



International Journal of
Cancer Research

ISSN 1811-9727



Academic
Journals Inc.

www.academicjournals.com

Analysis of Her2/neu Overexpression and Amplification in Urothelial Carcinoma of the Bladder Associated with Cox-2 Overexpression

¹Hanan Mohamed Abd ElMoneim, ¹Heba Mohamed Tawfik, ²Yaser Makram El Sherbiny and ³Ehab Rifat Tawfik

¹Department of Pathology,

²Department of Clinical Pathology,

³Department of Urology, Faculty of Medicine, Minia University, P.O. Box 61111, Egypt

Corresponding Author: Hanan M. Abd ElMoneim, Department of Pathology, Faculty of Medicine, El Minia University, Egypt

ABSTRACT

This study assessed 110 urothelial carcinomas to determine frequency of Her2/neu overexpression in relation with gene amplification and association between Her2/neu and Cox-2 status and clinicopathologic features. Paraffin-embedded tissues of transurethral resection or cystectomy were evaluated by immunohistochemistry, using antibodies against Her2/neu and Cox-2. All samples with Her2/neu overexpression were evaluated by Florescent *in situ* Hybridization. It was noticed that overexpression of Her2/neu was seen in 52 patients (47.3%). Her2/neu expression was positive in 19 (46.3%) of 41 squamous cell carcinoma and in 33 (47.8%) of 69 transitional cell carcinoma. Her2/neu overexpression in high grade tumors was statistically significant when compared with low grade ($p < 0.01$). Her2/neu gene amplification was found in 11.5% (6 of 52) of high grade transitional cell carcinoma with muscle invasion of highly expressing Her2/neu protein. Expression of Cox-2 was observed in 58 (52.7%) of the patients. Cox-2 was expressed in 34 (49.3%) of transitional cell carcinoma and in 24 (58%) of squamous cell carcinoma. Cox-2 expression was significantly positive in high grade bladder transitional cell carcinoma than in low grade ($p < 0.05$). Also, there was a significant difference in Cox-2 expression level between superficial and invasive tumors ($p < 0.05$). There is a significant correlation between Her2/neu protein expression and Cox-2.

Key words: Bladder, Cox-2, fluorescence *in situ* hybridization, Her2/neu, immunohistochemistry

INTRODUCTION

In Egypt, bladder cancer accounts for about 30% of all cancers, where it is the most common malignancy in men and the second most common malignancy in women after breast cancer (Ashley *et al.*, 2008). Many pathogenetic factors most commonly Bilharzial infestation which is an endemic infection in the Nile River Valley, was found to play an important role in bladder cancer development (El-Sebaie *et al.*, 2005). In 2006, there were 61420 new cases of UC, resulting in 13060 deaths in the United States (Jamal *et al.*, 2006). A unique distinction of UC is that the recurrence rate is the highest of any carcinoma (Hammerich *et al.*, 2008). The bladder cancer disease is characterized by two basically different disease courses: one in which the tumor is superficial and the other one showing muscle invasion (Ashley *et al.*, 2008). Urothelial Cell Carcinomas (UCC) represent more than 90% of bladder tumors are often papillary and are classified into non-muscle invasive and muscle invasive. Monitoring for tumor recurrence for

patients with this type of disease is very important and currently this is achieved by regular cystoscopic observation with relatively high morbidity and significant costs. Such patients would greatly benefit from non-invasive methods for disease monitoring and improved post-surgical intravesical therapies (Ashley *et al.*, 2008). High-grade tumors display a high risk of progression in spite that low grade tumors are characterized by frequent recurrences (approximately 70%) and infrequent progression to muscle-invasive tumors (Borden *et al.*, 2004). Hence, there is a need to better identify patients who have a low risk of recurrence, in order to avoid over treatment, as well as those who are likely to progress in order to treat them more aggressively. Recent studies have proposed that the progression from superficial-low grade/papillary and invasive-high grade tumors takes different molecular pathways. Molecular studies have identified distinct genetic, epigenetic and expression changes in these groups. It is predicted that multiple heritable changes are required for tumor development because bladder cancer is a disease of the middle to late decades of life. Thus it is surprising that so few genetic changes have been identified in low-grade tumours which represent the major group at diagnosis (Wu, 2005; Knowles, 2006). The ErbB subfamily of receptor tyrosine kinases consists of four homologous proteins: the epidermal growth factor receptor (also named ErbB1 or HER1), ErbB2 (HER2/NEU, c-Neu), ErbB3 (HER3) and ErbB4 (Estall *et al.*, 2009). Her2/neu, a 185 kDa transmembrane receptor tyrosine kinase, is a member of the Epidermal Growth Factor Receptor (EGFR) family localized to chromosome 17q encoding a glycoprotein with intrinsic tyrosine kinase activity. The Her2/neu-encoded protein molecule occupies a critical position in the biochemical pathways responsible for the transduction of mitogenic signals from a variety of growth factor receptors. It plays an important role in neoplastic cell growth in addition to its role in regulating normal cellular proliferation (Seshadri *et al.*, 1993; Press *et al.*, 1997). The incidence of overexpression of Her2/neu in bladder cancer is one of the highest among all human malignancies, ranging from 9 to 34% of cancers tested (Coombs *et al.*, 1991; Sato *et al.*, 1992). Her2/neu gene is amplified in 18% of all breast cancers (Yaziji *et al.*, 2004). In invasive urothelial bladder carcinomas, amplification and/or overexpression were also found. However, the true incidence of Her2/neu overexpression and/or amplification remains uncertain ranging from 23 to 80% for overexpression (Matsubara *et al.*, 2008) and from 0 to 32% for amplification (Matsubara *et al.*, 2008; Caner *et al.*, 2008). This could be explained by the small number of patients evaluated in each series and the heterogeneity in laboratory tests (Lae *et al.*, 2009) or in other way protein overexpression in urothelial carcinoma is not always the result of Her2/neu gene amplification. Amplification of Her2/neu can occur in isolation or be associated with coamplification of other known oncogenes (Simon *et al.*, 2003; Park *et al.*, 2005). It was also found that, although Her2/neu gene amplification has also been reported in bladder cancer using FISH (Sauter *et al.*, 1993), its amplification, development and progression and clinical significance is unknown. Molecular studies in bladder cancer have shown a discrepancy between gene amplification and overexpression of Her2/neu (Coombs *et al.*, 1991; Zhau *et al.*, 1990; Underwood *et al.*, 1995) and the clinical significance of Her2/neu gene amplification remains controversial (Lipponen, 1993; Mellon *et al.*, 1996). Structural and numerical chromosomal anomalies such as translocation, inversion, deletion and gain of chromosomes are usually associated with tumor aggressiveness and progression (Ohta *et al.*, 2001).

Cyclooxygenase (Cox) is a rate-limiting enzyme in the conversion of arachidonic acid to prostanoids. There are two forms of Cox: Cox-1 which is constitutive and Cox-2 which is inducible by multiple factors including cytokines, hormones and mitogens (Herschman, 1994; Smith *et al.*, 1996). The Cox-2 gene is 8 kilobase pairs in length and contains 10 exons. Cox-2 expression can

