

SHORT REPORT

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Gene expression pattern of the epidermal growth factor receptor family and LRIG1 in renal cell carcinoma

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Abstract

Background: Previous studies have revealed altered expression of epidermal growth factor receptor (EGFR)-family members and their endogenous inhibitor leucine-rich and immunoglobulin-like domains 1 (LRIG1) in renal cell carcinoma (RCC). In this study, we analyzed the gene expression levels of EGFR-family members and LRIG1, and their possible associations with clinical parameters in various types of RCC.

Methods: Gene expression levels of EGFR-family members and LRIG1 were analyzed in 104 RCC samples, including 81 clear cell RCC (ccRCC), 15 papillary RCC (pRCC), and 7 chromophobe RCC (chRCC) by quantitative real-time RT-PCR. Associations between gene expression levels and clinical data, including tumor grade, stage, and patient survival were statistically assessed.

Results: Compared to kidney cortex, *EGFR* was up-regulated in ccRCC and pRCC, *LRIG1* and *ERBB2* were down-regulated in ccRCC, and *ERBB4* was strongly down-regulated in all RCC types. *ERBB3* expression did not differ between RCC types or between RCC and the kidney cortex. The expression of the analyzed genes did not correlate with patient outcome.

Conclusions: This study revealed that the previously described up-regulation of *EGFR* and down-regulation of *ERBB4* occurred in all analyzed RCC types, whereas down-regulation of *ERBB2* and *LRIG1* was only present in ccRCC. These observations illustrate the need to evaluate the different RCC types individually when analyzing molecules of interest and potential biological markers.

Keywords: Renal cell carcinoma, EGFR, ERBB2, ERBB3, ERBB4, LRIG1, Survival

Findings

Background

Renal cell carcinoma (RCC) consists of various tumor types [1]; clear cell RCC (ccRCC) accounts for approximately 70-80 % of the RCCs, papillary RCC (pRCC) for 10-15 % of cases, chromophobe RCC (chRCC) for approximately 5 %, and collecting duct carcinoma for less than 1 % of RCCs. Approximately 4-5 % of RCCs do not fit the histopathological criteria and are referred to as unclassified carcinomas [1]. The RCC types represent tumor groups with different genetic and molecular properties, as reviewed in [2] and [3]. When RCC types are analyzed collectively, the results predominantly reflect the properties of ccRCC, since this type accounts

for the majority of RCC cases. Previous studies have revealed altered expression of epidermal growth factor (EGF) receptor (EGFR)-family members and their endogenous inhibitor leucine-rich and immunoglobulin-like domains 1 (LRIG1) in renal cell carcinoma (RCC). The EGFR family consists of four receptor tyrosine kinases, EGFR (ERBB1, HER1), ERBB2 (HER2, neu), ERBB3 (HER3), and ERBB4 (HER4) [4], of which downstream intracellular signaling pathways regulate cell proliferation, differentiation, and migration [5]. LRIG1 [6] negatively regulates all four members of the EGFR-family [7-10]. LRIG1 is also a negative regulator of the MET- and RET-receptor tyrosine kinases [11,12]. LRIG1 is down-regulated in several cancers and cancer cell lines, including breast cancer and squamous cell carcinoma of the skin and uterine cervix, where low LRIG1 expression correlates with poor patient survival [13-17].

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Table 1 Characteristics of the patients and tumors included in the study

Total no. of patients		104
Sex	male/female	56/48
Age in years	median (range)	65 (25–85)
Tumor diameter in mm	median (range)	80 (30–250)
Survival in months	Range	0–130
Tumor stage (WHO) (As derived from TNM)	I	26
	II	15
	III	30
	IV	33
Tumor grade (Fuhrman)	1	4
	2	14
	3	60
	4	26
RCC type by histology	Clear cell	81
	Papillary	15
	Chromophobe	7
	Unclassified	1
Patients with known metastasis at diagnosis		35
Patients who died from the disease		57
Patients dead from other causes		16
Patients alive at last follow-up (with disease)		31 (3)

Previously, we reported the expression of the EGFR-family members and LRIG1 in a limited number of RCC-patients [18,19]. Here, we extended these studies to a larger patient cohort, and analyzed the RCC types individually.

