Correlation Of The Expression Of Various Markers (i) Beta-HCG And P53, (ii) Beta-HCG And C-erb-b2, (iii) Beta-HCG And eGFR And (iv) C-erb-b2 And P53) In Human Urothelial Carcinomas

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Author(s): Venyo A , Herring D , Greenwood H , Maloney D

Abstract

Background:
There is no clear cut way of predicting which superficial urothelial tumours would recur and which muscle-invasive tumours would progress.

Objectives:
To study study the correlation of the expression of various markers (i) β-HCG and p53, ii) β-HCG and c-erb-B2, iii) c-erb-B2 and p53; iv) β-HCG and EGFr ) in urothelial carcinoma using an immunohistological (ABC) method in other to investigate whether or not the use of more than one tumour marker would be more advantageous in predicting outcome than the use of one tumour marker.

Methods:
Formalin fixed / paraffin embedded urothelial carcinoma specimens of 86 patients were used for the correlation of the expression of β-HCG and p53, β-HCG and c-erb-B2, c-erb-B2 and p53. Fresh frozen urothelial carcinoma specimens of 44 patients were used for the study of the correlation of the expression of β-HCG and EGFr. The expression of the various markers were analysed with regards to grade, stage and outcome.

Analysis:
The results were analysed using SPSS for windows

Results and conclusions:
All the superficial tumours that were positive for both markers in each group (β-HCG and p53, β-HCG and c-erb-B2, β-HCG and EGFr, or c-erb-B2 and p53) recurred; two thirds of the pTa and pT1 tumours that were negative for both markers did not recur. The difference between outcome of the double marker positive superficial tumours and double marker negative tumours was significant. However, the difference between the outcome of double marker and single marker positive superficial tumours was not significant. Regarding the muscle-invasive tumours, the best survival was observed in tumours that were negative for both markers and the worst outcome in tumours that were positive for both markers There is evidence to suggest even though anecdotal that muscle-invasive bladder tumours positively stained for β-HCG are aggressive tumours which are resistant to radiotherapy; survival after radical cystectomy for such tumours is poor; but such β-HCG positive muscle-invasive tumours even if they are inoperable have been shown to be sensitive to combination systemic chemotherapy therefore such patients should be offered combination systemic chemotherapy if they are fit to be under-go chemotherapy.

KEY WORDS. Urothelial Carcinoma, β-HCG, c-erb-B2, p53, EGFr, Immunohistochemistry, Bladder Cancer

Introduction

Studies regarding the immunohistological expression of β-HCG by urothelial cancers are few and these studies have suggested varying rates of expression[1, 2, 3, 4, 5,]. Martin and co-workers found that the expression of β-HCG correlated with tumours that did not respond to radiotherapy and the presence of squamous metaplasia. [5] Other workers have also suggested that tumours expressing β-HCG may be radioresistant.[6; 7; 8; 9,]. In a study of malignancies producing ectopic HCG, which included 2 bladder cancers Crawford and co-workers suggested such expression may indicate chemosensitivity [10]. Williams and co-workers noted response to chemotherapy of patients with advanced bladder cancer associated with production of Germ cell tumour markers [11]. The epidermal growth factor (EGF, or c-erb-B1) is a 53 - amino acid peptide of 6,000 daltons [12] which was isolated by Cohen from the submaxillary gland of the immature male mouse. This potent mitogen is widespread in human tissues with high levels also found in prostatic fluid and milk. Large quantities of EGF have been found in urine with low plasma levels of EGF suggesting that renal tubules probably secrete EGF. EGF has been reported to increase crypt cell proliferation in an infant with microvillus atrophy and it has been used clinically to accelerate corneal re-epithelialization following injury. EGF has been
noted to increase transcription of the nuclear proto-oncogene c-jun: a gene which forms part of the transcription factor AP1. The EGF receptor (EGFr) is mediated by binding to the external domain of a transmembrane receptor which is the product of the c-erb-B1 gene. The EGF receptor is a 175,000 d protein consisting of an extra cellular binding portion, including two cysteine rich domains, a small transmembrane domain containing the protein tyrosine kinase and three major auto-phosphorylation sites located on tyrosine residues. EGF receptors can be detected biochemically or by immunohistochemical methods. EGF receptors have been identified on a variety of cell types including: normal basal, urothelial cells, corneal cells, kidney cells and fibroblasts [13]. Expression of high levels of EGF receptors have been found in some tumours including breast carcinomas, squamous cell carcinomas, gynaecological tumours, gliomas, lung tumours and sarcomas [13; 14].

