Expression of LRIG proteins as possible prognostic factors in primary vaginal carcinoma

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Abstract

Background

Primary vaginal carcinoma (PVC) is a rare malignancy. Established prognostic factors include tumour stage and age at diagnosis. The leucine-rich repeats and immunoglobuline-like domains (LRIG)-1 protein functions as a tumour suppressor, but less is known about the functions of LRIG2 and LRIG3. The present study aimed to evaluate the expression of LRIG proteins and analyse their possible associations with clinical characteristics and survival in a cohort of PVC patients.

Methods

We used immunohistochemistry to investigate LRIG1, LRIG2, and LRIG3 expression in tumour samples from a consecutive cohort of 70 PVC patients. The association between LRIG protein expression and clinical characteristics and cancer-specific survival was investigated using univariate and multivariate analyses.

Results

The majority of PVC patients (72%) had >50% LRIG1- and LRIG2-positive cells, and no or low LRIG3-positive cells. HPV status was significantly correlated with LRIG1 expression (p = 0.0047). Having high LRIG1 expression was significantly correlated with superior cancer-specific survival in univariate and multivariate analyses. LRIG2 and LRIG3 expression did not significantly correlate with clinical characteristics or survival.
Introduction

Primary vaginal carcinoma (PVC) is a rare malignancy of the female genital tract. It has a poor prognosis and most commonly affects postmenopausal women over 60 years of age [1–3]. Survival rates depend on patient age and clinical stage of the disease, but overall survival is less than 50% [1,2]. The most common histological subtype of PVC is squamous cell carcinoma; adenocarcinomas are also seen, but other histological subtypes are very rare [3]. Studies on PVC are scarce and small due to the rarity of the disease, and increased knowledge of biological and prognostic factors is required in order to improve clinical strategies for these patients.

Studies on the treatment of PVC are also rare and have shown inconclusive results. Given the relative frequency of cervical cancer, as well as its anatomical, histological, and biological similarities with PVC, treatment strategies for PVC tend to be extrapolated from those for cervical cancer [4]. Therefore, most PVC patients are treated with radiotherapy [5] or definitive chemoradiotherapy [6,7], which sometimes results in substantial toxic side effects.

Similarly to other squamous cell carcinomas of the genital tract, the majority of PVC cases are related to human papillomavirus (HPV) infection [3,8–13]. Although it has been suggested that HPV could be a useful prognostic marker for PVC [8,12], another study was unable to confirm this [14], and yet another study confirmed it only in women with advanced stages of disease [15]. Other prognostic factors that have repeatedly been shown to be significant in PVC include clinical variables such as age, tumour size [16,17], and clinical stage [2,8]. Proposed prognostic biomarkers have included p16, Ki67, p53, and laminin-5 expression [14,16,17].

The expression of the human leucine-rich repeats and immunoglobulin-like domains (LRIG) proteins LRIG1, LRIG2, and LRIG3 has emerged as a new potential prognostic biomarker in different types of human cancer, including cervical cancer [18]. LRIG1, LRIG2, and LRIG3 encode transmembrane proteins involved in the regulation of growth factor signalling and cell proliferation. Accordingly, the expression of LRIG proteins is commonly dysregulated in human cancer. LRIG1 has been proposed to function as a tumour suppressor through negative regulation of oncogenic receptor tyrosine kinases, such as members of the ERBB family, MET and RET receptors, and PDGFRA [19]. LRIG1 expression is of prognostic significance and is associated with good prognosis in various human cancers, including cervical cancer [20,21], non-small-cell lung cancer [22,23], breast cancer [24], and others [18]. In cervical cancer, the variation in the number of LRIG1 gene copies and promoter methylation patterns are associated with patient survival [25]. Although we know that LRIG3 interacts with, and possibly opposes the function of, LRIG1 [26], we know little else about it, or its counterpart LRIG2.

