^{o}c for **2** hours, washed in distilled water and in PBS. Tissue sections were incubated with **P63** at a dilution of **1/100** in normal goat serum (NGS) at a dilution of **(1/100)** overnight at room temperature to block nonspecific interactions. After rinsing in PBS, tissue sections were treated with biotinylated goat serum for **10** min at room temperature. The slides were rinsed in PBS and peroxidase-labeled streptavidin was applied for **10** min at room temperature, rinsed again in

PBS and blotted. The slides were incubated with 1 micron chromogen to **25** micron diaminobenzidine (DAB) for **20** minutes, washed in distilled water and counter-stained using Myer's Hematoxylin. Tissue sections were washed in tap water dehydrated in ascending grades of alcohol, cleared in xylol, left to dry, then mounted with DPX, and cover slipped. Positive control was section from normal prostate. Negative control was sections of the examined tissues but with omission of the primary antibody.

Evaluation of immunostaining of p63

The entire sections were histologically examined by bright field microscope to evaluate the immunostaining positivity as the mean percentage of positive cells and staining intensity in at least three different fields. Cells stained positive for **p63** were identified by the presence of nuclear staining. Semiquantitation of nuclear **p63** immunoreactivity was calculated with a **12**-point weighted score system. First, the percentage of positive cells in each area was scored with a **5**-point scale: **0 for <5%, 1 for 5-25%, 2 for 25-50%, 3** for **50-75%,** and **4** for over **75%.** Second, the intensity of positive signal was scored with a **3**-point scale: **0** for negative, **1** for weak, **2** for medium, and **3** for strong staining, then, the weighted score for each area was calculated by multiplying the percentage of positive cells by the intensity of staining score. The results were scored as negative **(0-1),** weak **(2-3),** moderate **(4-6)** and strong **(8-12) (19).** Results were statistically analyzed using Statistical Package for Social Sciences (SPSS) for windows. The association of **p63** protein expression with the other bladder carcinoma variables was assessed by the Pearson's Chi-square test. P value less than **0.05** was considered significant.

Results

This study included **50** specimens of urothelial carcinoma of the urinary bladder included **38** cases were diagnosed as invasive UC and **12** cases were diagnosed as non-invasive UC, **33/50** of cases were high grade and **17** cases were low grade. The age range of the **50** studied patients was **20-80** years, mean age was **57.2** years, and median age was **56** years. **42/50 (84%)** were males and **8/50 (16%)** were females with male: female ratio **5.25:1.** Tissue specimens were obtained by TUR in **41** cases **(82%),** and by radical cystectomy in **9** cases **(18%).**

Histopathological findings

The collected **50** cases of bladder urothelial carcinoma were graded according to World Health Organization classification **(17**). Stage grouping and TNM pathological classification were carried out according to The American Joint Committee on Cancer (AJCC) **(18).** Twelve cases **(24%)** of non-invasive urothelial carcinoma were divided into three cases **(25%)** high grade, and nine cases **(75%)** low grade. Thirty eight cases **(76%) (38/50)** were invasive urothelial carcinoma; with thirty cases were high grade, and eight cases **(21%)** were low grade. Three cases **(3/12) (25%)** of non-invasive urothelial carcinoma and thirteen cases **(13/38) (34.2%)** of invasive urothelial carcinoma showed Bilharziasis. Four cases **(4/17)** of low grade UC and (**12/33)** of UC showed Bilharziasis.

Immunuohistochemical finding: P63 expression appeared as brownish nuclear staining in basal cells of the normal prostatic glands which were used as a positive control. **P63** was expressed in **28/38** cases of invasive UC (**73.7%)** with variation in its