Placenta and the A431 cell line originally derived from a vulval squamous cell carcinoma, have especially high concentrations of EGFr. In bladder cancer a correlation between positivity for EGFr and tumour grade and stage has been demonstrated by Neal and Co-workers [13; 14].

P53 is a 393 amino acid nucleo-phosphoprotein first identified as a result of binding of the large T antigen of DNA virus SV40 [15]. The P53 (TP53) which is located on chromosome 17p13.1 has been described as the most commonly altered gene in human cancer. Transfection studies have revealed that the wild type protein is able to suppress cell proliferation and transformation [16; 17]. A significant association has been found between p53 and the EGFr [18]. Strong staining for p53 has been found in 18% of primary human transitional cell carcinomas of the bladder with weaker staining in a further 36% by Wright and co-workers [19].

The c-erb-B2 gene (also known as HER2 or neu) located on chromosome 17q21 encodes a membrane bound glycoprotein (185 kDa transmembrane phosphoglycoprotein) which has sequence similarity with the epidermal growth factor receptor (c-erb-B1) [20]. Overexpression and amplification of c-erb-B2 in carcinoma of breast and ovary is reported to be associated with inferior prognosis [21; 22].

There is no clear cut way of predicting which pTa and pT1 urothelial cancers would recur and which recurrences would be of high stage. There is also no clear cut way of predicting which muscle invasive tumours would result in the death of a patient. In three separate studies Venyo and co workers observed that individually the expression of β-HCG, c-erb-B2 and p53 by pTa and pT1 tumours may be associated with tumours of high grade and high stage [23; 24; 25]. Venyo and co workers also observed that the expression of β-HCG, c-erb-B2 and p53 by superficial tumours is quite often associated with recurrences of higher stage. They also observed that the individual expression of these markers by muscle invasive urothelial tumours is associated with inferior prognosis. Considering the fact that no single tumour marker has been found which could be used to predict every carcinoma with the potential of progressing or not responding to treatment it was decided to carry out this study in order to find out if the simultaneous use of a second tumour marker would be of an additional advantage in selecting a) pTa and pT1 tumours with the potential to recur and b) muscle invasive tumours with grave prognosis.

**Patients and Methods**

Between 1990 and 1994, 86 patients (49 male and 37 female), with urothelial carcinomata, mean age 69.5 years (range 20 to 95 years) who were treated in Dryburn Hospital in Durham City united Kingdom (now called University Hospital of North Durham) were enrolled in the study of the correlation of the expression of a) β-HCG and p53, b) β-HCG and c-erb-B2 and c-erb-B2 and p53. These patients had a mean follow up time of 55.7 months. For the study of the correlation of the expression of β-HCG and EGFr 44 patients with urothelial carcinomata, mean age 70 years (range 51 to 85 years) who were treated in the Freeman Hospital in Newcastle upon Tyne were enrolled. These patients had a mean follow up of 40.6 months.

**Correlation of the expression of a) β-HCG and p53, b) β-HCG and c-erb-B2 and (c) c-erb-B2 and p53.**

Urothelial tumour samples were obtained from all the 86 patients requiring surgical excision or transurethral resection of their tumours. 55 of these patients required transurethral resections of bladder tumours and 31 patients had resections of bladder tumours followed by radiotherapy and / or laparotomy and cystectomy. In each case the tumours were staged based upon the TNM classification (UICC 1987), by a careful bimanual examination under anaesthesia at the time of surgery in combination with the histology report. The tumours were graded according to the system of Bergkvist et al., 1965, using routine haematoxilin and eosin (H&E) stained sections of formalin fixed-paraffin embedded tumour. In addition, sections of 8-12 weeks gestational age placenta were obtained for use as positive control specimen for immune-histochemistry for β-HCG. The patients were
followed up at regular intervals and any recurrent or persistent tumour carefully graded and staged (categorised). In the case of pTa and pT1 tumours these patients had 3 monthly check cystoscopies initially for 2 years and in the absence of recurrence, check cystoscopies were carried out at 6 monthly intervals for 2 years following which the patients were followed up at yearly intervals in the case of no recurrence but when a recurrent tumour was found the follow up interval was then reduced to 3 monthly intervals. Intravenous urography was performed at 2 yearly intervals and any recurrent or persistent tumour carefully graded and staged. The patients who had cystectomy were followed up in the out patients department (these patients had careful clinical examinations and appropriate investigations as was indicated; for example bone scan, chest x-ray, liver function tests, intravenous urography, biopsy of any recurrent tumour as well as any other investigation and management that was necessary). Those patients who had transurethral resection of their tumours and subsequent radiotherapy were followed up by regular check cystoscopies and bimanual examination. In the case of the patients with superficial bladder tumours, who had frequent superficial recurrences, these patients were treated by intra-vesical chemotherapy following trans urethral resection of their tumours.

