Prognostic Impact Of P53, EGFR And HER2/Neu Proteins On Renal Cell Carcinoma

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Citation

A Michael, K Relph, E Havranek, C Anderson, A Polychronis, C Corbishley, H Pandha. *Prognostic Impact Of P53, EGFR And HER2/Neu Proteins On Renal Cell Carcinoma*. The Internet Journal of Urology. 2005 Volume 4 Number 1.

Abstract

The expression of p53, HER2/neu and EGFR in renal cell carcinoma was studied using immunohistochemical methods in paraffin-embedded nephrectomy specimens from 40 patients. Expression of p53, EGFR and HER2/neu was observed in 22 (55%), 33 (82.5%) and 10 (25%) patients respectively. In the Cox regression analysis overall expression of p53 was not statistically significant with a p value of 0.5 for total expression, 0.6 for weak expression and 0.2 for strong p53 expression. The HER2 positivity was also not statistically significant with a p value of 0.2 and a hazard ratio of 0.4. Similar results were obtained for EGFR overexpression with a p value of 0.591 for overall positivity, 0.3 for weak staining and 0.56 for strong staining. Our results indicate that p53, EGFR and HER2/neu proteins may play a role in determining the prognosis in RCC. However the results were insignificant and a greater patient cohort is required before any true conclusions can be made.

INTRODUCTION

Renal cell carcinoma (RCC) is the most common malignancy of the adult kidney. The clinical outcome of RCC is generally determined by clinical stage and histologic grade and can vary considerably (Skinner at., 1971). However clinical staging is not entirely accurate in predicting the prognosis. Therefore several studies have focused on evaluating additional indicators of biologic aggressiveness of RCC. A variety of proteins and carbohydrates have been investigated for their use as prognostic tumour markers.

The tumour suppressor gene p53 encodes a nuclear phosphoprotein involved in detecting DNA damage and allowing DNA repair or apoptosis: As a result of DNA damage cellular progression from G1 to S phase is halted to allow time for repair to occur. In this way mutations are corrected and prevented from accumulating in the cell. Mutations in the p53 gene are one of the most common genetic abnormalities in malignancies. It is generally accepted that p53 hyperexpression is the consequence of an increase in the half-life of the non-functional protein which is usually related to mutation of the gene and is easily detectable by immunohistochemistry.

The HER-2/neu oncogene (also named c-erbB2) encodes a 185kDa transmembrane glycoprotein with tyrosine kinase activity and extended homology in structure and sequences to the EGFR (Coussens et al., 1985). It can heterodimerise with the EGFR to generate a more efficient mitogenic signal than either the EGFR itself or the HER2/neu homodimer (Dougall et al., 1994). HER2/neu amplification and overexpression has been associated with a number of malignancies including breast and ovarian tumours (Slamon et al., 1989), transitional cell carcinoma (Chow et al., 1997), colon carcinoma (Brossart 1998), prostatic adenocarcinoma (Kallakury et al., 1998), cervical carcinoma (Ndubisi et al., 1997), gastric carcinoma and non-small cell lung carcinoma (Yoshino et al., 1994).

The Epidermal growth factor receptor (EGFR) is a 170 kilodalton transmembrane cell-surface receptor which has tyrosine kinase activity. It is overexpressed in a third of all epithelial cancers and is frequently associated with a poor prognosis (Lieberman et al., 1985, Ozanne et al., 1986, Neal et al., 1990). Blockage of EGFR using monoclonal antibodies has been shown to promote inhibition of the malignant phenotype (Rubin Grandis et al., 1997)

The purpose of this retrospective study was to investigate expression of p53, HER2/neu and EGFR in 40 patients with RCC and correlate their expression with prognosis

MATERIALS AND METHODS

PATIENTS AND TUMOUR CHARACTERISTICS

This study used data from 40 patients (25 men, 15 women) who underwent surgery for RCC between 1990 and 2000 (table 1). The mean age of the patients was 65.2 years (range 25 to 85). The follow up period was at least 4 years. The main clinical and pathological characteristics included in our study are shown in table 1. Tissue samples from 40 patients were analyzed. Three of the patients had papillary RCC and the remaining samples were of the clear cell type. Of the 40 patients 14 had G1, 10 had G2, 6 had G3 and 10 G4 RCC. Three had stage T1a, 8 stage T1b, 9 stage T2, 14 stage T3a, 4 stage T3b and 1 stage T4.The histologic staging and grading were done according to the UICC TNM classification. In every case an experienced pathologist carefully examined the tumour.