Methods

In this study we analyzed tumor samples from 104 patients who underwent nephrectomy at the Department of Urology, Umeå University Hospital, between the years 1986 and 1999 (Table 1). These tumors included 81 ccRCC, 15 pRCC, 7 chRCC, and 1 unclassified carcinoma. Additionally, specimens of histologically verified non-neoplastic kidney cortex were obtained from 27 of the nephrectomized kidneys. RNA was prepared and quantitative real-time reverse transcription- (RT-) PCR of *EGFR*, *ERBB2*, *ERBB3*, *ERBB4*,

LRIG1, and RN18S1 (18 S rRNA) was performed as previously described [18,19]. To correct for differences in RNA quality and quantity, apparent levels of RN18S1 were used to normalize the *EGFR*, *ERBB2-4*, and *LRIG1* values in each respective RNA sample. To test the reliability of the analysis, all five protein encoding genes were analyzed five times for three different samples. The maximum coefficient of variation and the standard deviation, expressed as a percentage of the mean, was 22 %. Patients provided informed consent for the use of both their tumor material and clinical data for studies. This study was approved by the research ethics committee at Umeå University Medical Faculty (No 02–340).

Statistical analysis was performed using nonparametric statistics, as normal distribution of the data could not be assumed. For comparisons between two groups, the Mann–Whitney U-test was used. Comparisons of more than two groups were performed using the Kruskal-Wallis test. Comparisons between coupled samples were performed using the Wilcoxon signed-rank test. Correlations were analyzed according to Spearman's rank correlation. Survival analysis was performed by first comparing patients with mRNA expression levels either above or below the median and then subjecting the data to Kaplan-Meier analysis by log-rank test. All P-values were two-sided. All calculations were performed using SPSS 14.0 software.

Results

The expression of *EGFR* was higher in all RCC types combined compared to kidney cortex tissue ($P < 0.001$) (Table 2). This up-regulation was significant for both ccRCC ($P < 0.001$) and pRCC ($P = 0.016$), but not for chRCC ($P = 0.257$) (Figure 1A; Table 2). This is in line with earlier findings by us and others [18,20–24].

ERBB2 expression was significantly reduced in all RCC types combined compared to kidney cortex ($P = 0.003$); however, reduced expression was only significant in ccRCC ($P = 0.001$) and not in pRCC or chRCC (Figure 1B). These results indicate that the previously described down-regulation of *ERBB2* in RCC [19] is actually a result of down-regulation in the ccRCCs.

Table 2 Significant differences in mRNA expression between kidney cortex and the RCC types

	All RCC combined*	ccRCC*	pRCC*	chRCC*	Altered expression	Difference RCC types†
EGFR	< 0.001	0.001	0.016	NS	Up-regulation	NS
ErbB2	0.003	0.001	NS	NS	Down-regulation	< 0.001
ErbB3	NS	NS	NS	NS	-	NS
ErbB4	0.001	< 0.001	0.001	0.03	Down-regulation	NS
LRIG1	NS	0.015	NS	NS	Down-regulation	0.002

* P-values were calculated in comparison to kidney cortex using the Mann–Whitney U-test.

† Comparison of the significant differences between the RCC types was performed using the Kruskal-Wallis test.

NS, non-significant.