In general, high expression of LRIG1 and LRIG3 in tumours is associated with improved survival in cancer patients, whereas high expression of LRIG2 is associated with poor survival even in early-stage cervical cancer patients [27]. Previous studies on cervical cancer have implied that each LRIG protein may be of different prognostic value, depending on histology and stage of disease [20,21,27].
The LRIG proteins have not previously been studied in PVC. The present study aimed to evaluate the expression of LRIG proteins and analyse their possible associations with clinical characteristics and survival in a cohort of PVC patients.

Materials and methods

The present analysis used archived PVC samples collected from a consecutive cohort of 81 patients with PVC treated at Örebro University Hospital, and at the central hospitals in Eskilstuna, Västerås, and Karlstad between 1975 and 2002. In a previous study by Larsson et al.,[12] data on age, tumour site, FIGO stage, tumour localisation, histology (based on World Health Organisation criteria and included basaloid squamous cell carcinoma, non-keratinizing squamous cell carcinoma, keratinizing squamous cell carcinoma, verrucous squamous cell carcinoma, adenocarcinoma, sarcoma, and melanoma), tumour grade, and treatment for each patient were recovered from hospital records and were used in this analysis. We also used follow-up data from Larsson et al [12]. All patients were retrospectively followed up from the time of diagnosis and median follow-up time for patients who were alive at the end of the study was 121 months (range 44–290 months). Complete remission was defined as the disappearance of all clinical evidence of disease after primary treatment. Tumour recurrence was defined as the detection of cancer after a period of at least 6 months of initial complete remission. Finally, we used information on HPV status reported by Larsson et al [12]. In that study, all 81 samples were subjected to HPV testing and genotyping. Among the 81 PVC cases, 37 were HPV-positive, 34 were HPV-negative and 10 had insufficient material for HPV detection (Table 1). Of the 37 HPV-positive cases, 26 (70%) were HPV16-positive, whereas the remaining 11 were positive for other high-risk HPV genotypes.

The present study was approved by the regional Ethical Committee in Uppsala, Sweden (EPN, Dnr 2008/294). Patients were orally informed about the clinical research database, and after 2003 they were also informed about tissue biobanking according to the Swedish biobank act 2002:297. No specific informed consent was requested by the Ethical Committee.

Immunohistochemistry for LRIG protein expression

Seventy of the 81 PVC patients had sufficient material for immunohistochemical analyses. Immunohistochemical staining for LRIG1, LRIG2, and LRIG3 was carried out on formalin-fixed paraffin-embedded sections of 3.5–4 μm. Tissue sections were incubated for 60 minutes at 60˚C. Immunohistochemical staining was performed in an automated Ventana Benchmark XT apparatus (Ventana Medical Systems, Tucson, AZ, USA) using rabbit primary antibodies for LRIG1, LRIG2, and LRIG3 [28–31]. Epitope retrieval for LRIG1 and LRIG2 was done with Cell conditioning 1 (Product nr. 950–124, Ventana Medical Systems) for 60 minutes at 95˚C. For LRIG 3, Protease 1 (Product nr. 760–2018, Ventana Medical Systems) was used for 12 minutes at 37˚C. Automated immunohistochemical staining was performed using the ultraView Universal DAB Detection Kit (Product nr. 760–500, Ventana Medical Systems). The slides were deparaffinised and blocked for peroxidase using ultraView Universal DAB Inhibitor containing 3% hydrogen peroxide solution. Primary antibodies (LRIG1; 1:100, LRIG 2; 1:200, and LRIG 3; 1:50) were incubated for 32 minutes at 37˚C. UltraView Universal HRP Multimer, ultraView Universal DAB Chromogen, and ultraView Universal Copper were used according to the manufacturer’s instructions. The slides were counterstained with hematoxylin (Product nr. 760–2021, Ventana Medical Systems) for 4 minutes followed by bluing with Bluing Reagent (Product nr. 760–2037, Ventana Medical Systems) for 4 minutes, both at 37˚C.