Routinely formalin fixed paraffin wax embedded blocks of urothelial cancer were cut at 5μ and attached to poly-l-lysine coated slides. The sections were allowed to dry overnight at room temperature. The following Avidin-Biotin peroxidase (ABC) immunocytological procedure was then carried out: The sections were deparaffinised, rehydrated, rinsed in tap water for 5 minutes and then rinsed in distilled water. Endogenous peroxidase activity was blocked by placing the sections in 1% hydrogen peroxide/methanol solution for 20 minutes. The sections were next rinsed in running tap water for 5 minutes. In order to allow for ‘batch’ runs to be performed and to provide consistent reproducible results Shandons sequenza immuno-staining centre (see illustration 1) and its cover plate assembly was used for the subsequent intermediate steps of the immunohistochemistry. In fact the slides were put in the Shandons sequenza immunostaining centre and rinsed in phosphate buffered saline (PBS) PH 7.2. Incubation was then carried out in normal rabbit serum (DAKO X902) diluted 1/20 with PBS for 10 minutes. The slides were next transferred to the primary antisera (for β-HCG, β-HCG ANTIBODY) diluted 1/100 with PBS [Sera Lab, monoclonal AB β-HCG, AE8 319.], for c-erb-B2, c-erb-B2 antibody diluted 1/40(NOVO CASTRIA NCL-CB11 ANTI-c-erb-B2 ONCOPROTEIN MOUSE MONOCLONAL ANTIBODY), for p53, p53 ANTIBODY NOVO CASTRIA NCL-DO7 was used) and incubated overnight at 4 degrees centigrade. The slides were next rinsed in PBS for 5 minutes and then incubated in secondary antisera (DAKO E354 Rabbit anti-mouse immunoglobulins/Biotinylated) diluted with PBS for 5 minutes and then incubated in AB complex (DAKO K 355 AB Complex/HRP) diluted 1/50 with PBS for 45 minutes and rinsed in PBS for 5 minutes. Immunoreactivity was visualised with Diaminobenzidine Tetrachloride dihydrate (DAB) solution for 1 to 5 minutes. The slides were rinsed in PBS for 5 minutes and then removed from the sequenza immunostaining centre and rinsed in running tap water for 10 minutes. The cell nuclei were counter stained lightly in Mayers Haematoxylin. The slides were washed, dehydrated, cleared and mounted in DPX.

Sections of formalin fixed paraffin wax embedded placenta were also cut at 5μ and stained simultaneously with the urothelial tissues using the same steps as above for use as control slides for β-HCG (known c-erb-B2 and p53 positive breast cancer specimens were used for control for c-erb-B2 and p53). For purposes of negative control, sections of tumour specimens were processed and stained as above apart from the omission of the primary antisera. Microscopy (immunohistochemistry for β-HCG, c-erb-B2 and p53)

Routine microscopy of the immunohistochemistry slides was performed in order to assess each slide for the expression of β-HCG or c-erb-B2 or p53 (staining for β-HCG, c-erb-B2 or p53). Positive staining in the case of β-HCG was demonstrated by brownish coloration in the cytoplasm of tumour cells similar to the staining characteristics of the placenta. Positive staining for c-erb-B2 was mainly peripheral (membranous) staining of the tumour cells. Positive staining for p53 was demonstrated as nuclear staining of the tumour.

**Assessment of staining for β-HCG**

Staining was assessed taking into consideration the intensity of positive staining through out the section. Staining intensity was scored on a 4 point scale: Negative stain (No staining) (0), weak (1), moderate (2), and strong (3). The extent of staining was based upon the proportion of tumour cells positively stained: 0-25% (+); 25-50% (++); 50-75% (+++); 75-100%; - (++__). In the final analysis of data all tumours showing weak, moderate and strong staining were recorded as positive and those showing no staining (O) were recorded as negative. Tumour cytoplasmic staining was recorded as positive (see illustration 2). Assessment of staining for c-erb-B2
Intensity of staining was scored on a 4 point scale: negative (no stain) (0), weak stain (1), moderate (2), and strong (3). Assessment of the extent of staining was based upon the proportion of tumour cells positively stained as in the case of β-HCG. Staining was mainly peripheral. In the final analysis of data tumours which were moderately or strongly stained were considered positive (see illustrations 3 and 4) and the ones that were graded negative and weakly positive were scored as negative.

