Acta Oncologica, 2010; 49: 812–815

informa
healthcare

ORIGINAL ARTICLE

LRIG2 in contrast to LRIG1 predicts poor survival in early-stage squamous cell carcinoma of the uterine cervix

HÅKAN HEDMAN[1], ANNIKA K. LINDSTRÖM[2,3], TIBOR TOT[4], ULF STENDAHL[1], ROGER HENRIKSSON[1,5] & DAN HELLBERG[3,6]

[1]*Department of Radiation Sciences, Umeå University, Umeå, Sweden,* [2]*Ventrum Clinic, Bjursås, Sweden,* [3]*Center for Clinical Research, Falun, Sweden,* [4]*Department of Pathology and Clinical Cytology, Central Hospital, Falun, Sweden,* [5]*Oncology Clinic, Karolinska University Hospital, Stockholm, Sweden and* [6]*Department for Women's and Children's Health, Uppsala University, Uppsala, Sweden*

Abstract

Background. The human leucine-rich repeats and immunoglobulin-like domains (LRIG) protein family comprises LRIG1, 2, and 3. LRIG1 negatively regulates growth factor signaling and is a proposed tumor suppressor. In early stage uterine cervical carcinoma, expression of LRIG1 is associated with good survival. Less is known about the function and expression of LRIG2; it has not been studied in cervical carcinoma, previously. *Materials and methods*. LRIG2 expression was studied by immunohistochemistry in 129 uterine cervical squamous cell carcinomas and 36 uterine cervical adenocarcinomas. Possible associations between LRIG2 immunoreactivity and patient survival were evaluated. *Results*. In early-stage squamous cell carcinoma (stages IB–IIB), high expression of LRIG2 was associated with poor survival (Kaplan-Meier, log-rank, p=0.02). The 10-year survival rate for patients with high expression of LRIG2 was 60%, compared to 87% in patients with low expression (odds ratio 0.22, 95% CI 0.07–0.64). In multivariate analysis including the previously studied tumor suppressor LRIG1 and clinical stage, LRIG2 emerged as an independent prognostic factor (odds ratio 0.22, 95% CI 0.09–0.50). For patients with both high expression of LRIG2 and low expression of LRIG1, the 10-year survival rate was only 26% compared to 66% for the remaining study population. There was no correlation between LRIG2 expression and prognosis in the limited adenocarcinoma series. *Discussion and conclusion*. LRIG2 appears to be a significant predictor of poor prognosis in early-stage squamous cell carcinoma of the uterine cervix. A combination of high LRIG2 expression and low LRIG1 expression identified women with a very poor prognosis.

The human leucine-rich repeats and immunoglobulin-like domains (LRIG) gene family comprises *LRIG1*, *2,* and *3*. The *LRIG* genes are located at chromosome bands 3p14.3 (*LRIG1*) [1], 1p13 (*LRIG2*) [2], and 12q13 (*LRIG3*) [3]. All of these genomic regions have been implicated in human cancer (http://cgap.nci.nih.gov/Chromosomes/Mitelman). The *LRIG* genes encode integral membrane glycoproteins with extracellular or luminal domains containing a leucine-rich repeat domain and three immunoglobulin-like domains, a transmembrane domain, and a cytosolic tail. The amino acid sequence of LRIG1 is 47% identical to the sequences of both LRIG2 and LRIG3 [2,3]. LRIG1, 2, and 3 are expressed in most or all organs that have been analyzed [1–4].

Increasing evidence indicates that LRIG1 is a tumor suppressor in certain cancer types [reviewed in 5]. At the molecular level, LRIG1 negatively regulates oncogenic receptor tyrosine kinases, including EGFR [6,7], ERBB2 [6,8], MET [9], and RET [10]. The functions of LRIG2 and LRIG3 are still poorly understood. The subcellular localization of LRIG proteins seems important for their activity. In psoriatic lesions, LRIG proteins are redistributed compared to in normal skin [11], and in astrocytic tumors of the brain, perinuclear expression of LRIG proteins is associated with a good prognosis [12].

In accordance with the proposed role of LRIG1 as a tumor suppressor, we found a significant correlation between expression of LRIG1 and favorable outcome in early stage invasive cervical cancer [13].

