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Short Communication

Amplification of epidermal growth factor receptor gene in renal cell carcinoma

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ABSTRACT

Expression of epidermal growth factor receptor (EGFR) may be of prognostic value in renal cell cancer (RCC). Gene amplification of EGFR was investigated in a cohort of 315 patients with advanced RCC from a previously reported randomised study. Using fluorescent *in situ* hybridisation, only 2 patients (0.6%) had gene amplification; therefore gene amplification is of no prognostic value in RCC.

1. Introduction

The prognosis of renal cell carcinoma (RCC) remains poor, because one-third of patients already have metastatic disease at initial presentation and 30–40% who undergo surgery for primary tumour develop distant metastases.[1] The increasing understanding of the pathogenesis of RCC resulted in identification of relevant therapeutic targets.[2] In particular, Von

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Table 1 – Baseline patient characteristics of the 315 patients included in the analysis.

Characteristic	Lapatinib (n = 161)	HT (n = 154)	Total
Age (years)			
Median	60	62	61
Range	31–83	27–76	27–83
Sex, *n* (%)			
Male	110 (68)	114 (74)	224 (71)
Female	51 (32)	40 (26)	91 (29)
Stage, *n* (%)			
3	6 (4)	3 (2)	9 (3)
4	154 (96)	149 (97)	303 (96)
Histology, *n* (%)			
Clear cell	138 (86)	139 (90)	277 (88)
Papillary	10 (6)	6 (4)	16 (5)
Chromophobe	3 (2)	3 (2)	6 (2)
Collecting duct	0	0	0
Unclassified	10 (6)	6 (4)	16 (5)
No. of metastatic sites, *n* (%)			
⩽2	73 (45)	78 (51)	151 (48)
>2	88 (55)	76 (49)	164 (52)
Prior nephrectomy	151 (94)	145 (94)	296 (94)
Common sites of metastasis, *n* (%)			
Lung	128 (80)	124 (81)	252 (80)
Bone	56 (35)	46 (30)	102 (32)
Liver	38 (24)	36 (23)	74 (23)
Lymph nodes	88 (55)	65 (42)	153 (49)
IHC, *n* (%)			
0	3 (2)	1 (<1)	4 (1)
1+	16 (10)	17 (11)	33 (10)
2+	55 (34)	43 (28)	98 (31)
3+	87 (54)	93 (60)	180 (57)
Overall survival (weeks)			
Median	46	38.3	42.6
CI[a]	34.6–56.7	32.7–50.1	35.9–49.3
Time to progression (weeks)			
Median	12.7	15.4	15.1
CI[a]	9.1–16.9	10.7–18.0	10.6–16.7

HT, hormone therapy; IHC, immunohistochemistry; CI, confidence interval.
a Using Greenwood's formula for the standard error and the log–log transformation in calculating the confidence interval (SAS 9.2).

Hippel–Lindau (*VHL*) gene silencing occurs in the majority of non-inherited clear-cell RCC, activating the hypoxia-response pathway and inducing transcription of several genes, including vascular endothelial growth factor (VEGF).[3] Novel targeted agents such as the VEGF receptor inhibitors, sorafenib and sunitinib, and the VEGF-binding agent, bevacizumab, improve progression-free survival in patients with good prognosis.[4–6]

Other pathways that have been implicated in the pathobiology of RCC include epidermal growth factor receptor (EGFR).[7,8] However, because of insufficient clinical efficacy, controversy exists concerning the rationale for using EGFR inhibitors in patients with advanced RCC.[9] In a phase 3 study of lapatinib, a dual EGFR/human epidermal growth factor receptor 2 (HER-2) inhibitor, versus hormonal therapy (HT) in the second-line setting in patients with RCC, a significant improvement in overall survival (OS) was reported in a subset of a patient population with tumours that had increased EGFR expression (3+ by immunohistochemistry [IHC]; n = 241), with median OS of 46.0 weeks for lapatinib versus 37.9 weeks for HT (hazard ratio = 0.69; P = 0.02).[10]

Gene amplification of EGFR has been shown to have both prognostic and predictive value in non-small cell lung cancer (NSCLC).[11,12] Therefore, this study was undertaken to explore the relationship between expression of the EGFR protein assessed by IHC, gene copy number assessed by fluorescent *in situ* hybridisation (FISH), and their association to the prognosis of patients with RCC.