IMMUNOHISTOCHEMICAL ANALYSIS

Formalin fixed and paraffin-embedded tissue blocks from patients with RCC who underwent radical nephrectomy were studied. The tissue blocks were cut into 5um thick sections and placed on a Poly-L-Lysine treated glass slide. Representative sections were stained with hematoxylin and eosin and evaluated.

For detection of p53, EGFR and HER2/neu proteins primary antibodies were used to stain the sections: mouse monoclonal antiserum to p53 (DAKO) at a dilution of 1:100, mouse monoclonal antibody to EGFR (DAKO) dilution 1:200 and mouse monoclonal antiserum to HER2 (DAKO) at a dilution of 1:100. The sections were then deparaffinized and dehydrated. After washing with Tris-buffered saline the sections were incubated with each primary antibody for 1 hour at room temperature. The sections were washed and incubated with avidin-biotin peroxidase complex (DAKO) for 30 minutes. Peroxidase reaction was detected by addition of diamonobenzidine tetrahydrochloride. All slides were slightly counterstained with Haematoxylin. The positive control for EGFR staining was a section of human placenta known to be EGFR positive. The positive control for p53 and HER2/neu was a section of breast tumour tissue expressing both of these proteins. In evaluating the immunohistochemical results, only nuclear positivity was scored for p53 whereas membraneous staining was scored for HER-2 and EGFR. Positive reaction for either p53 of HER-2 protein was considered when more than 10% of cancer cells exhibited strong diffuse immunostaining. The staining results were examined in a blinded manner with no prior knowledge of the clinical data.

EVALUATION OF IMMUNOSTAINING

A tumour tissue section was examined and scored by two researchers with no prior knowledge of the pathologic data. The fraction of positive cells was determined under the microscope at a magnification of 20-40x. The nuclear intensity of p53 was evaluated by Quickscore (Detre et al., 1995). The quickscore method involved grading the entire tissue on two criteria, A (intensity) and B (quantity) then adding the result to get the score. The total sum was then classified as negative, low, and strong positive staining. The membraneous staining of HER2/neu was assessed as positive when the score was higher than 2 (0,1-negative, 2,3 – positive) and EGFR were classified as 0 for negative, 1 for intermediate and 2 for strong staining

STATISTICAL ANALYSIS

All statistical analysis was performed by a statistician. The Cox regression test was used to estimate the prognostic relevance of p53, HER2/neu and EGFR overexpression. All p values less than 0.05 reflected statistically significant differences.

RESULTS

CLINICAL CHARACTERISTICS

40 patients with RCC were analysed. Three of the specimens belonged to patients with papillary RCC, the remaining ones were of clear cell type. The mean age of the patients was 65.2 with a standard deviation of 11.5, range between 25 and 85. All samples were analysed according to the clinical tumour stage, nuclear grade, pathological grade, macrovascular invasion, macrocapsular invasion, microvascular invasion, microcapsular invasion.

At the time of the histological analysis 16 (40%) of the patients were deceased and 24 (60%) were alive and well. The summary of the Kaplan-Meier analysis which compares the survival distribution of the different levels of the variable using logrank statistics is shown in table1.

Table 1.

Figure 1

Variable	*Label*	*n*	*Mean*	*95% CI*	*Logrank statistic (p-value)*
Sex	Female	15	110	78 - 141	0.54 (0.462)
	Male	25	101	72 - 130	
Nuclear grade	high	13	84	41 - 126	2.14 (0.144)
	low	27	110	87 - 133	
pT	1	11	130	100 - 160	3.43 (0.180)
	2	9	97	56 - 138	
	3+	20	88	56 - 120	
Macro vascular invasion	No	34	115	91- 139	2.41 (0.121)
	Yes	6	46	15 - 78	
Macro capsular invasion	No	23	125	103 - 147	7.73 (0.005)
	Yes	17	71	37 - 106	
Micro vascular invasion	No	36	119	97 - 141	19.5 (0.000)
	Yes	4	5	1 - 10	
Micro capsular invasion	No	24	130	104 - 156	5.51 (0.019)
	Yes	10	70	38 - 102	
P53 expression	0	22	114	85 - 144	0.77 (0.682)
	1	12	97	62 - 132	
	2	6	78	18 - 138	
HER 2neu expression	neg	30	93	69 - 116	1.54 (0.215)
	pos	10	136	98 - 174	
EGFR expression	0	7	77	38 - 115	0.67 (0.717)
	1	18	105	74 - 136	
	2	15	109	72 - 146	

Of the histological variables macrocapsular invasion, microvascular invasion and microcapsular invasion have reached statistical significance with a p value of 0.005, 0.000 and 0.019 respectively. The 95% confidence intervals (CI) are shown in the table 1. The pathological stage (pT) as well as macrovascular invasion did not reach the statistical

significance most likely due to the small sample size.