Correspondence: Håkan Hedman, Department of Radiation Sciences, Umeå University, SE-90187 Umeå, Sweden. Tel: +46-90-785 2881. Fax: +46 90 785 2031. E-mail: hakan.hedman@onkologi.umu.se

(Received 18 January 2010; accepted 7 May 2010)

ISSN 0284-186X print/ISSN 1651-226X online
DOI: 10.3109/0284186X.2010.492789

Similarly, in cutaneous squamous cell carcinoma, down-regulation of LRIG1 protein level is associated with high tumor grade and poor survival [14]. LRIG2 expression, in contrast, was recently reported to be associated with poor survival in oligodendroglioma [15].

The purpose of the present study was to evaluate the expression of the LRIG2 protein in invasive squamous cell carcinoma and adenocarcinoma of the uterine cervix and its relationship with patient outcome.

Materials and methods

The study population comprised 129 women with invasive squamous cell carcinoma and 36 women with adenocarcinoma of the uterine cervix who were admitted to the Department of Gynecologic Oncology, Norrlands University Hospital, Umeå, during 1984–1990. Clinical staging was made according to FIGO [16]. The women were followed up for at least ten years. The treatment was radiotherapy and/or surgery in accordance with contemporary routines. The clinical history included reproductive events, smoking habits, and oral contraceptive use.

The tissue micro array (TMA) used in this study was the same as previously used to study expression of LRIG1 [13]. Briefly, three-micrometer sections of the original paraffin blocks were reviewed by one of the authors (TT) and the most representative area(s) was marked for TMA. Three-millimeter punch biopsies were taken from the original blocks and joined into TMA paraffin blocks, containing an average of 25 punch biopsies each. Immunohistochemical staining of LRIG2 was carried out with polyclonal rabbit antibodies against the cytoplasmic tail of the protein as previously described [12].

The stained TMA were evaluated by an external senior pathologist who was blinded for the clinical details. Expression was scored as high when 50% or more of the cancer cells were immunopositive and as low when less than 50% of the cancer cells were immunopositive. This cut-off was chosen because it showed the best explanatory power of the different cut-offs tested (0, 20, 50, and 100%).

A χ^2 test (likelihood ratio) was used to estimate p-values for categorical variables and the t-test was used for continuous variables. Logistic regression was used for estimation of odds ratios (OR) and 95% confidence interval. Logistic regression was also used in the multivariate analysis. Log rank test was used for Kaplan-Meier analyses.

All women provided written informed consent. The study was approved by the Research Ethical Committee, Medical Faculty, Umeå University.

Results

Patient characteristics and clinical parameters are shown in Table I. Clinical stage and age correlated with 10-year survival, but age was insignificant after adjustment for stage.

Specific LRIG2 immunoreactivity was generally only seen in the cytoplasm (Figure 1). In squamous cell carcinoma, there was a statistically insignificant tendency towards high LRIG2 expression being more common in early stage cancer than in later stages ($p=0.10$) (Table II). Kaplan-Meier analysis revealed a significantly shorter survival for squamous cell carcinoma stage IB/IIB patients with high vs. low expression of LRIG2 (log-rank test, $p=0.02$) (Figure 2). High LRIG2 expression was associated with poor 10-year survival in squamous cell carcinoma (Table III), but this association was not statistically significant when all stages were included. In stages IB–IIB there was, however, a significant association between high LRIG2 expression and poor 10-year survival. In the limited adenocarcinoma series, LRIG2 expression did not correlate with survival. There was no correlation between LRIG2 expression and smoking habits, reproductive history, or climacteric status.

In multivariate analysis of squamous cell carcinomas including expression of LRIG1 and clinical stage, expression of LRIG2 emerged as an independent and significant factor associated with poor 10-year survival (OR 0.22, 95% CI 0.09–0.50). LRIG1 expression was also an independent and significant factor, associated with a favorable outcome (OR 2.60, 95% CI 1.19–5.91). The combination of high LRIG2 and low LRIG1 expression ($n=23$) was associated with a very poor 10-year survival rate (26%) compared to the remaining study population (66%; $p=0.0004$) (not shown in Table). Despite the opposing effects of LRIG2 and LRIG1 on survival, there was a significant correlation between their expression ($p<0.001$).