be induced through multiple signaling pathways involving protein kinases A and C, tyrosine kinases, bacterial endotoxin (Smith *et al.*, 2000). Cox-2 converts arachidonic acid into prostaglandin H₂ which is then further metabolized to prostaglandin E2 (Dubois *et al.*, 1998). Cox-2 is important in carcinogenesis and this idea is supported by numerous studies. It is up-regulated in transformed cells and in various forms of cancer (Parett *et al.*, 1997; Ristimaki *et al.*, 1997) and plays a role in increasing proliferation (Tsuji and Du Bois, 1995), reducing apoptosis and inhibiting cyclooxygenase-2 so, inducing apoptosis (Souza *et al.*, 2000). Cox-2 has been found to be upregulated and overexpressed in tumors of the colon (Yoshimura *et al.*, 2005), stomach, pancreas and lung cancers as well as in bladder cancer, suggesting an important role for Cox-2 in their tumorigenesis (Mohammed *et al.*, 1999; Komhoff *et al.*, 2000; Yoshimura *et al.*, 2000). Epidemiologic studies showing a 40 to 50% reduction in the incidence of colorectal cancer in individuals taking nonsteroidal anti-inflammatory drugs and constitutive up-regulation of Cox-2 in tumor cells (Trifan and Hla, 2000; Brown and DuBois, 2005). Some studies have suggested that polymorphisms in the Cox-2 gene or chromosomal gain at the Cox-2 locus (Knosel *et al.*, 2004), correlates with the risk for colorectal cancer or with survival of colorectal cancer patients. Aberrant expression of Cox-2 and the potential chemotherapeutic role of Cox-2 selective nonsteroidal anti-inflammatory drugs have been shown in many other types of cancer, including breast and lung (Howe and Dannenberg, 2002; Koehne and DuBois, 2004; Sandler and Dubinett, 2004; Terry *et al.*, 2004). For the urinary bladder there have been several studies of Cox-2 upregulation in nonschistosomal bladder cancer and on the potential role of nonselective and selective Cox-2 inhibitors in suppressing experimental tumorigenesis. This tumor-specific expression of Cox-2 suggested the potential utility of this Cox-2 promoter for the construction of a novel replication-selective adenovirus for the treatment of bladder cancer (Okajima *et al.*, 1998; Shirahama, 2000; Dovedi *et al.*, 2005). Cox-2 is also stimulated by growth factors through activation of the Epidermal Growth Factor Receptor (EGFR) (Coffey *et al.*, 1997) and tumor promoters (Ding *et al.*, 2003). A link between Her-2/neu signaling and Cox-2 expression has been established thus, overexpression of Her-2/neu in the biliary epithelium of transgenic mice led to increased levels of Cox-2 (Kiguchi *et al.*, 2001). In addition, activation of the Her2/neu pathway induced Cox-2 in colorectal cancer cells (Vadlamudi *et al.*, 1999). The objective of the study is to find out the association between Her2/neu and Cox-2 expression and to give an idea that additional screening for their implication for patients' management be performed to better stratify patients and interpret clinical response to Her2/neu-targeted therapy. Appropriate patient screening for Her2/neu and Cox-2 status may yield important insights into patient response and may guide the development of useful therapies for patients with limited treatment and a poor prognosis.

MATERIALS AND METHODS

One hundred and ten patients were included in this study on the basis of tumor availability, of whom 86 underwent total cystectomy and 24 underwent transurethral resection. Their initial staging and evaluation included cystoscopy and Transurethral Resection of the Bladder Tumor (TURBT) from department of Urology, Minia University Hospital from the period (2004-2009). All patients had histologically confirmed muscle-invasive carcinoma of the bladder. Eligibility criteria included no clinical or radiographic evidence of lymph node and distant metastases (N0 M0). Staging was defined according to the TNM (tumor-node-metastasis) Staging Classification. Response was assessed using both clinical and pathological staging.

Immunohistochemical studies: Immunohistochemistry was performed on formalin-fixed paraffin-embedded tissues using an avidin-biotin complex immunoperoxidase technique. Serial tissue sections (4 μ m) were cut from selected blocks of representative tumor tissue and mounted on poly-L-lysine-coated slides, baked at 50°C for 1 h, dewaxed with xylene and rehydrated through a series descending from alcohol to distilled water. These sections were then immersed into a 10 mM citrate buffer (pH 6.0) and heated in a water bath at 98°C for 30 min. The heated sections were allowed to cool at room temperature for 20 min and then washed in running water. After the target retrieval, the sections were treated with 3% hydrogen peroxide in methanol for 15 min for blocking endogenous peroxidase activity. After blocking with horse serum at room temperature for 10 min, the primary antibodies were applied at 4°C in a humidified chamber. IHC analysis for Her2/neu protein expression was carried out using standard avidin-biotin technique with mouse monoclonal antibody (NCL-CB11 1:100 dilution; Novocastra) and Cox-2 protein expression was assessed by Cox-2 monoclonal antibody (C-20 at 1:100 dilutions; Santa Cruz Biotechnology Company, Santa Cruz, California). Sections then incubated for 30 min with avidin-biotin peroxidase complexes (Vector Laboratories). Diaminobenzidine was used as the chromogen and modified Harris hematoxylin as the counterstain. Colonic mucosa and cancer breast known to express Cox-2 and Her2/neu were used as a positive control, respectively.

Evaluation of staining: Her2/neu positivity was assessed using the ASCO scoring system, evaluating only membranous staining. The level of Her2 protein expression was assessed semiquantitatively by the intensity and percentage of staining and scored on a scale of 0 to 3+. Scores of 0 and 1+ are absent or faint equivocal and incomplete membranous staining in less than 10% of cells, both score 0 and 1 categorized as negative; score 2+ showed unequivocal complete membranous staining and score 3+ as strong membranous staining. Tumors with scores of 2 or 3 were considered positive. The evaluation was carried out only on the invasive component of the tumor. A cytoplasmic staining was considered nonspecific (Jimenez *et al.*, 2001; Vincent-Salomon *et al.*, 2003; Wolff *et al.*, 2007).

Cox-2 showed mostly cytoplasmic expression with condensation on the nuclear membrane. The percentage of positively stained cells was determined semiquantitatively by assessing the whole tumor section. Its expression was scored 0 (absent), 1+ (weak), 2+ (moderate) and 3+ (strong) based on the extent and intensity of staining. Cox-2 positivity was defined as a score 2 and 3 and negativity including score 0 and 1 (Ristimaki *et al.*, 2002; Shim *et al.*, 2003; Boland *et al.*, 2004).

The interpretation of the results was on the basis of negativity of normal tissues. The expression of Her2/neu and Cox-2 was measured in 10 successive high-power fields (\times 400). Immunostaining was evaluated by light microscopy blindly and independently by H.M.A and H.M.T and a consensus agreement was achieved.

FISH analysis: In order to test whether Her2/neu gene amplification occurred in bladder carcinoma, a FISH analysis was performed on a representative proportion of the tumors using the Path Vision kit. All samples presenting +ve Her2/neu protein expression were evaluated using labeled probes for both Her2/neu-specific DNA sequences (17q11.2-q12 region) and the centromere of chromosome 17, CEP17 (alpha satellite DNA located at locus 17p11.1q11.1), were used (Her2/neu FISH PharmDx kit; Dako). Samples with no staining were not tested. Formalin-fixed, paraffin-embedded 4 μ tumortissue sections were deparaffinized and dehydrated. Sections were incubated in pretreatment buffer at 95°C for 10 min, then in proteolytic solution at 37°C for 8 min.

Codenaturation of the probe and DNA of the tissue section was achieved by incubation at 82°C for 5 min using a Thermo-Brite automate (Abbott Molecular Diagnostics, INC, USA). This was followed by 15 h hybridization at 37°C and by post-hybridization washes, according to the protocol. Slides were mounted in di aminido phenyl indol (DAPI)/antifade. Internal and external controls were included in the experiments. Slides were viewed with a DAPI/rhodamine/fluorescein filter and images were captured with a charge-coupled device camera, filtered and processed with Applied Spectral Imaging System using a Leica Microsystems microscope. The slides were first scanned at low magnification, using the DAPI filter, in order to locate invasive areas. The number of green (corresponding to copies of chromosome 17) and red signals (corresponding to copies of Her2/neu gene) was counted in at least 100 nuclei of invasive tumor cells, in two distant areas of the section at high magnification (×1000). Tumors were classified as amplified when they showed a Her2/neu/centromere 17 ratio>2.2 and as non amplified when the Her2/neu/centromere 17 ratio was<2.

Statistical analysis: For statistical analysis, Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 16.0 for Windows software was used. The Chi-squared test was used to detect statistically significant differences between the groups, with a significance level of $p < 0.05$.

RESULTS

A total of 110 patients were evaluated-89 males and 21 females with mean age of 54.93 years (range 40-75). Malignant tumors were subclassified into 41 tumors of squamous cell carcinoma (SqCC), six of them were superficial tumors and 35 were invasive (pT2 + pT3). Ten of SqCC were low grade and 31 were high grade. While 69 tumors were transitional cell carcinoma (TCC) 18 of them were superficial tumors (pTa and pT1) and 51 were invasive tumors (pT2 and pT3), 32 of TCC were low grade and 37 were high grade. Totally 68 out of 110 (61.8%) of patients were presented with high-grade tumors. All cases of SqCC were associated with Bilharziasis while 22 cases of TCC were Bilharzial-associated, so 63 cases were totally bilharzial associated (Table 1).