expression as it was strongly expressed in **12/38 (31.6%),** moderately expressed in 8/38 **(21%),** weakly expressed in **8/38 (21%)** and was negative in **10/38 (26.3%)** of cases. P63 was expressed in all cases of non-invasive UC as it was strongly expressed in **7/12 (58.33%),** moderately expressed in **2/12 (16.6%),** weakly expressed in **3/12 (25%)** of cases **(Table 1, Figure 1). P63** showed variation in its expression in **24/33** cases **(72.7%)** of high-grade UC as it was strongly expressed in **8/33**, moderately expressed in **7/33,** weakly expressed in **9/33** and was not expressed in **9/33 (27.3%)** of cases. Expression of **p63** in **(16/17** cases) of low-grade UC and was strong in **11/17**, moderate in **3/17**cases, weak expression in **2/17** cases and no expression in **1/33** of cases **(Table 2).** There was a statistically significant correlation between **p63** expression and tumor grade, P value < **(0.034)** (decreased expression with increasing grade). There was no statistically significant correlation between **p63** expression and clinical parameters (age, sex, bilharziasis, and myoinvasion) although we found that **p63** immunostaining was decreased with deeper muscle invasion of the tumor.

(Table 1): P63 expression in non-invasive and invasive UC

Tumor type	No of cases	P63 expression				P value
		+++	++	+	-ve	
Non-invasive UC	12	7	2	3	-	0.0001*
		12/12				
Invasive UC	38	12	8	8	10	
		28/38 (73.7%)			10/38 (	

*Chi-square test was used, *= Significant*

(Table 2): P63 expression in UC according to tumor grading.

Tumor grade	No of cases	P63 expression				P value
		+++	++	+	-ve	
Low grade UC	17	11	3	2	1	0.034*
		16/17(94.1%)			1/17	
High grade UC	33	8	7	9	9	
		24/33(72.7%)			9/33(27.3%)	