Table I. Characteristics of the 129 cases of uterine cervical squamous cell and 36 cases of uterine adenocarcinoma included in the study.

	Squamous cell carcinoma	Adenocarcinoma
Mean age, years	59.6	58.0
Pregnancies, mean no.	3.1	2.3
Parity, mean no.	2.7	2.1
Climacteric status (%)	89 (69.0)	22 (62.9)
Clinical stage		
IB-IIA (%)	68 (52.7)	28 (80.0)
IIB (%)	19 (14.7)	3 (8.6)
III-IV (%)	42 (32.6)	4 (11.5)
Ten year survival (%)	53 (41.1)	10 (28.6)

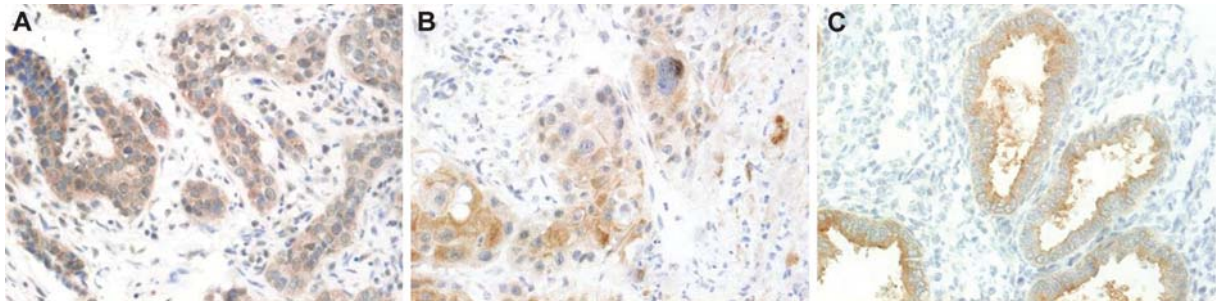

Figure 1. Immunohistochemical staining for LRIG2 in uterine cervical cancers. A, B, Squamous cell carcinomas. C, Adenocarcinoma.

Discussion

In the present study a high LRIG2 expression correlated with poor survival in invasive early-stage squamous cervical cancer. This contrasts with our previous finding regarding the homologous LRIG1 protein, whose expression correlates with good survival in the same patient series. Importantly, in multivariate analyses, expression of LRIG2 and LRIG1 both remained as prognostic factors independent of each other and clinical stage.

Recently, high LRIG2 expression was reported to be associated with poor survival in oligodendroglioma [15]. Thus, the emerging picture indicates that LRIG1 and LRIG2 may have opposite effects on patient survival in various tumor types. Notably, in squamous cell carcinomas, an association between LRIG1 expression and good survival has been shown for both cutaneous and cervical squamous cell carcinoma [13,14], and here, an association between LRIG2 expression and poor survival in cervical squamous cell carcinoma was shown. Our results on cervical carcinoma may be particularly relevant since the immunohistochemical analyses of LRIG1 and LRIG2 were performed simultaneously, by the same investigators, and on the same clinical material. The positive correlation between expression of LRIG1 and LRIG2 is intriguing. One speculation could be that it is the balance between the levels of LRIG1 and LRIG2 that is the important determinant, where a high ratio would imply a favorable prognosis and a low ratio a poor prognosis.

Table II. Expression of LRIG2 and LRIG1 in uterine cervical squamous cell carcinoma of different clinical stages.

	LRIG2[1]		LRIG1[2,3]	
Clinical stage	high	low	any	none
IB (n=53)	28 (52.8)	25 (47.2)	30 (57.7)	22 (42.3)
IIA-IIB (n=34)	20 (58.8)	14 (41.2)	17 (50.0)	17 (50.0)
III-IV (n=42)	15 (35.7)	27 (64.3)	14 (33.3)	28 (66.7)

[1]p (trend)=0.10.
[2]Data from Lindström et al. [13].
[3]p (trend)=0.06.