Expression of Her2/neu protein was positive in 52 patients (47.3%), while 58 tumors (52.7%) had a minimal or negative expression. Overexpression of Her2/neu protein were detected in 14 (33.3%) of 42 patients of low grade and 38 of 68 (55.9%) patients of high grade. Increased expression of Her2/neu protein in high grade tumors was statistically significant when compared

Table 1: Patients' characteristics

Patient characteristics	No.	(%)
Sex		
Male	89	80.9
Female	21	19.1
Median age (range) (years)		
Squamous cell carcinoma	41	37.3
Transitional cell carcinoma	69	62.7
Tumor grade		
Low grade	42	38.2
High grade	68	61.8
T stage		
Ta-T1	24	21.8
T2-T3	86	78.2

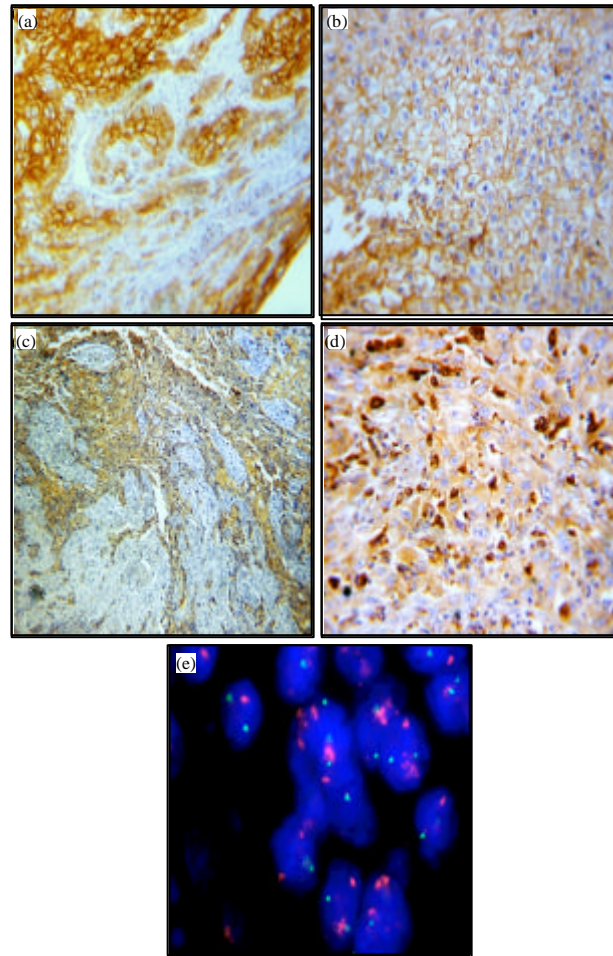


Fig. 1: Preserved membranous immunoreactivity for Her-2/neu expression in high grade squamous cell carcinoma; (a) Preserved membranous immunoreactivity for Her-2/neu expression in high grade Transitional carcinoma, (b) Preserved cytoplasmic immunoreactivity for Cox 2 expression in high grade squamous cell carcinoma, (c) Preserved Cytoplasmic immunoreactivity for Cox 2 expression in high grade Transitional cell carcinoma, (d) Fluorescent in situ hybridization HER-2 signals: Amplification clusters with multiple HER-2 gene (HER-2, Spectrum orange, Centromere 17 SpectrumGreen, 100X) (e)

with low grade ($p < 0.01$) (Fig. 1a). As regard Her2/neu protein expression in Bilharzial associated cases, expression was significantly higher in Bilharzial associated cases 37 of 63 (59.3%) than in non Bilharzial associated cases 15 of 47 (32.1%) ($p < 0.005$). Her2/neu protein expression was positive in 19 (46.3%) of 41 SqCC cases and in 33 (47.8%) of 69 TCC cases. Overexpression of Her2/neu protein were positive in 9 (37.5%) of 24 superficial (Ta-T1) tumor specimens and 43 (50%) of 86 invasive (T2-T3) tumor specimens (Fig 1b). No significant correlation was found between Her2/neu protein expression and tumor type or stage. There was also no correlation between Her2/neu protein expression and either patient gender or age (Table 2).

Table 2: Immunohistochemistry staining results and Her2/neu and Cox-2

IHC results for	No.	%
Her2/neu		
Positive	52	47.3
Negative	58	52.7
Low grade	14	33.3
High grade	38	55.9
TCC	33	47.8
SqCC	19	46.3
Superficial	9	46.3
Invasive	43	50.0
Cox-2		
Positive	58	52.7
Negative	52	47.3
Low grade	17	40.5
High grade	41	60.3
TCC	34	69.3
SqCC	24	58.5
Superficial	8	33.3
Invasive	50	58.1

Table 3: Correlation between Cox-2 and Her2/neu immunohistochemistry staining results

Her2/neu IHC status	Cox-2 IHC status	
	Positive	Negative
Positive	34 (65.4%)	18 (34.6%)
Negative	24 (41.4%)	34 (58.6%)

Expression of Cox-2 was observed in 58 (52.7%) of the patients. Cox-2 was positive in 34 TCC (49.3%) and in 24 SCC (58.5%). The expression of Cox-2 positivity is markedly heterogeneous in low grade tumors of TCC and SqCC, in which the percentage and intensity of positive staining markedly varied from one field of the tumor to another, whereas homogeneous staining was usually obtained in tumors of higher grade. Cox-2 expression was significantly higher in high grade than in low grade. Overexpression of Cox-2 was found in 17 (40.5%) of 42 patients of low grade and 41 of 68 (60.3%) patients of high grade ($p < 0.05$). Also, there was a significant difference in Cox-2 expression level between superficial and invasive tumors. Positivity was detected in 8 of 24 superficial (T_a-T₁) tumor cases and 50 (58.1%) of 86 invasive (T₂-T₃) tumor cases ($p < 0.05$). Cox-2 was positive in 38 of 63 (60.3%) of Bilharzial associated cases while it was detected in 20 of 47 (42.6%) in non bilharzial cases and this show significant correlation ($p < 0.05$). There was also no correlation between Cox-2 expression and either patient gender or age (Fig. 1c and Table2).

There is a significant correlation between Her2/neu protein expression and Cox-2. Out of the 52 positive Her2/neu protein, 34 (65.4%) were positive for Cox-2. While Cox-2 positivity were 24 of 58 (41.4%) Her2/neu protein negatively stained cases ($p = 0.01$) (Table 3).

Fifty two samples scored 2 and 3+ were analyzed by FISH to evaluate HER2 gene copy number. Her2/neu gene amplification was found in 6 of 52 (11.5%) cases highly expressing Her2/neu protein. These cases were high grade transitional cell carcinoma with muscle invasion. Relative increase in Her2/neu gene copy number was found in 22 of 52 (42.3%). It was identified in 13 of 38 (34.2%) of high grade tumors and 12 of 43 (27.9%) of high-stage ($\geq pT_2$) tumors. There was a

significant correlation between relative increase in Her2/neu gene copy number and tumor grade ($p = 0.0436$) in bladder cancers but no correlation between relative increase in Her2/neu gene copy number and tumor stage (Fig. 1d).

DISCUSSION

It is well known that, ErbB expression may correlate with prognosis, or predict responses to chemotherapy, hormonal therapy or radiation (Yarden and Sliwkowski, 2001; Arteaga, 2002). At the same time, ErbB inhibitor therapies if targeted to select patients with demonstrated overexpression of the receptors may produce optimal results. The best available evidence for this is that Herceptin therapy provides clinical benefit almost exclusively to patients with demonstrated ErbB2 overexpression (Vogel *et al.*, 2002). Moreover, fundamental studies have shown that Her-2/neu overexpression induces cell transformation and that Her-2/neu-positive tumors are more aggressive. With regard to the distribution of Her-2/neu in normal tissues, Her-2/neu is slightly expressed only in the bile duct, liver, gastrointestinal tract, genital organs, skin and urinary tract, with limited expression in most normal tissues (Natali *et al.*, 1990; Press *et al.*, 1990; Gorgoulis *et al.*, 1995). Therefore, the potential for molecular targeted therapy targeting Her2 is of great interest. It has been indicated that examination of gene amplification rather than protein overexpression is a more reliable method to identify patients with Her-2/neu-positive breast cancer (Dybdal *et al.*, 2005; Mass *et al.*, 2005).