**Assessment of staining for p53**

Intensity of staining and extent of staining in the case of p53 was also based upon a 4 point scale like the other two markers. In the final analysis tumours that were weakly, moderately and strongly positive stained were scored as positive (see illustration 5).

Correlation of the expression of β-HCG and EGFr

Samples of fresh frozen urothelial tumour specimens were used for this study.

Immunohistochemistry for Beta Human Chorionic Gonadotrophin (β-HCG) and Epidermal Growth Factor Receptor (EGFr)

Beta Human Chorionic Gonadotrophin (β-HCG)

Fresh frozen urothelial tumours which were cut at 6 μm were air dried and fixed in acetone for 10 minutes. The subsequent steps of the immunohistochemistry for β-HCG were similar to that described for formalin fixed-paraffin embedded specimens.

Epidermal Growth Factor Receptor (EGFr)

Sections of the same fresh frozen urothelial tumours which were stained for β-HCG were also cut at 5 μm, air dried and fixed in acetone for 10 minutes. The protein products of EGFr were identified using an indirect immunoperoxidase technique using monoclonal antibody EGFr1 (Amersham international plc) which recognises an epitope on the external domain of the EGFr and this was applied at a dilution of 1:40 for 30 minutes at room temperature. After incubation; with the primary antibody, the sections were washed in tris-phosphate buffered saline (TBS) and covered with peroxidase -conjugated rabbit anti mouse immunoglobulin (Dakopatts) diluted 1:20 for 30 minutes. The peroxidase reaction was developed using diaminobenzidine as chromogen and then section counter stained with haematoxylin. For positive control purposes sections of normal human skin and placenta were included with each staining run. For each run negative controls were prepared by staining duplicate sections of each tumour using the method described above but omitting the primary antibody.

Assessment of staining for β-HCG and EGFr

Assessment of staining for β-HCG using fresh urothelial tumour was similar to that described for formalin fixed urothelial tumour. Assessment of staining for EGFr using fresh urothelial tumour was similar to that described for c-erb-B2.

**Statistical analysis**

Statistical analysis of the results was done using SPSS for windows to calculate chi square tests of the various tumour groups and the outcome.

**Results**

The results of the histological immunohistochemical analysis are summarised in Illustrations 6 to 10 (see tables 1, 2, 3, 4 and 5). For the correlation of the expression of a) β-HCG and p53, b) β-HCG and c-erb-B2 and c) c-erb-B2 and p53 86 tumours were studied. Fifty of the tumours were pTa or pT1 (superficial tumours) and 31 tumours were muscle invasive (T2 to T4 tumours). In the case of the correlation of the expression of β-HCG and EGFr 44 tumours different from the earlier study were used. Of the 44 tumours 3 were G1, 19 were G2 and 22 were G3 tumours respectively. Twenty of this group of 44 tumours were pTa and pT1 tumours and 24 were muscle invasive tumours (T2 to T4 tumours).

Staining characteristics and outcome of the tumours.

Correlation of the expression of β-HCG and p53 (see illustration 6)

Of the 86 tumours studied 20 were positive for β-HCG as well as positive for p53 (5 pTa and pT1 tumours and 15 muscle invasive tumours). Thirteen of the tumours were β-HCG positive and p53 negative (8 pTa and pT1 tumours and 5 muscle invasive tumours). Twenty four tumours were β-HCG negative and p53 positive (15 pTa and pT1 tumours and 9 muscle invasive tumours). Twenty nine tumours were both β-HCG and p53 (27 pTa and pT1 tumours and 2 muscle invasive tumours). Regarding outcome, all the pTa and pT1 tumours that were positive for both β-HCG and p53 recurred but in comparison most of the tumours (17/27) that were negative for both β-HCG and p53 did not recur. The difference in the outcome of these two groups of tumours was significant. (p β-HCG and c-erb-B2 (see illustration 7)

All the 9 patients with superficial tumours positive for both β-HCG and c-erb-B2 had tumour recurrences at review cystoscopy. In comparison nearly two thirds (15/22) of patients whose tumours were negative for both β-HCG and c-erb-B2 had no evidence of recurrence at review cystoscopies. The difference in the outcome of the two groups was significant. (p C-erb-B2 and p53 (see illustration 8)