A senior pathologist (MK), blinded to all clinical data, evaluated the immunostaining. The percentage of positive cells was based on a 4-point semi-quantitative scale: 0 = 0% positive.
Table 1. Patient and tumour characteristics (n = 81).

| Mean (range)          |
|----------------------|
| Age (years)          | 69 (37–90)  |
| Tumour size (cm)     | 2.5 (1.0–4.0) |
| FIGO stage N (%)     |             |
| I                    | 25 (31)     |
| II                   | 34 (42)     |
| III                  | 9 (11)      |
| IV                   | 13 (16)     |
| Localisation         |             |
| Upper vagina         | 23 (28)     |
| Middle vagina        | 14 (17)     |
| Middle-lower         | 2 (3)       |
| Lower vagina         | 26 (32)     |
| Entire length of vagina | 16 (20) |
| Histology            |             |
| Squamous cell carcinoma | 72 (89)  |
| Basaloid             | 28          |
| Non-keratinizing     | 24          |
| Keratinizing         | 9           |
| Verrucous            | 2           |
| Adenocarcinoma       | 7 (8.5)     |
| Other                | 2 (2.5)     |
| Tumour grade         |             |
| 1                    | 13 (16)     |
| 2                    | 30 (37)     |
| 3                    | 27 (33)     |
| Unknown              | 11 (14)     |
| HPV status (n = 71)  |             |
| Negative             | 34 (48)     |
| Positive             | 37 (52)     |
| HPV 16               | 26          |
| Other HPV types      | 11          |
| Treatment            |             |
| EBRT*                | 10 (12.5)   |
| EBRT+BT              | 48 (59)     |
| BT**                 | 13 (16)     |
| Other                | 10 (12.5)   |

*EBRT = external beam radiotherapy, **BT = vaginal brachytherapy

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cells, 1 = 1–25% positive cells, 2 = 25–50% positive cells, and 3 = >50% positive cells. Cells were considered positive regardless of whether the staining was cytoplasmic or nuclear (see S1 Table for description of LRIG protein expression and HPV status for all patients).

**Statistical analysis**

Associations between ordinal variables were tested using a Pearson chi-square or Fisher’s exact test. Patient and tumour characteristics, HPV status, and LRIG1, LRIG2, and LRIG3 expression were illustrated in a Kaplan-Meier graph to reflect possible prognostic factors for cancer-specific survival, and a log-rank test was used to compare different groups. Age, tumour size, FIGO stage, and HPV status were significant in the univariate analysis, and thus were included in a Cox regression multivariate analysis. All significance testing was carried out at the 0.05 level using SPSS 19 software.

**Results**

**LRIG protein expression, patient and tumour characteristics, and HPV status**

A majority of the 70 tumours showed LRIG1 and LRIG2 expression in >50% of cells and demonstrated no or low LRIG3 expression (Table 2). Fig 1 shows examples of the evaluation of staining intensity.

High LRIG1 expression was significantly correlated with HPV positivity (Pearson chi-square; p = 0.0047). No other statistically significant correlations were found between LRIG expression, tumour size, FIGO stage, localisation, histology, tumour grade or HPV status.

Among non-keratinizing tumours, 66.7% were HPV-positive, compared to only 22.8% of keratinizing tumours (Pearson chi-square: p = 0.022).