In this study Her2/neu overexpression was statistically significant in high grade tumors when compared with low grade and no correlation was found between Her2/neu and tumor stage. While Her-2/neu overexpression was observed in 12 to 71% of urothelial cancers (Lipponen *et al.*, 1991; Gandour-Edwards *et al.*, 2002), it was correlated with grade and stage in some studies (Miyamoto *et al.*, 2000) but not in others (Koyuncuoglu *et al.*, 1998; Ioachim *et al.*, 2000). Early studies of the expression of Her-2/neu protein in bladder carcinoma found a correlation between increased Her-2/neu expression and both higher tumor stage and grade (Moriyama *et al.*, 1991; Moch *et al.*, 1993; Gorgoulis *et al.*, 1995). Others suggested that the overexpression of Her-2/neu was an independent variable in determining patient survival (Korkolopoulou *et al.*, 1997). Several recent reports found a statistically significant difference in Her2/neu protein overexpression detected by IHC in grade III TCC compared with grade I and II and in invasive TCC (T2 or T3) versus superficial TCC (Ta or T1), with more prevalent overexpression in the high grade and invasive specimens (Jimenez *et al.*, 2001; Gandour-Edwards *et al.*, 2002; Kruger *et al.*, 2002b).

It is widely accepted that, the variability in these studies was that several different antibodies were used for IHC and that the criteria for IHC positivity were based on cytoplasmic and/or membrane staining patterns. Her2/neu protein overexpression is believed to be due to a combination of two possible mechanisms. The first is gene amplification which as several reports suggests that it is not a common mechanism in bladder cancer (Coombs *et al.*, 1991; Kruger *et al.*, 2002b). The other mechanism is upregulated transcription which is possibly reflective of the nature of the Her2/neu protein (as a growth factor). Increased Her2/neu protein overexpression is noticed to be due to high levels of transcription factors, even in the absence of gene amplification (Latif *et al.*, 2004). It has been indicated that examination of gene amplification rather than antigen expression is a more reliable method to identify patients with Her2/neu positive breast cancer while it is inconclusive whether this result can similarly be applied to patients with cancer other than breast cancer, for example, patients with bladder cancer (Dybdal *et al.*, 2005; Mass *et al.*, 2005). Evidence from breast cancer indicates that only tumors with Her2/neu gene

amplification respond to an anti- Her2/neu-targeted therapy, such as Trastuzumab. Using the same principle, 5% of muscle-invasive urothelial bladder carcinomas should be suitable for such treatment. But unluckily, the potential involvement of Her2/neu in the proliferation of urothelial carcinoma led to the initiation of anti-Her2/neu-targeted therapy protocols in advanced disease (Lae *et al.*, 2009). Hence, we performed this analysis using both IHC staining and FISH. Our series indicate that 11.5% of invasive urothelial bladder carcinomas presented with Her2/neu amplification as assessed by FISH which was lower than rates observed in breast cancers. These results are in agreement with those described in the literature ranging from 0 to 32% (Latif *et al.*, 2003; De Pinieux *et al.*, 2004; Caner *et al.*, 2008; Matsubara *et al.*, 2008). In the literature on comparison between IHC and FISH, Her2/neu gene amplification was detected in only 7% of patients with urothelial cancer whereas, 43% of tumors were Her2/neu positive by IHC (Sauter *et al.*, 1993). In addition, 203 patients with urothelial cancer were studied revealing 37% of patients were HER-2 positive by IHC whereas, HER-2/neu gene amplification was detected in only 5% of patients (Kruger *et al.*, 2002a). Moreover, 23% of patients with invasive urothelial cancer were Her2/neu positive, while HER-2/neu gene amplification was detected in 28% of patients (De Pinieux *et al.*, 2004). In this study, 47.3% of patients were Her2/neu positive by IHC and 11.5% of patients were positive for gene amplification by FISH (6/52). In comparison of Her2/neu expression by IHC and amplification by FISH, it was suggested that the dissociation between gene amplification and overexpression could be related to a point mutation that leads to protein overexpression, translocation or transcriptional upregulation (Sauter *et al.*, 1993; Matsubara *et al.*, 2008). Alterations in Her2/neu by gene amplification or protein overexpression have been well-characterized in many tumor types, with Her2/neu gene amplification corresponding to worsened outcomes in many studies, including those in urothelial carcinoma (Chakravarti *et al.*, 2005; Rabindran, 2005). Furthermore, studies on Her2/neu are of particular interest in light of Her2/neu targeted therapies. Despite several studies on the overexpression and amplification of Her2/neu in urothelial carcinoma have found that, the benefits of Her2/neu directed therapy in urothelial carcinoma remain unclear. Current chemotherapeutic regimens provide only a 4 to 15% long-term survival rate for patients with advanced urothelial carcinoma (Garcia and Dreicer, 2006).

Studies involving a range of human malignancies have shown significant overexpression of cyclooxygenase (Cox)-2 in tumors compared with normal tissues, with an increase in the level of its downstream prostaglandin (PG) products. Cox catalyzes the conversion of arachidonic acid to PGs by 2 different Cox isoforms, Cox-1 and Cox-2. Cox-1 is constitutively expressed in most tissues and mediates the synthesis of PGs required for normal physiologic functions, whereas Cox-2 is primarily responsible for PGs produced in inflammatory sites and undetectable in most tissues (normal tissues) under physiologic conditions but it is induced by cytokines, growth factors, oncogenes and tumor promoters. The PGs produced by Cox-2 promote tumor development by stimulating cell proliferation and angiogenesis and by suppressing programmed cell death and immune defense (Hammam *et al.*, 2008).

The overexpression of Cox-2 in tumor cells can be attributed to transcriptional and posttranscriptional factors. The Cox-2 gene promoter contains transcriptional regulatory elements linked to multiple signaling pathways downstream of growth factors and cytokines (Subbaramaiha *et al.*, 1997). inducers of Cox-2 expression include the followings, the proinflammatory cytokines interleukin-1, Tumor Necrosis Factor (TNF)-alpha and Transforming Growth Factor (TGF)-beta which are produced by activated macrophages at the sites of chronic

inflammation (Huang *et al.*, 2000) and may contribute to mechanisms by which chronic inflammation initiates transformation of urothelial cells in Schistosomal associated bladder cancer. However, the inflammatory reaction alone cannot account for upregulated Cox-2 expression in Schistosomal associated bladder cancer because the nonmalignant epithelium exposed to the same inflammatory stimuli had a significantly lower level of Cox-2. Growth factors with tyrosine kinase second messengers, as Epidermal Growth Factor (EGF) and TGF-alpha, are also known to be inducers of Cox-2 (Saha *et al.*, 1999) and both are involved in progression of bladder neoplasia, including Schistosomal associated bladder cancer (Tungekar and Linehan, 1998) and N-nitrosamine-induced bladder cancer (Marjou *et al.*, 2000).