All the 14 patients with either pTa or pT1 (superficial) tumours whose tumours were positive for both c-erb-B2 and p53 had recurrences at review. On the
other hand nearly two thirds (14/20) of patients whose pTa and pT1 tumours were negative for both c-erb-B2 and p53 had no evidence of recurrent tumour at review cystoscopies (6/20 had recurrences). The difference in the outcome of these two groups was significant. (p

β-HCG and EGFr (see illustrations 9 and 10)

Only one out of the 20 superficial tumour group ( pTa and pT1 group) was positive for EGFr and the same tumour was also positive for β-HCG. This observation would make it impossible to make any interpretation of the out come of the superficial tumours. Even though 44 patients were enrolled in the study. Records were available for only 37 patients, 16 in the pTa and pT1 tumour group and 21 in the muscle invasive group of tumours. The numbers involved in this study are so small that a meaningful comparison of the various subgroups cannot be made. The only superficial tumour that was positive for both β-HCG and EGFr recurred. Four of the 7 tumours positive for β-HCG alone recurred and the recurrences were of higher stage. (3 did not recur). Of the 8 tumours negative for both β-HCG and EGFr, 3 did not recur but 5 had recurrences of the same grade and stage. Four patients out of 21 with muscle invasive tumours who were either treated by radiotherapy, radical resection of tumour alone, cystectomy or combination chemotherapy were alive without evidence of tumour by the end of the study. Two patients responded to treatment without any evidence of tumour but died of unrelated cause. Three patients had partial response to treatment but subsequently died of their tumours. Twelve patients with muscle invasive tumours were observed not to have had any response to various forms of treatment and died of their tumours. In the case of muscle invasive tumours anecdotal findings were observed in the analysis of the outcome of patients with regard to the various treatment subgroups and the staining characteristics as follows:

Cystectomy

Three patients with muscle invasive tumours had cystectomy. Of these, one had a β-HCG positive and EGFr negative tumour (G3T3N+), he died as a result of his tumour after 2 years (1 year and 8 months after the cystectomy). The second patient whose tumour was positive for both β-HCG and EGFr died one year later. The third patient whose tumour was negative for both β-HCG and EGFr died after 2 years and 3 months.

Inoperable cases treated by partial resection of tumour (TURBT).

Four patients had tumours which were considered to be inoperable and had TURBT only. All the 4 patients died as a result of their tumours. Two of these patients had tumours positive for both β-HCG and EGFr. Of the remaining 2 patients one had a tumour negative for both markers and the other had a tumour negative for β-HCG but positive for EGFr.

Radical Resection alone for muscle invasive tumour.

One patient with a small G2T2 tumour which was positive for both β-HCG and EGFr was treated by radical resection of the tumour only. This patient was alive and well after five and half years without any evidence of tumour.

Radiotherapy

Five patients with muscle invasive tumours were treated by radiotherapy alone. Three of these patients had tumours positive for both β-HCG and EGFr. None of these patients responded to radiotherapy, they died as a result of their tumours 11 months, 1 year and 2 years later. On the other hand one patient with a G3T3 tumour which was negative for both β-HCG and EGFr responded to radiotherapy but died as a result of carcinoma of prostate 17 months later. The last person with a G3T3 tumour which was negative for β-HCG but positive for EGFr was alive and well and five and half years later. (There was only one G2pTa recurrent tumour which was resected after 2 years and no further recurrence).

Combination systemic chemotherapy.