*Chi-square test was used, *= Significant.*

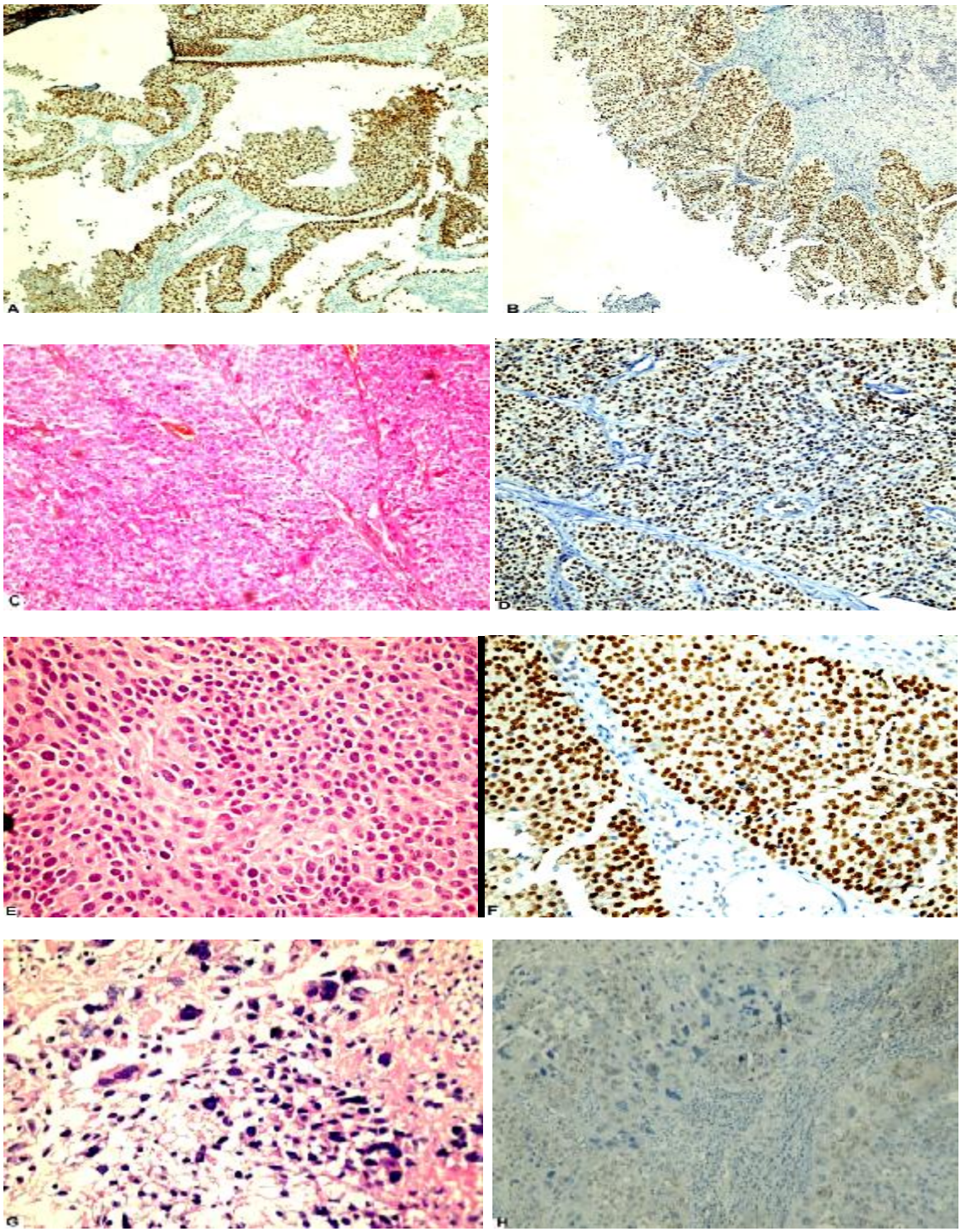

Figure (1): A&B: P**63** strong expression in non-invasive, Low Grade UC, **C:** Invasive LG UC stained by H&E. **D:** p**63** expression in invasive, LG UC. **E**: Invasive High Grade UC stained by H&E. **F**: strong p**63** immunoexpression. **G**: invasive High Grade UC stained by H&E. **H**: Weak p**63** immunostaining.

Discussion

Despite advances in surgical techniques as well as in systemic chemotherapy; up to **50%** of patients in Egypt with invasive urothelial carcinoma suffered from disease progression, recurrence, and eventual death. **P63** has emerged as a critical player in embryonic development, epithelial stem cell maintenance and differentiation. In cancer biology **(20**). The gene encoding the tumor protein **p63** is a member of the **p53** family and like other family members contains two different promoters that generate two classes of **p63** proteins. The transactivating TAp**63** and the NH**2**-terminal truncated ΔNp**63.** TAp**63** contains an NH**2**-terminal transactivation domain that is absent in ΔNp**63** (**9**). **P63** regulates many genes involved in DNA repair, ΔNp**63** binds to the promoters of the RAD**51**, BRCA**2,** and MRE**11** genes which are involved in homologous recombination, one of the most important pathways for repair of double-strand breaks. In addition, **p63** interacts with ATM, a key kinase involved in the recognition of DNA double-strand breaks **(21).** Analysis of biopsy samples have shown that reduced **P63** expression is associated with progression and advanced stages of breast cancer (**22 & 23)** cancer bladder (**14)** and melanoma (**24).** **P63** was expressed in all normal and hyperplastic urothelium in the areas adjacent to the tumor in our study cases. An earlier study at **2001** revealed that **p63** is proposed to be critical for the proliferation and maintenance of epithelial progenitor cells population that give rise to the differentiated stratified squamous epithelial cells rather than for the differentiation process itself **(25).** On the other hand, a study group reported that **P63** expression was correlated with the degree of differentiation in the superficial lesion and with the number of cell layers which covered the tumor papillae in muscle-invasive urothelial carcinoma **(26).** Several reports which tried to evaluate the role of **p63** in the process of tumergenesis suggest that **p63** is involved in cell migration and adhesion and thus also in processes connected with these cell abilities such as metastasis and wound healing. Those studies that performed on SCC lines have demonstrated that disruption of **p63** causes upregulation of genes associated with a higher potential to metastasize and invade **(27).** while a more recent study showed that **TAp63** suppresses metastasis by regulating micro RNA processing complex **(28)**. In our study, **P63** was expressed in **(73.7%)** of invasive UC and in all cases of non-invasive UC with a statistically significant relation (p value **<0.001).** In agreement with the authors **(29)** who found that **P63** was expressed in all cases of non-invasive papillary urothelial carcinoma and the immune-reaction was strong in low grade papillary superficial carcinoma **(93%)** than in high grade urothelial carcinoma **(68%)** that showed a significant reduction in **P63** positivity. Koyuncuer in his study on the other

hand found no statistically significant relation was observed between invasive and non -invasive **(pT1-PT2)** urothelial carcinoma for **P63** expression (**30).**

P63 showed positive expression in **(72.7%)** of high-grade UC and in (**16/17** cases) of low-grade UC with a statistically significant correlation between **p63** expression and tumor grade (P value < **0.034)** (decreased expression with increasing grade).