In the current work, we found that there was a statistically significant positive correlation between Cox-2 expression and tumor grade. Cox-2 expression was significantly higher in high grade TCC than in low grade. Correlation between Cox-2 expression and progression of bladder TCC was observed; there was a significant difference in Cox-2 expression level between the superficial TCC and invasive TCC. In the present study, Cox-2 was expressed in muscle-invasive bladder tumors that are at a more advanced stage than superficial-type tumors; this is in accordance with the results of other recent studies Wadhwa *et al.* (2005), Gurocak *et al.* (2006), Margulis *et al.* (2007). Again these results are in accordance with El-Sheikh *et al.* (2001) who confirmed Cox-2 overexpression in Schistosomal associated bladder cancer and a significant correlation between Cox-2 and tumor grade. There was no correlation between the stage of Schistosomal associated bladder cancer and Cox-2 reactivity in their study. A previous study of non-Schistosomal associated bladder cancer showed that Cox-2 is highly expressed in malignant bladder tumors but not in benign bladder tissues; also, they detected no correlation between Cox-2 expression and the individual clinical stages pT1-pT4, although such a correlation was present when stages pT1-pT4 were combined and compared with noninvasive TCC (Komhoff *et al.*, 2000). In a study of cases with intrahepatic cholangiocarcinoma, high levels of Cox-2 expression associated with reduced apoptosis and increased proliferation of tumor cells and this study concluded that Cox-2 expression is an independent prognostic factor in resected intrahepatic cholangiocarcinoma, offering a potential adjuvant to therapeutic approach with Cox-2 inhibitors (Schmitz *et al.*, 2007). There is much evidence that the Cox-2 gene is involved in features of tumor aggressiveness, such as invasiveness and metastasis. For example, Cox-2 increases adhesion to the extracellular matrix and reduces the level of the cell-adhesion molecule, E-cadherin, in rat intestinal epithelial cells (Huang *et al.*, 2000). The mechanism of elevated Cox-2 expression in tumor cells may depend on the activation of oncogenes. Activation of the K-ras oncogene is associated with an elevated expression of Cox-2 (Hermanek and Sobin, 1992; Krajewski *et al.*, 1997; Huang *et al.*, 2000) and the K-ras oncogene is frequently activated in bladder tumors (Agoff *et al.*, 2000). This particular mechanism may help explain the level of Cox-2 expression found in bladder tumors.

Cox-2 levels were increased in Her2/neu overexpressing human mammary epithelial cells and breast cancers. The induction of Cox-2 by HER-2/neu was mediated by the Ras pathway. Ras can regulate gene expression by stimulating MAPK activities (Vojtek and Der, 1998). Several lines of evidence suggest that Her2/neu induced Cox-2 via activation of ERK, JNK and p38 MAPKs. First, the activities of ERK1/2, JNK and p38 were increased in Her2/neu transformed cells. Second, inhibitors of MAPK kinase and p38 decreased amounts of Cox-2 in Her2/neu transformed cells. Third, overexpression of dominant negatives for ERK1, JNK and p38 suppressed the induction of Cox-2 promoter activity by HER-2/neu. Her2/neu status was a determinant of Cox-2 expression in

human breast tumors. Both the frequency and magnitude of Cox-2 overexpression were markedly enhanced in HER-2/neu-positive breast cancers (Subbaramaiah *et al.*, 2002). HER-2/neu induces the expression of vascular endothelial growth factor (Petit *et al.*, 1997). Cox-2 derived prostaglandins enhance the production of vascular endothelial growth factor (Williams *et al.*, 2000). It is reasonable to postulate, therefore, that the increased levels of vascular endothelial growth factor and angiogenesis in Her2/neu positive breast cancers are a consequence, in part, of Her2/neu mediated induction of Cox-2 and prostaglandins biosynthesis. Another important issue concerns the role of nonsteroidal anti-inflammatory drugs, prototypic inhibitors of Cox, in preventing cancer. The finding that Cox-2 is undetectable in most cases of Her2/neu negative breast cancer may help to explain why non-steroidal anti-inflammatory drugs have not been shown consistently to protect against breast cancer (Schreinemachers and Everson, 1994; Egan *et al.*, 1996). Present findings also imply that selective Cox-2 inhibitors may be useful in preventing or treating the subset of cancers in which Her2/neu is overexpressed. In support of this idea, treatment with a selective Cox-2 inhibitor reduced the growth rate of a HER-2/neu-positive colon cancer cell line *in vitro* and *in vivo* (Moss *et al.*, 2001). Future studies will be needed to determine whether selective inhibitors of Cox-2 have a role in preventing or treating Her2/neu positive human breast cancers (Subbaramaiah *et al.*, 2002). Both the EGFR and the prostaglandins signaling pathways promote cell survival through activation of the mitogen-activated protein kinase and phosphatidylinositol 3-kinase/Akt signaling pathways. Overexpression of Her2/neu enhances the EGFR signaling response (Yarden and Sliwkowski, 2001, Citri *et al.*, 2003), resulting in activation of the Ras/Raf/mitogen-activated protein kinase, phosphatidylinositol 3-kinase and phospholipase C pathways and up-regulates Cox-2 expression (Ristimaki *et al.*, 2002; Benoit *et al.*, 2004). Prostaglandins E₂ was observed to transactivate EGFR in NMF11.2 cells. This evidence provides a strong rationale for targeting both EGFR and Cox-2 in Her2/neu overexpressing breast cancer (Lanza-Jacoby *et al.*, 2006).

There may be a link between association of Cox-2 expression with Her2/neu expression and the development and progression of urothelial carcinoma. Additional investigations are needed to determine whether Cox-2 expression has any prognostic value and whether Cox-2 inhibitors are useful for chemoprevention and cancer treatment of bladder tumors. Additional work is required to elucidate the possible downstream molecular targets of Cox-2 products and to assess the potential role of selective Cox-2 inhibitors in preventing and treating bladder cancer. Her2/neu amplification can be assessed by a standardized IHC, using FISH in the ambiguous cases. These findings may have clinical implications for the management of patients with Her2/neu positive locally advanced or metastatic bladder cancer. In practice, all muscle-invasive urothelial bladder carcinomas should be tested for Her2/neu status according to the recommendations for breast carcinoma.

REFERENCES

- Agoff, S.N., T.A. Brentnall, D.A. Crispin, S.L. Taylor and S. Raaka *et al.*, 2000. The role of cyclooxygenase 2 in ulcerative colitis-Associated neoplasia. *Am. J. Pathol.*, 157: 737-745.
- Arteaga, C.L., 2002. Epidermal growth factor receptor dependence in human tumors: More than just expression. *Oncologist*, 7: 31-39.
- Ashley, S.F., S.A. Soliman, H. Khaled, S.M. Zaghloul and B. Mousumi *et al.*, 2008. The changing patterns of bladder cancer in Egypt over the past 26 years. *Cancer Causes Control*, 19: 421-429.
- Benoit, V., B. Relic, X. De-Leval, A. Chario, M.P. Merville and V. Bours, 2004. Regulation of HER-2 oncogene expression by cyclooxygenase-2 and prostaglandin E₂. *Oncogene*, 23: 1631-1635.