Eight patients with muscle invasive tumours were treated by combination chemotherapy. A 67 year old man with a G3T3 tumour which was strongly positive for β-HCG as well as positive for EGFr had resection of his tumour followed by radiotherapy and 4 cycles of CMV chemotherapy because he was not fit to undergo radical surgery. This patient had a partial response to treatment and died 2 years later as a result of his tumour. An 80-years-old patient with a G3T4B β-HCG positive and EGFr positive tumour had 6 cycles of CMV chemotherapy and was alive and well without any evidence of tumour five and half years later. A 74-years-old patient was thought to have a G3T3 tumour but was found at laparotomy to have an inoperable tumour. The patient was treated by radiotherapy and 6 cycles of CMV chemotherapy. This patient was alive without evidence of tumour five years later but died of cerebro vascular accident, heart failure and lung collapse. A 72-years-old patient who was thought to have a G3T3(4)a tumour (β-HCG positive and EGFr negative) underwent laparotomy and was found to have an inoperable tumour. This patient had radiotherapy and 6 cycles of CMV chemotherapy. The patient had a partial response in that the tumours initially disappeared but after 3 years liver metastases developed and the patient died. A 51 year old patient with a G2T3b tumour and hydroureter, whose tumour was positive for β-HCG but negative for
EGFr had 2 cycles of EPIC M treatment but did not respond and died 17 months later as a result of tumour. A 74-years-old patient with a G3T3b tumour (β-HCG negative and EGFr positive tumour) had a deep resection of tumour down to fat followed by 5 cycles of Cisplatinum and methotrexate chemotherapy. This patient was alive and well with no evidence of tumour five and half years later. A 58 year old patient with a mixed G3T3 tumour (transitional cell carcinoma and adenocarcinoma of bladder) which was negative for β-HCG but positive for EGFr was treated by 2 cycles of EPIC M, radiotherapy and cystectomy. The transitional cell carcinoma elements responded to treatment but the patient died 45 months later due to the persistence and spread of a combination of the adenomatous and squamous elements of the carcinoma. A 70-years-old patient whose G3T3 tumour was negative for both β-HCG and EGFr had 4 cycles of chemotherapy and cystectomy but died 6 months later as a result of the tumour.

Discussion

Immuno-histological detection of p53 assessed by polyclonal antibody CMI (≥10% nuclear staining) was found to be highly significant in predicting progression in 25 pT1 bladder tumours, although grade remained the most statistically significant predictor of progression [26]. However a similar study of the three antibodies (CMI, Pab 1801 and D07) did not find that p53 immunoreactivity was associated with outcome [27]. In another study in which 243 patients were treated by radical cystectomy, p53 immuno-reactivity was associated with outcome [28]. Venyo and Co-workers [25] observed that the expression of p53 in urothelial carcinomas is more commonly associated with tumours of high grade and high stage as well as recurrence of the same grade, higher grade and higher stage in pTa and pT1 tumours. They also observed that the expression of p53 alone could not be used to predict every tumour with the potential to progress [25].

Venyo and others [23] observed that the expression of β-HCG by urothelial carcinomas is more commonly associated with tumours of high grade and high stage. They observed tumour recurrences of the same grade, higher grade and higher stage in pTa and pT1 tumours positive for β-HCG. Other additional observations include the association of decreased survival with β-HCG positive muscle invasive tumours and the fact that expression of β-HCG alone cannot be used to predict every tumour with the potential to progress.

In carcinoma of the breast and ovary over expression of c-erb-B2 was reported to be associated with gene amplification and poor outcome [21; 29; 30]. Venyo and co-workers [24] observed that the expression of c-erb-B2 in urothelial carcinomas was more commonly associated with tumours of high grade, high stage and tumour recurrence and progression in pTa and pT1 tumours. They also observed inferior outcome in muscle invasive tumours. An additional observation was the fact that expression of c-erb-B2 was not always associated with progression in pTa and pT1 tumours and the fact that not every patient with a c-erb-B2 positive muscle invasive tumour died.

In the study of pTa and pT1 tumours it was observed that all the tumours that were positive for both markers (β-HCG & p53, β-HCG & c-erb-B2, or c-erb-B2 & p53) recurred. This finding may be important. The finding that nearly two thirds of all the pTa and pT1 tumours that were negative for both markers (17/27 of β-HCG & p53-, 15/22 of β-HCG- & c-erb-B2- and 14/20 p53- & c-erb-B2-) did not recur may be another important finding. Nevertheless, the observation that there was no significant difference between the outcome of tumours that were positive for both markers and tumours that were positive for one marker in most cases may suggest that there may be no advantage in the simultaneous use of two markers. Some of the tumours that progressed were positive for both markers. Other tumours that progressed were positive for one marker alone or negative for both markers. These findings may indicate that there may be no added advantage in the simultaneous use of two different markers to select tumours that have the potential to progress. However, the finding that superficial tumours that are positively stained for one marker alone could not be used to predict every tumour with the potential to progress [25].

Venyo and others [23] observed that the expression of β-HCG by urothelial carcinomas is more commonly associated with tumours of high grade and high stage. They observed tumour recurrences of the same grade, higher grade and higher stage in pTa and pT1 tumours positive for β-HCG. Other additional observations include the association of decreased survival with β-HCG positive muscle invasive tumours and the fact that expression of β-HCG alone cannot be used to predict every tumour with the potential to progress.
positive for β-HCG did not respond to cystectomy alone. 4) Some tumours positive for β-HCG responded to combination systemic chemotherapy. 5) Some tumours positive for EGFr responded to combination systemic chemotherapy. 6) Response to combination systemic chemotherapy was observed in tumours considered to be inoperable.