P53, EGFR AND HER2/NEU IMMUNOSTAINING

The immunohistochemical expression of p53, EGFR and HER2/neu was observed in 22 (55%), 33 (82.5%) and 10 (25%) patients respectively.

In the Cox regression analysis the overall expression of p53 was not statistically significant with a p value of 0.5 for total expression, 0.6 for weak expression and 0.2 for strong p53 expression (see table 1). The HER2 positivity was also not statistically significant with a p value of 0.2 and a hazard ratio of 0.4. Similar results were obtained for EGFR overexpression with a p value of 0.591 for overall positivity, 0.3 for weak staining and 0.56 for strong staining. The 95% CI for each of the variables are shown in table 1. The Kaplan-Meier survival curves for all the above markers are shown in figure 2.

Figure 2

Figure 2a: Survival curves in relation to p53 (a), HER2/neu (b) and EGFR (c) overexpression. Axis X represents survival in % and axis Y-time in days, the legend numbers relate to the intensitiy of the staining with 0-no staining, 1-intermediate staining, 2-strong positive staining

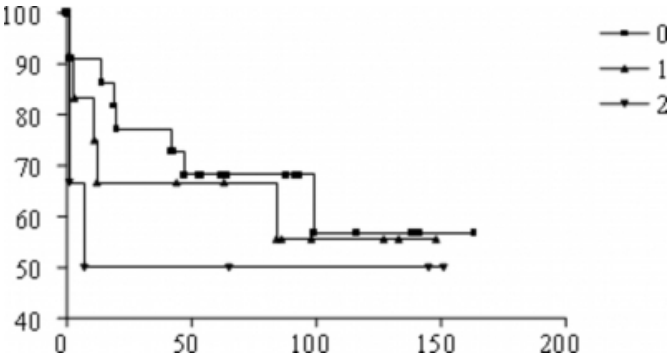


Figure 3

Figure 2b

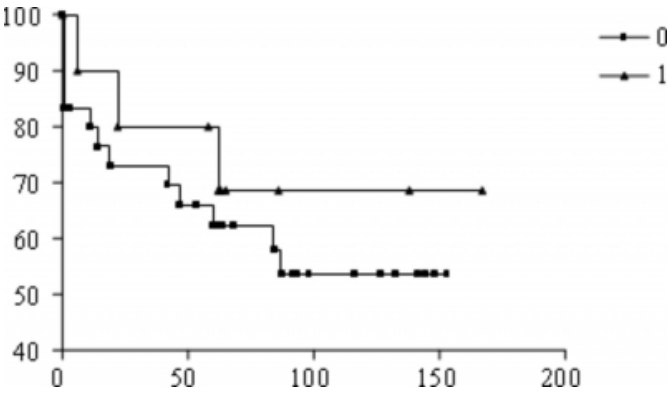
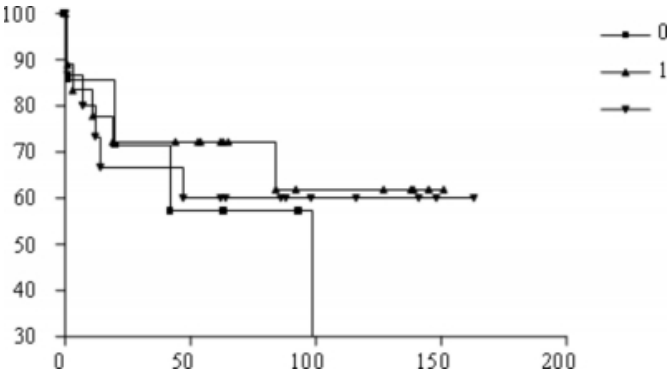


Figure 4

Figure 2c



DISCUSSION

In this study we investigated immunohistochemically the prognostic relevance of over expression of the p53, EGFR and HER2/neu proteins in a homogenous series of RCC tumour samples. Although neither of the p53, EGFR or