- Boland, G.P., I.S. Butt, R. Prasad, W.F. Knox and N.J. Bundred, 2004. Cox-2 expression is associated with an aggressive phenotype in ductal carcinoma *in situ*. *Br. J. Cancer*, 90: 423-429.
- Borden, L.S., P.E. Clark and M.C. Hall, 2004. Bladder cancer. *Curr. Opin. Oncol.*, 16: 257-262.
- Brown, J.R. and R.N. Du Bois, 2005. Cox-2: A molecular target for colorectal cancer prevention. *J. Clin. Oncol.*, 23: 2840-2855.
- Caner, V., N.S. Turk, F. Duzcan, N.L.S. Tufan and E.C. Kelten *et al.*, 2008. No strong association between HER-2/neu protein overexpression and gene amplification in high-grade invasive urothelial carcinomas. *Pathol. Oncol. Res.*, 14: 261-266.
- Chakravarti, A., K. Winter and C.L. Wu, 2005. Expression of the epidermal growth factor receptor and Her-2 are predictors of favorable outcome and reduced complete response rates, respectively, in patients with muscle-invasive bladder cancers treated by concurrent radiation and cisplatin-based chemotherapy: A report from the radiation therapy oncology group. *Int. J. Radiat. Oncol. Biol. Phys.*, 62: 309-317.
- Citri, A., K.B. Skaria and Y. Yarden, 2003. The deaf and the dumb: The biology of ErbB-2 and ErbB-3. *Exp. Cell Res.*, 284: 54-65.
- Coffey, R., C. Hawkey, L. Damstrup, R. Graves-Deal and V. Daniel *et al.*, 1997. Epidermal growth factor receptor activation induces nuclear targeting of cyclooxygenase-2, basolateral release of prostaglandins and mitogenesis in polarizing colon cancer cells. *Proc. Natl. Acad. Sci. USA.*, 94: 657-662.
- Coombs, L.M., D.A. Pigott, E. Sweeney, A.J. Proctor, M.E. Eydmann, C. Parkinson and M.A. Knowles, 1991. Amplification and over-expression of c-erbB-2 in transitional cell carcinoma of the urinary bladder. *Br. J. Cancer*, 63: 601-608.
- De Pinieux, G., D. Colin, A. Vincent-Salomon, J. Couturier, D. Amsellem-Ouazana, P. Beuzebec and A. Vieillefond, 2004. Confrontation of immunohistochemistry and fluorescent *in situ* hybridization for the assessment of HER-2/neu (c-erbB-2) status in urothelial carcinoma. *Virchows Arch.*, 444: 415-419.
- Ding, X.Z., R. Hennig and T.E. Adrian, 2003. Lipoxigenase and cyclooxygenase metabolism: New insights in treatment and chemoprevention of pancreatic cancer. *Mol. Cancer*, 2: 10-10.
- Dovedi, S.J., J.A. Kirby, H. Atkins, B.R. Davies and J.D. Kelly, 2005. Cyclooxygenase-2 inhibition: A potential mechanism for increasing the efficacy of bacillus calmette-guerin immunotherapy for bladder cancer. *J. Urol.*, 174: 332-337.
- Dubois, R.N., S.B. Abramson, L. Crofford, R.A. Gupta, L.S. Simon, L.B.V.D. Putte and P.E. Lipsky, 1998. Cyclooxygenase in biology and disease. *FASEBJ.*, 12: 1063-1073.
- Dybdal, N., G. Leiberman, S. Anderson, B. McCune and A. Bajamonde *et al.*, 2005. Determination of HER2 gene amplification by fluorescence *in situ* hybridization and concordance with the clinical trials immunohistochemical assay in women with metastatic breast cancer evaluated for treatment with trastuzumab. *Breast Cancer Res. Treat.*, 93: 3-11.
- Egan, K.M., M.J. Stampfer, E. Giovannucci, B.A. Rosner and G.A. Colditz, 1996. Aspirin use and potential mechanisms for colorectal cancer prevention. *J. Natl. Cancer Inst.*, 88: 988-993.
- El-Sebaie, M., M.S. Zaghoul, G. Howard and A. Mokhtar, 2005. Squamous cell carcinoma of the bilharzial and non-bilharzial urinary bladder a review of etiological features, natural history and management. *Int. J. Clin. Oncol.*, 10: 20-25.
- El-Sheikh, S., S. Madaan and A. Alhasso, 2001. Cyclooxygenase-2: A possible target in schistosoma-associated bladder cancer. *BJU Int.*, 88: 921-927.

- Estall, J.L., J.L. Ruas, C.S. Choi, D. Laznik and M. Badman *et al.*, 2009. PGC-1alpha negatively regulates hepatic FGF21 expression by modulating the heme/Rev-Erb(alpha) axis. *Proc. Natl. Acad. Sci. USA.*, 106: 22510-22515.
- Gandour-Edwards, R., P.N. Lara, A.K. Folkins, J.M. LaSalle and L. Beckett *et al.*, 2002. Does HER2/neu expression provide prognostic information in patients with advanced urothelial carcinoma. *Cancer*, 95: 1009-1015.
- Garcia, J.A. and R. Dreicer, 2006. Systemic chemotherapy for advanced bladder cancer: Update and controversies. *J. Clin. Oncol.*, 24: 5545-5551.
- Gorgoulis, V.G., C. Barbatis, I. Poulis and A.M. Karameris, 1995. Molecular and immunohistochemical evaluation of epidermal growth factor receptor and c-erb-B-2 gene product in transitional cell carcinomas of the urinary bladder: A study in Greek patients. *Mod Pathol.*, 8: 758-764.
- Gurocak, S., S. Sozen, O. Erdem, S. Ozkan and Y. Kordan *et al.*, 2006. Relation between cyclooxygenase-2 expression and clinicopathologic parameters with patient prognosis in transitional cell carcinoma of the bladder. *Urol. Int.*, 76: 51-56.
- Hammam, O.A., A.A. Aziz, M.S. Roshdy and A.M. Abdel Hadi, 2008. Possible role of cyclooxygenase-2 in schistosomal and non-schistosomal-associated bladder cancer. *Medscape J. Med.*, 10: 60-60.
- Hammerich, K.H., G.E. Ayala and T.M. Wheeler, 2008. Application of immunohistochemistry to the genitourinary system (prostate, urinary bladder, testis and kidney). *Arch. Pathol. Lab. Med.*, 132: 432-440.
- Hermanek, P. and L. Sobin, 1992. TNM Classification of Malignant Tumours. 4th Edn., Springer-Verlag Berlin, Germany.
- Herschman, H.R., 1994. Regulation of prostaglandin synthase-1 and prostaglandin synthase-2. *Cancer Metastasis Rev.*, 13: 241-256.
- Howe, L.R. and A.J. Dannenberg, 2002. A role for cyclooxygenase-2 inhibitors in the prevention and treatment of cancer. *Semin. Oncol.*, 29: 111-119.
- Huang, Z.F., J.B. Massey and D.P. Via, 2000. Differential regulation of cyclooxygenase-2 (Cox-2) mRNA stability by interleukin-1 beta (IL-1 beta) and tumor necrosis factor-alpha (TNF-alpha) in human *in vitro* differentiated macrophages. *Biochem. Pharmacol.*, 59: 187-194.
- Ioachim, E., A. Charchanti, N.E. Stavropoulos, A. Skopelitou, E.D. Athanassiou and N.J. Agnantis, 2000. Immunohistochemical expression of retinoblastoma gene product (Rb), p53 protein, MDM2, c-erbB-2, HLA-DR and proliferation indices in human urinary bladder carcinoma. *Histol. Histopathol.*, 15: 721-727.
- Jamal, A., R. Siegel, E.L. Ward, T. Murray, J. Xu, M. Smigal and J. Thun, 2006. *Cancer Statistics. CA Cancer J. Clin.*, 56: 106-130.
- Jimenez, R.E., M. Hussain, F.J. Jr. Bianco, U. Vaishampayan and P. Tabazcka *et al.*, 2001. Her-2/neu overexpression in muscle-invasive urothelial carcinoma of the bladder: Prognostic significance and comparative analysis in primary and metastatic tumors. *Clin. Cancer Res.*, 7: 2440-2447.
- Kiguchi, K., S. Carbajal, K. Chan, L. Beltran and L. Ruffino *et al.*, 2001. Constitutive expression of ErbB-2 in gallbladder epithelium results in development of adenocarcinoma. *Cancer Res.*, 61: 6971-6976.
- Knosel, T., Y. Yu, U. Stein, H. Schwabe and K. Schluns *et al.*, 2004. Overexpression of cyclooxygenase-2 correlates with chromosomal gain at the cyclooxygenase-2 locus and decreased patient survival in advanced colorectal carcinomas. *Dis. Colon Rectum*, 47: 70-77.