2. Materials and methods

This prospective, open-label, randomised, phase 3 trial of lapatinib versus HT was conducted in patients with advanced RCC whose disease over-expressed EGFR or HER-2 and had progressed on first-line cytokine therapy.[10] The primary end-point of the study was time to tumour progression (TTP); secondary end-points included tumour response rate,

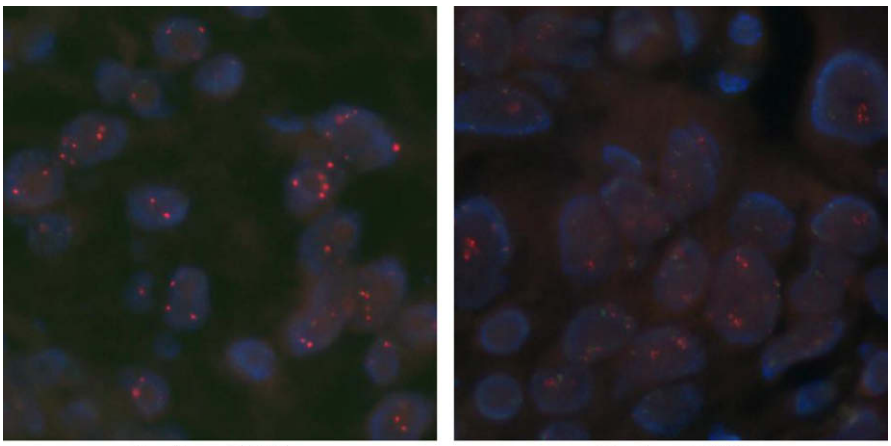

Fig. 1 – IHC and FISH of the 2 patients with EGFR amplification. Patient 1672: EGFR/CEP7 ratio 2.10, EGFR IHC 3+. Patient 2878: EGFR/CEP7 ratio 2.83, EGFR IHC 3+.

overall survival (OS), and correlative analysis of EGFR/HER-2 protein expression with TTP and OS, and gene amplification, which is reported here. Between December 2002 and February 2005, a total of 416 patients from 116 centres in 11 countries were randomised to receive lapatinib ($n = 209$) or HT ($n = 207$). All patients gave written informed consent, and the study was approved by competent authorities and independent local ethics committees. No formal statistical analysis was planned.

Archival tumour samples were collected and FISH analysis was performed centrally by Targeted Molecular Diagnostics (TMD) according to their standard operating procedure. Briefly, the Vysis LSI EGFR Dual Colour Probe Hyb Set was used containing two probes: one being labelled in SpectrumOrange covering an approximately 300-kb region that contains the entire *EGFR* gene (7p12) and the second *CEP 7* control probe, labelled in SpectrumGreen, whcih hybridises to the alpha satellite DNA located at the centromere of chromosome 7 (7p11.1–q11.1). Hybridised slides were counterstained with DAPI to visualise cell nuclei and examined under a fluorescence microscope. A minimum of 25 cells with bright and distinct signals in both colours were counted. Gene amplification was assigned if gene:chromosome ratio was >2.

IHC for EGFR (PharmDx; Dako, Glostrup, Denmark) expression was performed centrally in a blinded, independent manner by Quest Diagnostics (Madison, NJ). Moderate to strong complete membrane staining in more than 10% of the tumour cells was classified as 3+ strongly positive.

3. Results and discussion

To our knowledge, this is the first study to investigate *EGFR* gene amplification in a cohort of advanced RCC. A total of 315 samples, collected from a prospective, randomised study were analysed for EGFR amplification by FISH. The randomised study included 416 patients. Therefore, a total of 101 samples were not included in our analysis because either they failed to hybridise ($n = 59$), had FISH-incompatible fixative ($n = 26$), or an inadequate amount of tumour tissue was available ($n = 16$).

Table 1 shows the baseline characteristics of the patient population. Of the 315 patients, only 2 (0.6%) had increased *EGFR* gene amplification (FISH images from these 2 patients are provided in Fig. 1). Both of these patients had stage 4 clear-cell carcinoma with metastasis to the lung and showed EGFR 3+ IHC staining. Both had HT and survived 32 and 72 weeks, respectively. Because there were only two cases with increased gene copy number, a correlation with clinico-pathological factors was not possible. Therefore, gene copy number is not a useful prognostic or predictive marker in advanced RCC. A total of 180 of the 315 patients (57%) had a disease with EGFR over-expression of 3+ by IHC. The lack of positive correlation between EGFR protein expression and gene copy number suggests that mechanisms unrelated to gene amplification are involved in activating EGFR, for example, post-translational modification, mutation, possible transactivation by other genes, or chromosome 7 polysomy, which has previously been reported in RCC.[13] Additionally, there is mounting evidence to suggest that *VHL* mutations, which are common in RCC, may regulate EGFR expression and signalling pathways.[7]

These results are in sharp contrast to the positive correlation between protein expression and gene amplification of *EGFR* in NSCLC.[12] Moreover, *EGFR* amplification is of both prognostic and predictive value, which is clearly not the case for protein expression.[14,15]

Recent data have shown that the presence of mutations within the *EGFR* gene can distinguish responders from non-responders to EGFR tyrosine kinase inhibitors in NSCLC, with an incidence of 10% in adenocarcinoma histology.[16,17] Gene mutation is thought to be an earlier event than gene amplification in tumour progression.[18] Examination of *EGFR* gene mutations was not considered in this study, given the very low gene amplification rate in our work and the absence of *EGFR* mutations reported by others in RCC.[19]

In conclusion, EGFR expression may be important as a predictive marker of response to EGFR-targeted therapy in RCC.

Unfortunately, this is not the case for gene amplification, which occurs infrequently, making it prognostically irrelevant. This should be taken into consideration for future studies.

Conflict of interest statement

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