Conclusions and Recommendation:
All the superficial tumours that were positive for both markers in each group (β-HCG and p53, β-HCG and c-erb-B2, β-HCG and EGFr, or c-erb-B2 and p53) recurred; two thirds of the pTa and pT1 tumours that were negative for both markers did not recur. The difference between the recurrence rates of superficial tumours that were doubly positive in each group was significant when compared with tumours that were negatively stained for both markers in each group. However, the difference between the outcome of double marker and single marker positive tumours in each group was not significant. Regarding the muscle-invasive tumours, the best survival was observed in tumours that were negative for both markers and the worst outcome in tumours that were positive for both markers.

There is evidence (even though anecdotal) to suggest that β-HCG positive muscle-invasive tumours are chemo-sensitive tumours and such tumours should be treated by combination systemic chemotherapy even if they are found to be inoperable. There is the need to conduct a further larger study on the correlation of the expression of β-HCG, C-erb-B2, P53 and Epidermal growth factor receptor in human urothelial cancer in order to determine the biological behaviour of urothelial carcinomas in relation to the various markers and to ascertain the statistical significance of the biological behaviour of the tumours with regard to their tumour marker status.

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Professor D. E. Neal formerly of Department of Surgery University of Newcastle Upon Tyne and Freeman Hospital Department of Urology in the United Kingdom (Professor D E Neal is currently Professor of Surgical Oncology in Cambridge United Kingdom) and Dr Chris Wright Senior Lecturer Department of Pathology University of Newcastle Upon Tyne United Kingdom for allowing us to use their patients in this study. Mr Peter J English formerly consultant Urologist at Dryburn Hospital of Durham City (now called the University Hospital of North Durham Durham City) in the United Kingdom (Mr Peter J English is now Consultant Urologist in Sunderland Royal Infirmary in the United Kingdom) for allowing us to recruit some of his patients into the study.

Ethical Approval
The study was designed by Mr Anthony Kodzo-Grey Venyo who applied for Ethical approval for the project. Ethical approval for this research project was obtained from the North Durham Ethics Committee.

Conflict of interest
We do not have any conflict of interest to declare. The study was designed undertaken by Mr Anthony Kodzo-Grey Venyo. The project was supervised by Dr Douglas John Lindsay Maloney, Mr David William Herring and supervisory support was also provided by Professor David Neal of the Department of Surgery in the University of Newcastle upon Tyne (Professor Neal is now Professor of Surgical Oncology in Cambridge). Mr Anthony Kodzo-Grey Venyo had the initial guidance of Dr Chris Wright Senior Lecturer in Pathology who showed him slides of the staining characteristics of some of the tumours that were studied previously in Newcastle upon Tyne by Dr Chris Wright, Professor David Neal and their associates. Professor David Neal and Dr Chris Wright kindly gave frozen specimens of some of the tumours previously studied at Newcastle upon Tyne for the expression of some tumour markers including Epidermal growth factor receptor to Mr Anthony Kodzo-Grey Venyo to take to Durham City for storage and for the project in Durham City. These fresh frozen bladder cancer specimens were stored in Dryburn hospital in Durham City in United Kingdom and used only for the study of the correlation of the expression of β-HCG and EGFr. The study in Durham was carried out independently without the involvement of the Newcastle upon Tyne group. The clinical outcome of patients enrolled in the study in Durham were independently recorded and outcome independently analysed. The staining characteristics of tumours for EGFr were the same as in previous Newcastle upon Tyne studies except that the total numbers involved were different. Professor David Neal also supervised the project but purely on the academic side to ensure we were up to date with current literature.

Previous Presentation of this Paper at International Meetings
A paper entitled Correlation of the expressions of β-HCG; P53 and C-erb-B2 in human urothelial carcinomas (excluding EGFr) was presented by the corresponding author on behalf of our team at: The Biennial Congress of the South African urological Surgeons Association in Pretoria South Africa in 1994 The World Congress of the International College of Surgeons at Queen Elizabeth Ii Conference Centre in London in 1995

References


Illustrations

Illustration 1

Illustration 1: Shandons Sequenza Immuno-staining centre

Illustration 2

Illustration 2: Bladder Tumour Strongly Positively Stained for Beta Human Chorionic Gonadotrophin (?-HCG)
Illustration 3

Illustration 3 Bladder tumour Strongly positively stained for C-erb-B2

Illustration 4

Illustration 4: Bladder tumour moderately positively stained for C-erb-B2
Illustration 5