- Knowles, M.A., 2006. Molecular subtypes of bladder cancer: Jekyll and Hyde or chalk and cheese. *Carcinogenesis*, 27: 361-373.
- Koehne, C.H. and R.N. DuBois, 2004. Cox-2 inhibition and colorectal cancer. *Semin. Oncol.*, 31: 12-21.
- Komhoff, M., Y. Guan, H.W. Shappell, L. Davis and G. Jack, 2000. Enhanced expression of cyclooxygenase-2 in high-grade human transitional cell bladder carcinomas. *Am. J. Pathol.*, 157: 29-35.
- Korkolopoulou, P., P. Christodoulou, P. Kapralos, M. Exarchakos and A. Bisbiroula *et al.*, 1997. The role of p53, MDM2 and c-erb B-2 oncoproteins, epidermal growth factor receptor and proliferation markers in the prognosis of urinary bladder cancer. *Pathol. Res. Pract.*, 193: 767-775.
- Koyuncuoglu, M., A. Kargi, S. Cingoz and Z. Kirkali, 1998. Investigation of p53, c-erbB-2, PCNA immunoreactivity, DNA content, AgNOR and apoptosis in bladder carcinoma as prognostic parameters. *Cancer Lett.*, 126: 143-148.
- Krajewski, S., M. Krajewska, J. Ehrmann, M. Sikorska, B. Lach, J. Chatten and J.C. Reed, 1997. Immunohistochemical analysis of Bcl-2, Bcl-X, Mcl-1 and Bax in tumors of central and peripheral nervous system origin. *Am. J. Pathol.*, 150: 805-814.
- Kruger, S., G. Weitsch, H. Buttner, A. Matthiensen and T. Bohmer *et al.*, 2002a. HER2 overexpression in muscle-invasive urothelial carcinoma of the bladder: Prognostic implications. *Int. J. Cancer*, 102: 514-518.
- Kruger, S., G. Weitsch, H. Buttner, A. Matthiensen and T. Bohmer *et al.*, 2002b. Overexpression of c-erbB-2 oncoprotein in muscle-invasive bladder carcinoma: Relationship with gene amplification, clinicopathological parameters and prognostic outcome. *Int. J. Oncol.*, 21: 981-987.
- Lae, M., J. Couturier, S. Oudard, F. Radvanyi, P. Beuzebec and A. Vieillefond, 2009. Assessing HER2 gene amplification as a potential target for therapy in invasive urothelial bladder cancer with a standardized methodology: Results in 1005 patients. *Ann. Oncol.*, 21: 815-819.
- Lanza-Jacoby, S., R. Burd, F.E. Rosato, K. McGuire, J. Little, N. Nougibilly and S. Miller, 2006. Effect of simultaneous inhibition of epidermal growth factor receptor and cyclooxygenase-2 in HER-2/Neu-positive breast cancer. *Clin. Cancer Res.*, 12: 6161-6169.
- Latif, Z., A.D. Watters, I. Dunn, K.M. Grigor, M.A. Underwood and J.M.S. Bartlett, 2003. HER2/neu overexpression in the development of muscle-invasive transitional cell carcinoma of the bladder. *Br. J. Cancer*, 89: 1305-1309.
- Latif, Z., A.D. Watters, I. Dunn, K. Grigor, M.A. Underwood and J.M. Bartlett, 2004. HER2/neu gene amplification and protein overexpression in G3 pT2 transitional cell carcinoma of the bladder: A role for anti-HER2 therapy. *Eur. J. Cancer*, 40: 56-63.
- Lipponen, P., M. Eskelinen, S. Syrjanen, A. Tervahauta and K. Syrjanen, 1991. Use of immunohistochemically demonstrated c-erbB-2 oncoprotein expression as a prognostic factor in transitional cell carcinoma of the urinary bladder. *Eur. Urol.*, 20: 238-242.
- Lipponen, P., 1993. Expression of c-erbB-2 oncoprotein in transitional cell bladder cancer. *Eur. J. Cancer*, 29: 749-753.
- Margulis, V., S.F. Shariat, R. Ashfaq, M. Thompson, A.I. Sagalowsky, J.T. Hsieh and Y. Lotan, 2007. Expression of cyclooxygenase-2 in normal urothelium and superficial and advanced transitional cell carcinoma of bladder. *J. Urol.*, 177: 1163-1168.

- Marjou, A., A. Delouvee, J. Thiery and F. Radvanyi, 2000. Involvement of epidermal growth factor receptor in chemically induced mouse bladder tumor progression. *Carcinogenesis*, 21: 2211-2218.
- Mass, R.D., M.F. Press and S. Anderson, 2005. Evaluation of clinical outcomes according to HER-2 detection by fluorescence in situ hybridization in women with metastatic breast cancer treated with trastuzumab. *Clin. Breast Cancer*, 6: 240-246.
- Matsubara, H., Y. Yamada, K. Naruse, K. Nakamura and S. Aoki *et al.*, 2008. Potential for HER-2/neu molecular targeted therapy for invasive bladder carcinoma: Comparative study of immunohistochemistry and fluorescent *in situ* hybridization. *Oncol. Rep.*, 17: 57-63.
- Mellon, J.K., J. Lunec, C. Wright, C.H.W. Horne, P. Kelly and D.E. Neal, 1996. C-erbB-2 in bladder cancer: molecular biology, correlation with epidermal growth factor receptors and prognostic value. *J. Urol.*, 155: 321-326.
- Miyamoto, H., Y. Kubota, S. Noguchi, K. Takase and J. Matsuzaki *et al.*, 2000. C-ERBB-2 gene amplification as a prognostic marker in human bladder cancer. *Urology*, 55: 679-683.
- Moch, H., G. Sauter, D. Moore, M.J. Mihatsch, F. Gudat and F. Waldman, 1993. P53 and erbB-2 protein overexpression are associated with early invasion and metastasis in bladder cancer. *Virchows Arch. Pathol. Anat. Histopathol.*, 423: 329-334.
- Mohammed, S.I., D.W. Knapp, D.G. Bostwick, K.R.S. Foster and K.N.M. Khan *et al.*, 1999. Expression of cyclooxygenase-2 (Cox-2) in human invasive Transitional Cell Carcinoma (TCC) of the urinary bladder. *Cancer Res.*, 59: 5647-5650.
- Moriyama, M., T. Akiyama, T. Yamamoto, T. Kawamoto and T. Kato *et al.*, 1991. Expression of c-erbB-2 gene product in urinary bladder cancer. *J. Urol.*, 145: 423-427.
- Moss, M., H. Sheng, J. Shao, C.S. Williams, P.I. Pisacane, M.X. Sliwkowski and R.N. DuBois, 2001. Cyclooxygenase-2 is overexpressed in HER-2/neu breast cancer. *Gastroenterology*, 120: 1713-1719.
- Natali, P.G., M.R. Nicotra, A. Bigotti, I. Ventura, D.J. Slamon, B.M. Fendly and A. Ullrich, 1990. Expression of the p185 encoded by HER2 oncogene in normal and transformed human tissues. *Int. J. Cancer*, 45: 457-461.
- Ohta, J., Y. Miyoshi, H. Uemura, K. Fujinami and K. Mikata *et al.*, 2001. Fluorescence *in situ* hybridization evaluation of c-erbB-2 gene amplification and chromosomal anomalies in bladder cancer. *Clin. Cancer Res.*, 7: 2463-2467.
- Okajima, E., A. Denda, S. Ozono, M. Takahama and H. Akai *et al.*, 1998. Chemopreventive effects of nimesulide, a selective cyclooxygenase-2 inhibitor, on the development of rat urinary bladder carcinomas initiated by N-butyl-N-(4-hydroxybutyl) nitrosamine. *Cancer Res.*, 58: 3028-3031.
- Parett, M.L., R.E. Harris, F.S. Joarder, M.S. Ross, K.P. Clausen and F.M. Robertson, 1997. Cyclooxygenase-2 gene expression in human breast cancer. *Int. J. Oncol.*, 10: 503-507.
- Park, K., K. Kwak, J. Kim, S. Lim and S. Han, 2005. MYC amplification is associated with HER2 amplification and closely linked with cell proliferation in tissue microarray of nonselected breast cancers. *Hum. Pathol.*, 36: 634-639.
- Petit, A.M.V., J. Rak, M.C. Hung, P. Rockwell, N. Goldstein, B. Fendly and R.S. Kerbel, 1997. Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor production by tumor cells *in vitro* and *in vivo*: Angiogenic implications for signal transduction therapy of solid tumors. *Am. J. Pathol.*, 151: 1523-1530.
- Press, M.F., C. Cordon-Cardo and D.J. Slamon, 1990. Expression of the HER-2/neu proto-oncogene in normal human adult and fetal tissues. *Oncogene*, 5: 953-962.