There was no statistically significant correlation between **p63** expression and deep myoinvasion and poor prognosis in the present study although we found that **p63** immunostaining was decreased with muscle invasion of the tumor. In agreement with two studies concluded that **P63** expression diminished in high grade invasive urothelial carcinoma **(31& 32).** A recent study group at **2012** concluded that it is impossible to prospectively identify the lethal muscle-invasive tumors; however, accumulating evidence suggests that molecular reprogramming characteristic of a developmental process known as epithelial-to mesenchymal transition (EMT) is involved. Muscle-invasive cancers are characterized by down-regulation of E-cadherin and **p63**, two epithelial markers uniformly expressed in normal urothelium and in non-muscle-invasive cancers. These changes are accompanied by up-regulation of mesenchymal markers Zeb-1, Zeb-**2,** vimentin, and MMP9, leading to increased invasion and migration. The most superficial bladder cancers have excellent long-term survival (near **100%),** and uniformly express high levels of E-cadherin and **p63.** On the other hand, loss of **p63** is restricted to a subset of the muscle-invasive tumors, and muscle invasive disease is typically associated with worse clinical outcomes as compared to superficial, non-invasive cancer. In addition, the commercial antibody **(4A4)** that is most commonly used to measure **p63** in tissue sections cannot distinguish the two major **p63** isoforms (TA and ΔN), so the relationship between ΔN **p63** expression and poor outcome in muscle-invasive bladder cancers could not be recognized in previous IHC-based studies that employed this reagent **(33).** Another study group generated an anti-**p63** antibody that is specific for the ΔN isoforms and used it on an independent cohort of muscle-invasive tumors to show that ΔN **p63** protein levels also identify a lethal subset of cancers, whereas their results with the **4A4** antibody were inconclusive **(34).** **P63** was expressed in **87.5%** of UC with bilharziasis and in **76.5%** of UC cases with no bilharziasis with no statistically significant relation between bilharziasis and **p63** expression. A single study on **p63** and bilharziasis in -agreement with our study-found a tendency for a statistically significant decrease in the immunoreactivity in bilharzial cystitis **(p < 0.05)** but in malignant tumors, bilharziasis had no apparent effects on the pattern of p63 expression **(35)**.

CONCLUSION

P63 can be used as a diagnostic factor specific for urothelial carcinoma and as a prognostic factor for high grade UC in our community.

References

1- Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in **2008**: GLOBOCAN **2008.** Int J Cancer. **2010; 127(12): 2893-917**

2- Huang W, Williamson SR, Rao Q, Lopez-Beltran A, Monitroni R, Eble JN, Grignon DJ, Idrees MT, Emerson RE, Zhou XJ, Zhang S, Baldridge LA, Hahn

NM, Wang M, Koch MO, Cheng L. Novel markers of squamous differentiation in the urinary bladder. Hum Pathol. **2013; 44: 1989-1997.**

3- Gouda I, Mokhtar N, Bilal D, El-Bolkainy T, El-bolkainy NM. Bilharziasis and bladder cancer: a time trend analysis of **9843** patients. J Egypt Natl Canc Inst**. 2007; 19: 158-162.**

4- Khaled H. Schistosomiasis and cancer in Egypt: review. Medical Oncology, National Cancer Institute, Cairo University, Cairo **11796**, Egypt. J Advanced Res **.2013; 4: 461-466.**

5- Shariat SF, Karakiewicz PI, Palapattu GS, Lotan Y, Rogers CG, Amiel GE, Vazina A, Gupta A, Bastian PJ, Sagalowsky AI, Schoenberg MP, Lerner SP. Outcomes of radical cystectomy for transitional cell carcinoma of the bladder: a contemporary series from the bladder cancer Research consortium. J Urol **2006; 176: 2414-2422**.