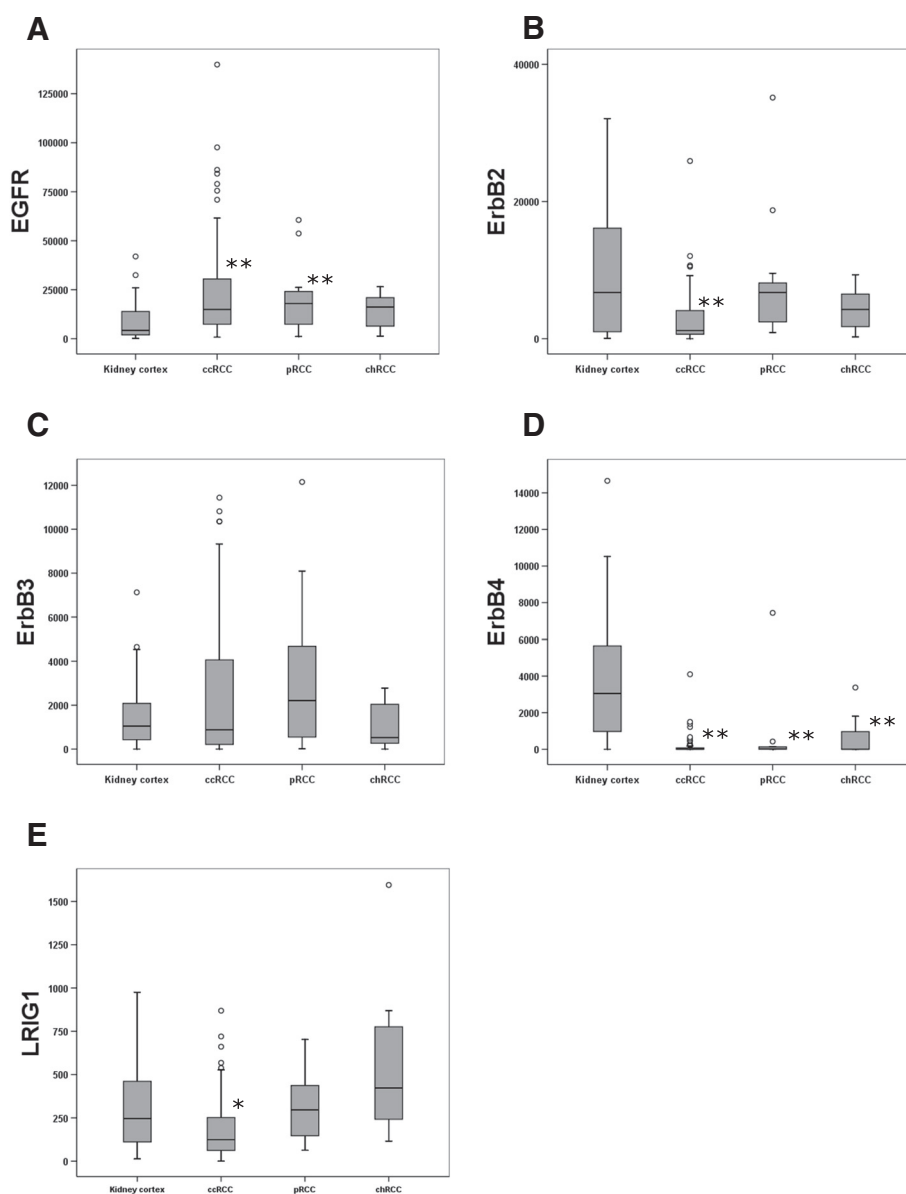


Figure 1 Boxplots of the relative mRNA expression of the *EGFR*-family members and *LRIG1* in both the kidney cortex and the RCC types. Relative mRNA expression of *EGFR*, *ERBB2*, *ERBB3*, *ERBB4*, and *LRIG1* was quantified in kidney cortex (n = 27), ccRCC (n = 81), pRCC (n = 15), and chRCC (n = 7). (A) *EGFR* mRNA expression was elevated in ccRCC and pRCC compared to kidney cortex. (Increased expression in chRCC was not significant, but expression levels were similar to other RCC groups.) (B) *ERBB2* mRNA expression was significantly lower in ccRCC compared to kidney cortex. In pRCC and chRCC, expression did not significantly differ from kidney cortex. (C) *ERBB3* mRNA expression was not significantly different between any of the RCC types compared to the kidney cortex. (D) *ERBB4* mRNA expression was significantly reduced in all RCC types compared to kidney cortex. (E) *LRIG1* mRNA expression was significantly lower in ccRCC compared to kidney cortex. In pRCC and chRCC, *LRIG1* expression did not significantly differ from kidney cortex. Outlier values are marked °. Significant differences compared to expression in the kidney cortex are labeled (*) for P < 0.05 and (**) for P < 0.01.

ERBB3 expression was similar in RCC and non-neoplastic kidney cortex, and there was no significant expression difference between RCC types (Figure 1C).

ERBB4 expression was markedly lower in all the different RCC types than in kidney cortex (P < 0.001). There was no difference in *ERBB4* expression between the

RCC types (Figure 1D). Thus, the earlier described down-regulation of *ERBB4* in RCC [19] was here shown to be prominent in all RCC types analyzed. In fact, no *ERBB4* expression could be detected in 51 out of the 104 tumors analyzed. This pronounced down-regulation of *ERBB4* may suggest an important

role for this receptor tyrosine kinase in inhibiting the development of RCC.

The expression of *LRIG1* was reduced in ccRCC compared to kidney cortex ($P = 0.020$). The expression of *LRIG1* in pRCC and chRCC was not significantly different from that of kidney cortex (Figure 1E). Thus, the previously described down-regulation of *LRIG1* in RCC [18] was restricted to ccRCC. This finding could indicate a tumor suppressive role for *LRIG1* in the context of ccRCC that is not present or of reduced importance in other types of RCC.