**Primary cure rate and cancer-specific survival rates**

High LRIG1 expression (>50% vs <50% positive cells) correlated with a higher primary cure rate (Pearson chi-square, p = 0.0004). High LRIG1 expression (>50% vs <50% positive cells)

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**Table 2. Immunohistochemical staining of LRIG1, LRIG2, and LRIG3 (n = 70).**

| Immunohistochemical score * | N (%) |
|---------------------------|-------|
| **LRIG1**                 |       |
| 0                         | 0 (0) |
| 1                         | 0 (0) |
| 2                         | 12 (17)|
| 3                         | 58 (83)|
| **LRIG2**                 |       |
| 0                         | 0 (0) |
| 1                         | 0 (0) |
| 2                         | 12 (17)|
| 3                         | 58 (83)|
| **LRIG3**                 |       |
| 0                         | 37 (53)|
| 1                         | 29 (41)|
| 2                         | 4 (6)  |
| 3                         | 0 (0)  |

*Protein score: 0 = 0%, 1 = 1–25%, 2 = 25–50%, 3 = >50% of positive cells.

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was also associated with a significantly better survival rate (log-rank test: \( p = 0.011 \)), and this difference was most pronounced in HPV-negative PVC (log-rank test: \( p = 0.027 \)). There was no statistically significant correlation between LRIG2 or LRIG3 expression and patient survival (Table 3, Fig 2).

Significant parameters were included in a Cox regression multivariate analysis, but the only factors that showed an independent, statistically significant association with patient survival were HPV status and LRIG1 expression (Table 4).

Discussion

In the present study, the expression of LRIG1, LRIG2, and LRIG3 was evaluated in a cohort of 70 PVC patients. To the best of our knowledge, this is the first study on the expression of LRIG proteins in PVC. Intriguingly, high LRIG1 expression was associated with superior cancer-specific survival in the PVC patients in our study.

LRIG proteins have previously been studied in cervical cancer, a disease with many similarities to PVC. In accordance with the present study, Lindstrom et al [20] showed that LRIG1 expression might be a predictor of improved survival in early-stage cervical carcinoma. In contrast to our results, where no differences between stages could be seen, LRIG1 expression appeared to decrease with increasing cervical cancer stage. Furthermore, increasing LRIG1 expression has been correlated with increasing grade of cervical intraepithelial neoplasia [32] and has also been observed as a prognostic marker in cervical adenocarcinoma [21]. Thus LRIG1 expression seems to play an important role in both precancerous and cancerous lesions.

| Variable                              | Hazard ratio | (95% CI)      | p-value |
|---------------------------------------|--------------|---------------|---------|
| Age (per year)                        | 1.04         | (1.01–1.07)   | 0.015   |
| Tumour size                           | 1.41         | (1.04–1.90)   | 0.026   |
| FIGO stage (III-IV vs I-II)           | 2.54         | (1.62–5.54)   | 0.019   |
| Localisation (whole vs upper vagina)  | 3.47         | (1.42–8.47)   | 0.010   |
| Tumour grade (3 vs 1)                 | 1.80         | (0.66–4.89)   | ns      |
| Histology (adeno vs SSC)              | 0.62         | (0.19–2.02)   | ns      |
| HPV status (negative vs positive)     | 4.01         | (1.96–8.21)   | <0.001  |
| LRIG1 (>50% vs <50% positive cells)  | 0.35         | (0.68–0.73)   | 0.011   |

Table 3. Univariate analyses with CCS as end-point.
The literature has only scarce information on the possible functions of LRIG2 and LRIG3. In 129 cases of cervical squamous cell carcinoma, LRIG2 expression was a predictor of poor prognosis in early-stage disease [27]. In addition, the combination of high LRIG2 expression and low LRIG1 expression identified women with a very poor prognosis. In contrast, no correlation was observed between LRIG2 expression and clinical parameters in cervical adenocarcinoma [21]. In the present study, no significant association was seen between LRIG2 expression and patient survival.

In an earlier study of the expression of LRIG3 in squamous cell cervical cancer, no correlation was seen with 10-year survival; however, LRIG3 expression was associated with a number of molecular events in cervical intraepithelial neoplasia [33]. It was concluded that LRIG3 was less clinically important. However, in the study by Muller et al [21] on cervical adenocarcinoma, having a high fraction of LRIG3-positive cells was associated with improved patient survival. In our study, LRIG3 showed no statistically significant association with patient survival.