We only saw cytoplasmic expression of LRIG2 in the present study. Previously, we found that perinuclear LRIG protein localization was associated with good prognosis in patients with astrocytic tumors [12] and cytoplasmic expression of LRIG2 was associated with poor prognosis in patients with oligodendroglioma [15]. The former could imply a requirement for perinuclear protein localization for proper tumor-suppressive functions of the LRIG proteins. Alternatively, the results are also consistent with a scenario where cytoplasmic localization is required for a tumor-promoting function of LRIG2. These models are not mutually exclusive; however, a better understanding of the physiological and molecular functions of the LRIG proteins is needed to resolve this issue.

Regarding the molecular functions of the LRIG proteins, several studies have shown that LRIG1 is a negative regulator of growth factor signaling in different contexts [6–10]. For squamous cell carcinoma, the function of LRIG1 as a negative regulator

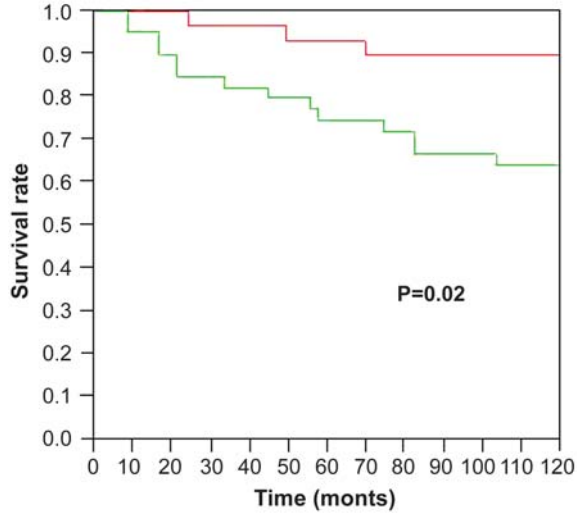

Figure 2. Kaplan-Meier curves showing survival of cervical squamous cell carcinoma stage IB-IIB patients in relation to LRIG2 expression. Red line, low LRIG2 expression (expression in <50% of cancer cells). Green line, high LRIG2 expression (expression in ≥50% of cancer cells). Log-rank test, p=0.02.

Table III. LRIG2 expression and overall 10-year survival in invasive uterine cervical cancer

	Survival No. (%)			
	LRIG2 high	LRIG2 low	Odds ratio	95% CI
Squamous cell carcinoma – all stages (n=129)	32 (50.8)	44 (66.7)	0.52	0.25-1.05
– stage IB-IIB (n=87)	29 (60.4)	34 (87.2)	0.22	0.07-0.64
– stage III-IV (n=42)	3 (20.0)	10 (37.0)	0.43	0.08-1.74
Adenocarcinoma or adenosquamous cancer (n=36)	11 (68.8)	15 (75.0)	0.73	0.16-3.24

of EGFR may be particularly relevant since EGFR signaling is implicated in the etiology of these tumors. The mechanism behind the possible tumor-promoting effect of LRIG2 seems less clear, however. One possibility is that the LRIG proteins have different functions, and that LRIG1 and LRIG2 also counteract each other at the molecular level. Opposite or different functions among members of the same protein family is not uncommon as exemplified by the BCL-2 protein family, where some members, e.g. BCL-2 and BCL-XL, are anti-apoptotic, whereas other members, e.g. BAX and BAK, have opposing activities and are pro-apoptotic [17]. For the LRIG family, however, the molecular functions of LRIG2 and LRIG3 in relation to LRIG1 remain unexplored.

In summary, we provide evidence that LRIG2 is an independent prognostic factor associated with poor survival in early-stage squamous cell carcinoma of the uterine cervix. This result, together with our previously reported finding that high expression of LRIG1 correlates with good survival in the same patient series, indicates that LRIG1 and LRIG2 may be opposing and important molecular determinants of the malignancy of cervical cancer.

Acknowledgements

We thank Kerstin Bergh for help with the immunohistochemical staining. This work was supported by grants from the Swedish Research Council, the Swedish Cancer Society, and the Cancer Research Foundation in Northern Sweden.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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