6- Lee R and Droller MJ. The natural history of bladder cancer. Implications for therapy. Urol Clin North Am. **2000; 27: 1-13.**

7- Higgins JP, Kaygusuz G, Wang L, Montgomery K, Mason V, Zhu SX, Marinelli RJ, Presti JC Jr, Van de Rijn M, Brooks JD. Placental **S100** (S100P) and GATA**3**: Markers for transitional epithelium and urothelial carcinoma discovered by complementary DNA microarray. Am J Surg Pathol. **2007; 31: 673-680.**

8- Hodges KB, Lopez-Beltran A, Emerson RE, Montironi R, Cheng L. Clinical utility of immunohistochemistry in the diagnoses of urinary bladder neoplasia. Appl Immunohistochem Mol Morphol. **2010; 18: 401-410.**

9- Pignon J C, Grisanzio C, Geng Y, Song J, Shivdasani RA, Signoretti S. **P63-**expressing cells are the stem cells of developing prostate, bladder, and colorectal epithelia. Proc Natl Acad Sci U S A. **2013; 110 (20): 8105-8110.**

10- Joerger, AC, Rajagopalan, S, Natan, E, Veprintsev, DB, Robinson, CV and Fersht, AR Structural evolution of **p53**, p63, and **p73:** Implication for heterotetramer formation. Proc. Natl. Acad. Sci. USA **106 (2009): 17705-17710.**

11- Gressner O, Schilling T, Lorenz K, Schulze-Schleithoff E, Koch A, Schulze-Bergkamen H, Lena AM, Candi E, Terrinoni A, Catani MV, Oren M, Melino G, Krammer PH, Stremmel W, and Muller M. TAp**63α** induces apoptosis by activating signaling via death receptors and mitochondria. EMBO J **2005; 24: 2458-2471.**

12- Mundt HM, Stremmel W, Melino G, Krammer PH, Schilling T, and Muller M. Dominant negative ΔN**p63**α induces drug resistance in hepatocellular carcinoma by interference with apoptosis signaling pathways. Biochem Biophys Res Commun **2010; 369: 335-341.**

13- Park, B. J., Lee SJ, Kim JI, Lee SJ, Lee CH, Chang SG, Park JH, Chi SG. Frequent alterations of **p63** expression in human primary bladder carcinomas. Cancer Res. **2000; 60: 3370-3374.**

14- Koga, F., Kawakami S, Kumagai J, Takizawa T, Ando N, Arai G, Kageyama Y, Kihara K. Impaired TN**p63** expression characterizes aggressive phenotypes of urothelial neoplasms. Br J Cancer **2003; 88: 740-747.**

15- Hibi, K., Trink, B., Patturajan, M, Westra WH, Caballero OL, Hill DE, Ratovitski EA, Jen J, Sidransky D. AIS are an oncogene amplified in squamous

cell carcinoma. Proc Natl Acad Sci. **2000; 97: 5462-5467.**

16- Nekulova M, Holcakova J, Coates P, Vojtesek B. The role of p63 in cancer, stem cells and cancer stem cells. Cell Mol Biol Lett. **2011; 16: 296-327.**

17- Lopez-Beltran A, Sauter G, Gasser T, Hartmann A, Schmitz-Drager BJ, Helpap B, Ayala AG, Tamboli P, Knowles MA, Sidransky D, Cordon-Cardo C, Jones PA, Cairns P, Simon R, Amin MB, Tyczynski JE. Infiltrating urothelial carcinoma: In World Health Organization classification of tumors. Pathology and Genetics of tumors of the urinary system and male genital organs. Lyon, IARCC Press. **2004; PP: 91-94.**

18- Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, and Trotti A. AJCC cancer staging manual (**7th** ed). New York, NY: Springer **2010.**

19- Chan WY, Cheung KK, Schorge JO, Huang LW, Welch WR, Bell DA, Berkowitz RS, Mok SC. Bcl-**2** and **p53** protein expression, apoptosis, and **p53** mutation in human epithelial ovarian cancers. Am J Pathol. **2000; 156: 409-417.**

20- Melino G. P63 is a suppressor of tumergenesis and metastasis interacting with mutant **P53.** Cell Death Differe. **2011; 18 (9): 1487-1499.**