The role of the LRIG proteins in cancers and their association with HPV infection has been previously investigated. In our PVC samples, HPV-positive tumours showed significantly higher scores for LRIG1 than HPV-negative tumours. Lindquist et al showed the same positive

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\text{Fig 2. Cancer-specific survival rate versus LRIG1 expression in immunohistochemical staining (score 3 vs. score 0–2). Log-rank test showed a statistically significant difference (p = 0.011).}
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![Log-rank test](https://doi.org/10.1371/journal.pone.0183816.g002)

| Variable   | Hazard ratio | (95% CI)   | p-value |
|------------|--------------|------------|---------|
| Age        | 1.014        | (0.98–1.05)| ns      |
| Tumour size| 1.323        | (0.95–5.64)| ns      |
| FIGO stage | 2.268        | (0.91–5.64)| ns      |
| HPV status | 3.863        | (1.81–8.25)| <0.001  |
| LRIG1      | 0.408        | (0.18–0.91)| 0.029   |

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correlation between HPV status and LRIG1 expression in oropharyngeal cancer [30]. However, we did not observe the inverse correlation between LRIG2 and HPV status that Lindquist reported. In both PVC and oropharyngeal cancer, a high expression of LRIG1 was identified and LRIG1 expression was revealed as an independent, positive prognostic factor. In addition, Lindquist et al demonstrated that HPV-positive tumours with high LRIG1 expression correlated with a very good prognosis in terms of disease-free survival and overall survival. This correlation between LRIG1 expression, HPV positivity, and patient survival was not significant in PVC.

In the study by Lindstrom and Hellberg, high LRIG3 expression was correlated with HPV positivity in both normal cervical epithelium and precancerous cervical lesions [33]. It was therefore suggested that LRIG3 might be involved in the development of precancerous lesions.

The most common histological type of PVC is squamous cell carcinoma, which made up the majority of our samples. In agreement with our results, previous studies on squamous cell carcinomas of the cervix and oropharynx, breast [24], skin [34], and non-small cell lung cancer [22,23] showed that high LRIG1 expression is associated with improved prognosis. In a study of squamous cell carcinoma of the skin, LRIG1 showed the highest expression in well-differentiated tumours, and these patients also proved to have the best survival [34]. In the present study, the association between LRIG expression and differentiation could not be shown, and non-keratinizing tumours were observed to have a higher LRIG1 expression, which was associated with a better prognosis. Additionally, we found that non-keratinising tumours correlated to HPV positivity. Thus our results indicate that patients with HPV-positive, non-keratinizing tumours with high LRIG1 expression might have the best prognosis.

Furthermore, it has been shown that high LRIG1 expression correlates with increased sensitivity to platinum-based chemotherapy [35], thus LRIG might be of interest as a potential predictor and target for treatment in PVC patients to minimise the risk of overtreatment.

It has been reported that LRIG ectodomains may be shed and suppress growth factor signalling in neighbouring cells, suggesting that LRIG1 ectodomains can suppress growth factor signalling in a paracrine manner [36]. This non-cell autonomous inhibition of growth factor signalling could be an interesting strategy for the treatment of PVC and other cancers. However, whether cervical and vaginal cancers depend on growth factor signalling for their growth, and whether LRIG1 can inhibit this growth, remain to be determined.

A better understanding of the role of different molecular markers in the genesis of PVC is important to optimise rational treatment interventions. Molecular markers, such as the LRIG proteins studied here, may help us to develop more efficient clinical strategies for PVC patients. Thus, analysis of LRIG expression in precursor lesions will be important to determine the potential of LRIG proteins as molecular markers for early detection and progression of invasive disease.

In summary, LRIG immunoreactivity was of prognostic importance in PVC. This provides additional knowledge about the possible clinical value of LRIG proteins in gynaecological malignancies, where LRIG1 has emerged as an independent, positive prognostic marker. LRIG proteins may be important determinants of PVC prognosis, which justifies further studies of their diagnostic and prognostic potential in this disease.