Illustration 5: Bladder tumour strongly positively stained for P53
**Illustration 6**

**Illustration 6, 7, 8, 9, 10**

**Table 1 (a and b)**

**Correlation of the expression of β-HCG and p53 as well as outcome in superficial (pTa and pT1) and muscle-invasive (T2 – T4) human urothelial carcinomas**

1a. pTa and pT1 tumours

<table>
<thead>
<tr>
<th></th>
<th>β-HCG+ P53+</th>
<th>β-HCG+ P53-</th>
<th>β-HCG- P53+</th>
<th>β-HCG- P53-</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>No recurrence</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>Recurrence of same grade and stage</td>
<td>1</td>
<td>5</td>
<td>6</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>Recurrence of higher grade and same stage</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Recurrence of higher stage</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>5</strong></td>
<td><strong>8</strong></td>
<td><strong>15</strong></td>
<td><strong>27</strong></td>
<td><strong>55</strong></td>
</tr>
</tbody>
</table>

1b. T2 to T4 tumours (Muscle invasive tumours)

<table>
<thead>
<tr>
<th></th>
<th>β-HCG+ P53+</th>
<th>β -HCG+ P53-</th>
<th>β -HCG- P53+</th>
<th>β -HCG- P53-</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alive</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>
Illustration 7: Tables 2 (a and b)

Correlation of the expression of β-HCG and c-erb-B2 as well as outcome in human urothelial carcinomas

2a: pTa and pT1 tumours

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No recurrence</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Recurrence of same grade &amp; stage</td>
<td>4</td>
<td>2</td>
<td>10</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>Recurrence of higher grade &amp; same stage</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Recurrence of higher stage</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>9</strong></td>
<td><strong>4</strong></td>
<td><strong>20</strong></td>
<td><strong>22</strong></td>
<td><strong>55</strong></td>
</tr>
</tbody>
</table>

2b: T2 to T4 tumours (Muscle invasive tumours)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Alive</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Died as a result of tumour</td>
<td>15</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>15</strong></td>
<td><strong>5</strong></td>
<td><strong>7</strong></td>
<td><strong>4</strong></td>
<td><strong>31</strong></td>
</tr>
</tbody>
</table>
Recurrence of same grade & stage  | 5  | 2  | 9  | 5  | 21  
Recurrence of higher grade & same stage | 5  | 2  | 1  | 0  | 8  
Recurrence of higher stage            | 4  | 0  | 1  | 1  | 6  
Totals                                | 14 | 6  | 15 | 20 | 55  

3b T2 to T4 tumours (Muscle invasive tumours) 

<table>
<thead>
<tr>
<th></th>
<th>p53+</th>
<th>p53+</th>
<th>p53-</th>
<th>p53-</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>c-erb-B2+</td>
<td>c-erb-B2-</td>
<td>Cerb-B2+</td>
<td>Cerb-B2-</td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Died (as a result of tumour)</td>
<td>16</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>Totals</td>
<td>18</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>31</td>
</tr>
</tbody>
</table>

Illustration 9: Table 4 (a and b)

Correlation of the expression of β-HCG and EGFr in carcinoma of the urinary bladder: Grade and Stage

4a Grade

<table>
<thead>
<tr>
<th>Grade</th>
<th>β-HCG+ p53+</th>
<th>β-HCG+ EGFr-</th>
<th>β-HCG- EGFr+</th>
<th>β-HCG- EGFr-</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>G2</td>
<td>4</td>
<td>8</td>
<td>1</td>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>G3</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>22</td>
</tr>
</tbody>
</table>
Illustration 10: Table 5 (a and b)

Correlation of the expression of \( \beta \)-HCG and EGFr in human urothelial carcinoma

<table>
<thead>
<tr>
<th>Outcome</th>
<th>( \beta )-HCG+</th>
<th>( \beta )-HCG-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EGFr+</td>
<td>EGFr-</td>
</tr>
<tr>
<td>No Recurrence</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Recurrence of same grade &amp; stage</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Recurrence of higher grade &amp; same stage</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Recurrence of higher stage</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Totals</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

5b Outcome of T2 to T4 tumours (outcome following all types of treatment)

(Note records available for 37 patients and not 44)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>( \beta )-HCG+</th>
<th>( \beta )-HCG-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EGFr+</td>
<td>EGFr-</td>
</tr>
<tr>
<td>Alive and no tumour</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Was alive no tumour died of unrelated cause</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>
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