21- Craig AL, Holcakova J, Finlan LE, Nekulova M, Hrstka R, Gueven N, DiRenzo J, Smith G, Hupp TR, and Vojtesek B. Delta Np63 transcriptionally regulates ATM to control **p53** serin-**15**-phosphorylation. Mol Cancer **2010; 9: 195.**

22- Stefanou D, Batistatu A, Nonni A, Arkoumani E, Agnantis NJ. **P63** expression in benign and malignant breast lesions. Histol Histopathol **2004; 19(2): 465-471.**

23- Wang X, Mori I, Tang W, Nakamura M, Nakamura Y, Sato M, Sakurai T, and Kakudo K. **P63** expression in normal, hyperplastic and malignant breast tissues. Breast Cancer **2002; 9(3): 216: 219.**

24- Haqq C, Nosrati M, Sudilovsky D, Crothers J, Khodabakhsh D, Pulliam BL, Federman S, Miller JR **3rd**, Allen RE, Singer MI, Leong SP, Ljung BM, Saqebiel RW, Kashani-Sabet M. The gene expression signatures of melanoma progression. Proc Natl Acad Sci USA **2055; 102(7): 6092-6097.**

25- Pellegrini G, Dellambra E, Golisano O, Martinelli E, Fantozzi I, Bondanza S, Ponzin D, McKeon F and DeLuca M. **P63** identifies keratinocytes stem cells. Pro Natl Acad Sci USA **2001; 98: 3156-3161.**

26- Stepan A, Margaritescu CL, Simionescu C, Ciurea R. E-cadherin and **P63** immunoexpression in dysplastic lesions and urothelial carcinoma of the bladder. Romanian J of Morphology and Embryology **2009; 50 (3): 461-465.**

27- Barbieri CE, Tang LJ, Brown KA, and Pietenpol JA. Loss of **p63** leads to increased cell migration and upregulation of genes involved in invasion and metastasis. Cancer **Res 2006; (66): 7589-7597.**

28- Su X, Chakravarti D, Cho MS, Liu L, Gi YJ, Lin YL, Leung ML, El-Naggar A, Creighton CJ, Suraokar MB, Wistuba I and Flores ER. TA**p63** suppresses metastasis through coordinate regulationof Dicer and mi RNAs. Nature **2010; 467: 986-990.**

29- Raheem SA, Saied AN, Al Shaer R, Mustafa O, Ali AH. The role of CK20, **P53** and **P63** in differentiation of some

urothelial lesions of urinary bladder, immunohistochemical study. Open Journal Of Pathology **2014; 4: 181-193.**

30-Koyuncuer A. Immunohistochemical expression of **P63** and **P53** in urinary bladder carcinoma. Indian J Pathology & Microbiology **2013; 56 (1): 10-15.**

31- Urist MJ, Di Como CJ, Lu ML, Charytonowicz E, Verbel D, Crum CP, Ince TA, Mckeon FD, Cordon-Cardo C. Loss of **P63** expression is associated with tumour progression in bladder cancer. Am J Patholo **2002; 16 (4): 1199-1206.**

32- Koga F, Kawakami S, Fuji Y, Sato K, Ohtsuka Y, Iwai A, Ando N, Takizawa T, Kaqeyama Y, Kihara K. Impaired p63 expression associated with poor prognosis and Uroplakin III expression in invasive urothelial carcinoma of the bladder. Clin Cancer Res **2003; 9(15): 5501-5507.**

33- Choi W, Shah JB, Tran M, Svatek R, Marquis L, Lee L, Yu D, Adam L, Wen S, Shen Y, Dinney C, McConkey DJ, Siefker-Radtke A. P63 expression defines lethal subsets of muscle-invasive bladder cancer. Plosone **2012; 7 (1): e30206.**

34- Karni-Schmidt O, Castillo-Martin M, Hvai Shon T, Gladoun N, Domingo-Domenech J, Sanchez-Carbayo M, Li Y, Lowe S, Prives C, Cordon-Cardo C. Distinct expression profile of **P63** variants during urothelial development and bladder cancer progression. Am J Patholo **2011; 178: 159-165.**

35- Mursi K, Agag A, Hammam O, Riad A and Daw M. The expression of p63 in bladder cancer vs chronic bilharzial bladder. Arab J Urol **2013; 11(1): 106-112.**