- Press, M.F., L. Bernstein, P.A. Thomas, L.F. Meisner and J.Y. Zhou *et al.*, 1997. Her-2/neu gene amplification characterized by fluorescence *in situ* hybridization: Poor prognosis in node negative breast carcinomas. *J. Clin. Oncol.*, 15: 2894-2904.
- Rabindran, S.K., 2005. Antitumor activity of HER-2 inhibitors. *Cancer Lett.*, 227: 9-23.
- Ristimaki, A., N. Honkanen, H. Jankala, P. Sipponen and M. Harkonen, 1997. Expression of cyclooxygenase 2 in human gastric cancer. *Cancer Res.*, 57: 1276-1280.
- Ristimaki, A., A. Sivula, J. Lundin, M. Lundin and T. Salminen *et al.*, 2002. Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. *Cancer Res.*, 62: 632-635.
- Saha, D., P.K. Datta, H. Sheng, J.D. Morrow and M. Wada, 1999. Synergistic induction of cyclooxygenase-2 by transforming growth factor-beta 1 and epidermal growth factor inhibits apoptosis in epithelial cells. *Neoplasia*, 1: 508-517.
- Sandler, A.B. and S.M. Dubinett, 2004. Cox-2 inhibition and lung cancer. *Semin Oncol.*, 31: 45-52.
- Sato, K., M. Moriyama, S. Mori, M. Saito and T. Watanuki *et al.*, 1992. An immunohistologic evaluation of cerbB-2 gene product in patients with urinary bladder carcinoma. *Cancer*, 15: 2493-2498.
- Sauter, G., H. Moch, D. Moore, P. Carroll and R. Kerschmann *et al.*, 1993. Heterogeneity of erbB-2 gene amplification in bladder cancer. *Cancer Res.*, 53: 2199-2203.
- Schmitz, K.J., H. Lang, J. Wohlschlaeger, H. Reis, G.C. Sotiropoulos, K.W. Schmid and H.A. Baba, 2007. Elevated expression of cyclooxygenase-2 is a negative prognostic factor for overall survival in intrahepatic cholangiocarcinoma. *Virchows Arch.*, 450: 135-141.
- Schreinemachers, D.M. and R.B. Everson, 1994. Mechanisms underlying non steroidal anti-inflammatory drug induced P27/kip 1 expression. *Epidemiology*, 5: 138-146.
- Seshadri, R., F.A. Firgaira, D.J. Horsfall, K. McCaul, V. Setlur and P. Kitchen, 1993. Clinical significance of HER-2/neu oncogene amplification in primary breast cancer. The South Australian breast cancer study group. *J. Clin. Oncol.*, 11: 1936-1942.
- Shim, V., M.L. Gauthier, D. Sudilovsky, K. Mantei and K.L. Chew *et al.*, 2003. Cyclooxygenase 2 expression is related to nuclear grade in ductal carcinoma in situ and is increased in its normal adjacent epithelium. *Cancer Res.* 63: 2347-2350.
- Shirahama, T., 2000. Cyclooxygenase-2 expression is up regulated in transitional cell carcinoma and its preneoplastic lesions in the human urinary bladder. *Cancer Res.*, 6: 2424-2430.
- Simon, R., R. Atefy, U. Wagner, T. Forster and A. Fijan *et al.*, 2003. HER-2 and TOP2A coamplification in urinary bladder cancer. *Int. J. Cancer*, 107: 764-772.
- Smith, W.L., R.M. Garavito and D.L. DeWitt, 1996. Prostaglandin endoperoxide H synthases (Cyclooxygenases)-1 and -2. *J. Biol. Chem.*, 27: 33157-33160.
- Smith, W.L., D.L. De Witt and R.M. Garavito, 2000. Cyclooxygenases: Structural, cellular and molecular biology. *Ann. Rev. Biochem.*, 69: 145-182.
- Souza, R.F., K. Shewemake, D.G. Beer, B. Cryer and S.J. Spechler, 2000. Selective inhibition of cyclooxygenase 2 suppresses growth and induces apoptosis in human esophageal adenocarcinoma cells. *Cancer Res.*, 60: 5767-5772.
- Subbaramaiah, K., D. Zakin, B.B. Welkaler and J.A. Dannenberg, 1997. Inhibition of cyclooxygenase: A novel approach to cancer prevention. *Proc. Soc. Exp. Bio. Med.*, 216: 201-210.
- Subbaramaiah, K., L. Norton, W. Gerald and A.J. Dannenberg, 2002. Cyclooxygenase-2 is overexpressed in HER-2/neu-positive breast cancer. *J. Biol. Chem.*, 277: 18649-18657.

- Terry, M.B., M.D. Gammon, F.F. Zhang, H. Tawfik and S.L. Teitelbaum *et al.*, 2004. Association of frequency and duration of aspirin use and hormone receptor status with breast cancer risk. *J. Am. Med. Assoc.*, 291: 2433-2440.
- Trifan, O.C. and T. Hla, 2000. Cyclooxygenase-2 modulates cellular growth and promotes tumorigenesis. *J. Cell Mol. Med.*, 7: 207-222.
- Tsujii, M. and R.N. Du Bois, 1995. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell*, 83: 493-501.
- Tungekar, M. and J. Linehan, 1998. Patterns of expressions of transforming growth factor and epidermal growth factor receptor in squamous cell lesions of the urinary bladder. *J. Clin. Pathol.*, 51: 583-587.
- Underwood, M., J. Bartlett, J. Reeves, D.S. Gardiner, R. Scott and T. Cooke, 1995. C-erbB-2 gene amplification: A molecular marker in recurrent bladder tumors. *Cancer Res.*, 55: 2422-2430.
- Vadlamudi, R., M. Mandal, L. Adam, G. Steinbach, J. Mendelsohn and R. Kumar, 1999. Regulation of cyclooxygenase-2 pathway by HER2 receptor. *Oncogene*, 18: 305-314.
- Vincent-Salomon, A., G. Macgrogan and J. Couturier, 2003. Calibration of immunohistochemistry for assessment of HER2 in breast cancer: Results of the French multicenter GEFPICS study, 2003. *Histopathology*, 42: 337-347.
- Vogel, C.L., M.A. Cobleigh, D. Tripathy, J.C. Gutheil and L.N. Harris *et al.*, 2002. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J. Clin. Oncol.*, 20: 719-726.
- Vojtek, A.B. and C.J. Der, 1998. Increasing complexity of the Ras signaling pathway. *J. Biol. Chem.*, 273: 19925-19928.
- Wadhwa, P., A.K. Goswami, K. Joshi and S.K. Sharma, 2005. Cyclooxygenase-2 expression increases with the stage and grade in transitional cell carcinoma of the urinary bladder. *Int. Urol. Nephrol.*, 37: 47-53.
- Williams, C.S., M. Tsujii, J. Reese, S.K. Dey and R.N. DuBois, 2000. Loss of cyclooxygenase-2 retards decidual growth but does not inhibit embryo implantation or development to term. *J. Clin. Invest.*, 105: 1589-1594.
- Wolff, A.C., M.E. Hammond and J.N. Schwartz, 2007. American society of clinical oncology/college of American pathologists guideline recommendations for human epidermal growth factor receptor2 testing in breast cancer. *J. Clin. Oncol.*, 25: 118-145.
- Wu, X.R., 2005. Urothelial tumorigenesis A tale of divergent pathways. *Nat. Rev. Cancer*, 5: 713-725.
- Yarden, Y. and M.X. Sliwkowski, 2001. Untangling the ErbB signalling network. *Nat. Rev. Mol. Cell Biol.*, 2: 127-137.
- Yaziji, H., L.C. Goldstein, T.S. Barry, R. Werling and H. Hwang, 2004. HER-2 testing in breast cancer using parallel tissue-based methods. *JAMA.*, 291: 1972-1977.
- Yoshimura, R., H. Sano, C. Masuda, M. Kawamura and Y. Tsubouchi *et al.*, 2000. Expression of cyclooxygenase-2 in prostate carcinoma. *Cancer*, 89: 589-595.
- Yoshimura, R., M. Matsuyama, K. Tsuchida, Y. Takemoto and T. Nakatani, 2005. Relationship between cyclooxygenase (Cox)-2 and malignant tumors. *Nippon Rinsho*, 63: 1839-1848.
- Zhau, H.E., X. Zhang, A.C.V. Eschenbach, K. Scorsone, R.J. Babaian, J.Y. Ro and M.C. Hung, 1990. Amplification and expression of the c-erb B-2/neu proto-oncogene in human bladder cancer. *Mol. Carcin.*, 3: 254-257.