The expression levels of *EGFR*, *ERBB2-4*, and *LRIG1* correlated significantly with each other in most cases (Table 3). This was possibly due to *LRIG1* expression being up-regulated by receptor activation [7] or due to methodological issues, e.g. due to variation in the expression of the reference gene, *RN18S1*. There was a non-significant trend to an inverse correlation ($R = -0.166$ $P = 0.058$) between the expression levels of *EGFR* and *ERBB4*. This results is in line with *EGFR* being up-regulated and *ERBB4* being down-regulated in tumors.

The expression levels of *ERBB3* within ccRCC were inversely correlated with tumor grade and tumor size ($R = -0.287$, $P = 0.009$ and $R = -0.244$, $P = 0.027$, respectively). As expression of *ERBB3* mRNA was low and did not differ between tumors and kidney cortex or between RCC types, the biological significance of this finding is highly uncertain. No other significant correlation was observed between the expression of *EGFR*-family members or *LRIG1* and the size, grade, or stage of the tumors. Survival analysis comparing patients with tumors expressing above or below median mRNA values of the five genes revealed no significant difference in overall survival or cancer specific survival, neither in ccRCC patients or all RCC patients combined (Additional file 1 Figure S1). The patient groups for the other RCC types

were too small for meaningful survival analyses. The previously described and non-significant association between *LRIG1* expression and tumor grade and patient survival [18] was not confirmed in the present and larger study. Therefore, it appears that although *LRIG1* may possess a tumor suppressive function in ccRCC, it does not appear to be an important prognostic factor in RCC.

Conclusions

This study demonstrated that the expression of *EGFR*, *ERBB3* and *ERBB4* was similar throughout different RCC types, whereas the expression of *ERBB2* and *LRIG1* differed between the various types of RCC. This demonstrates potentially important differences and similarities in the expression of the *EGFR*-family members and *LRIG1* genes between different RCC types. Up-regulated gene expression of *EGFR* compared to kidney cortex was found in all RCC types analyzed. A strong down-regulation of *ERBB4* was observed in all RCC types analyzed, while down-regulation of *ERBB2* and *LRIG1* was found only in ccRCC. The biological and clinical significance of these differences in gene expression warrants further study.

Additional file

Additional file 1: Figure S1 Kaplan-Meier survival curves of cancer specific survival comparing patients with tumors above and below median expression of all five genes.

Abbreviations

EGFR: Epidermal growth factor receptor; *ERBB*: from avian erythroblastosis oncogene B, gene encoding for *EGFR*; *HER*: Human EGF receptor; *Neu*: old designation given to *ERBB2* gene when first found in neural mouse tumors; *LRIG1*: Leucine-rich repeats and immunoglobulin-like domains-1; *MET*: Gene encoding the hepatocyte growth factor receptor; *RCC*: Renal cell carcinoma; *ccRCC*: Clear cell (also called conventional) RCC; *chRCC*: Chromophobe RCC; *pRCC*: Papillary RCC; *RET*: A receptor tyrosine kinase, receptor for members of the glial cell line-derived neurotrophic factor (*GDNF*) family.

Competing interests

The authors declare that they have no competing interests.

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Author's contribution

MT performed RT-PCR analysis, statistical analysis, and drafted the manuscript. HH provided support for the RT-PCR analyses and helped draft the manuscript. BL supervised collection of tumor samples, RNA extracts and clinical data. RH coordinated the study. All authors participated in the conception and design of the study. All authors have read and approved the final manuscript.

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Table 3 Correlation of mRNA expression levels between the analyzed genes

		<i>EGFR</i>	<i>ErbB2</i>	<i>ErbB3</i>	<i>ErbB4</i>	<i>LRIG1</i>
<i>EGFR</i>	CC	1.000	0.274*	0.425*	-0.166	0.370*
	P-value	-	0.002	<0.001	0.058	<0.001
<i>ErbB2</i>	CC	0.274*	1.000	0.586*	0.375*	0.456*
	P-value	0.002	-	<0.001	<0.001	<0.001
<i>ErbB3</i>	CC	0.425*	0.586*	1.000	0.152	0.456
	P-value	<0.001	<0.001	-	0.082	0.000
<i>ErbB4</i>	CC	-0.166	0.375*	0.152	1.000	0.277*
	P-value	0.058	<0.001	0.082	-	0.001
<i>LRIG1</i>	CC	0.370*	0.456	0.456	0.277*	1.000
	P-value	<0.001	<0.001	<0.001	0.001	-

CC, Correlation coefficient according to Spearman.

*Significant with P-value <0.05.

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