HER2/neu markers were shown to be statistically significant, over expression of p53 is likely to be associated with poor prognosis and shortened survival as shown on the survival graphs. The statistical analysis has been affected by the small sample size and therefore in the Cox regression analysis the p53 overexpression did not reach statistical significance. These results are consistent with those of several groups. Rioux-Leclercq et al. (2000) demonstrated that p53 immunostaining correlated with a poor prognosis whilst Ljungberg et al. (2001) found that patients with high p53 immunoreactivity had shorter cancer specific survival (p=0.003) than those with normal p53 protein expression. Likewise, Uchida et al. (2002) used multivariate analysis to demonstrate that p53 expression demonstrated a significant effect on prognosis. However in contrast some groups have found no correlation between p53 expression levels and prognosis. Recently several groups have all reported infrequent p53 expression in RCC samples (Vasavada, Novick et al. 1998), (Tomita, Bilim et al. 1996), (Kanamaru, Li et al. 1999) (Hsueh, Wang et al. 2002). Hofmockel et al. (1996) did report 32% rate of p53 expression detected histochemically but no difference was noted in the survival or clinical stage of the patients.

HER2/neu overexpression has been identified in a number of cancers. Moreover HER2/neu overexpression has been associated with poor prognosis in both intraductal cancinomas of the breast (20-40% cases) and ovarian cancer (30% cases) (Hofmockel, Wittmann et al. 1996), (Berchuck, Kamel et al. 1990). Despite the fact that HER2/neu overexpression has been linked to a poor prognosis in variety of other carcinomas very few studies have analysed the expression of this gene in RCC in relation to the course of the diseases and patient survival. We have found that HER2/neu overexpression is associated with an increased survival time when compared to the cases that stained negatively for HER2/neu although the difference was not statistically significant. To date most studies analysing HER2/neu overexpression have found the opposite – that HER2/neu overexpression is associated with a poor prognosis. One study carried out by (Zhang, Takenaka et al. 1997) studied 70 patients with RCC and demonstrated HER2/neu over expression in 40% of the cases. They concluded that there is significant association between HER2/neu overexpression and tumour stage in RCC. Seliger et al. (2000) found that immunohistochemical analysis of kidney tumours revealed a distinctive pattern of HER2/neu expression in RCC with the highest frequency in chromophilic and chromophobic RCC but neither associated with disease stage or grade. Whilst (Rotter, Block et al. 1992) examined 24 RCC and detected lower expression of HER2/neu in clear-cell and compact tumour subtypes whilst chromophilic, chromphobic and tubulopapillary subtypes did not show significant difference when compared to non-neoplastic tissue. No association was found with tumour stage. A study conducted by (Latif, Watters et al. 2002) examined 27 tumours from RCC patients and found that HER2 over-expression was uncommon.

The epidermal growth factor receptor (EGFR) is expressed in a wide variety of solid tumours. It has been demonstrated that the EGFR-associated signaling pathway plays an important role in carcinogenesis and cancer progression. Indeed, overexpression of EGFR has been associated with shorter survival times in several types of carcinoma (Libermann, Nusbaum et al. 1985), (Ozanne, Richards et al. 1986), (Neal, Sharples et al. 1990), (Veale, Kerr et al. 1993) including RCC (Uhlman, Nguyen et al. 1995), (Yoshida, Hosoya et al. 1997). In this study we analysed the association between EGFR expression and the prognosis of RCC. Our results did not demonstrate that positive expression of EGFR is associated with a better or worse prognosis however lack of conclusive results are likely to be explained by the small sample size.

Of all the histopathological prognostic factors tumour stage, histological type and nuclear (Fuhrman grade) have been identified as independent prognostic factors (Medeiros, Gelb et al. 1988), (Thrasher and Paulson 1993),. Microvascular invasion has also been proposed as an important prognostic parameter mainly for low clinical stage renal cell carcinoma (Goncalves, Srougi et al. 2004). Our study has confirmed the importance of microvascular invasion however nuclear grade has not reached the statistical significance most likely due to the small sample size. The importance of other factors such as microcapsular and macrocapsular invasion is still controversial.

In conclusion although the p53 overexpression did not reach the statistical significance it was associated with a trend to shorter survival time however neither HER2/neu nor the EGFR overexpression appered to have an impact on survival. Amongst the histopathological data the tumour stage, microvascular invasion and histological type remain the clinically important prognostic parameters.

ACKNOWLEDGMENTS

We would like to thank Clare McDonald for her help with tissue preparation.

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