**Supporting information**

S1 Table. LRIG protein expression and HPV status.

(DOCX)
Author Contributions

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References

1. Beller U, Benedet JL, Creasman WT, Ngan HY, Quinn MA, Maisonneuve P, et al. Carcinoma of the vagina. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet 2006; 95 Suppl 1: S29–S42.

2. Gunderson CC, Nugent EK, Yunker AC, Rocconi RP, Graybill WS, Erickson BK, et al. Vaginal cancer: the experience from 2 large academic centers during a 15-year period. J Low Genit Tract Dis 2013; 17: 409–413. https://doi.org/10.1097/LGT.0b013e3182800ee2 PMID: 23609592

3. Hellman K, Silfversward C, Nilsson B, Hellstrom AC, Frankendal B, Pettersson F. Primary carcinoma of the vagina: factors influencing the age at diagnosis. The Radiumhemmet series 1956–96. Int J Gynecol Cancer 2004; 14: 491–501. https://doi.org/10.1111/j.1048-891x.2004.014310.x PMID: 15228423

4. Orton A, Boothe D, Williams N, Buchmiller T, Huang YJ, Suneja G, et al. Brachytherapy improves survival in primary vaginal cancer. Gynecol Oncol 2016; 141: 501–506. https://doi.org/10.1016/j.ygyno.2016.03.011 PMID: 27036631

5. Hacker NF, Eifel PJ, van der Velden J. Cancer of the vagina. Int J Gynaecol Obstet 2015; 131 Suppl 2: S84–S87.

6. Chang JH, Jang WI, Kim YB, Kim JH, Kim YS, Kim YS, et al. Definitive treatment of primary vaginal cancer with radiotherapy: multi-institutional retrospective study of the Korean Radiation Oncology Group (KROG 12–09). J Gynecol Oncol 2016; 27: e17. https://doi.org/10.3802/jgo.2016.27.e17 PMID: 26768782

7. Rajagopalan MS, Xu KM, Lin JF, Sukumvanich P, Krivak TC, Beriwal S. Adoption and impact of concurrent chemoradiation therapy for vaginal cancer: a National Cancer Data Base (NCDB) study. Gynecol Oncol 2014; 135: 495–502. https://doi.org/10.1016/j.ygyno.2014.09.018 PMID: 25281493

8. Alonso I, Felix A, Torne A, Fusté V, del Pino M, Castillo P, et al. Human papillomavirus as a favorable prognostic biomarker in squamous cell carcinomas of the vagina. Gynecol Oncol 2012; 125: 194–199. https://doi.org/10.1016/j.ygyno.2011.12.449 PMID: 22226684
9. De Vuyst H, Clifford GM, Nascimento MC, Madeleine MM, Franceschi S. Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta-analysis. Int J Cancer 2009; 124: 1626–1636. https://doi.org/10.1002/ijc.24116 PMID: 19115209

10. Fuste V, del Pino M, Perez A, Garcia A, Torne A, Pahisa J, et al. Primary squamous cell carcinoma of the vagina: human papillomavirus detection, p16(INK4A) overexpression and clinicopathological correlations. Histopathology 2010; 57: 907–916. https://doi.org/10.1111/j.1365-2559.2010.03727.x PMID: 21166704

11. Giuliano AR, Tortolero-Luna G, Ferrer E, Burchell AN, de Sanjose S, Kjaer SK, et al. Epidemiology of human papillomavirus infection in men, cancers other than cervical and benign conditions. Vaccine 2008; 26 Suppl 10: K17–K28.

12. Larsson GL, Helenius G, Andersson S, Sorbe B, Karlsson MG. Prognostic impact of human papillomavirus (HPV) genotyping and HPV-16 subtyping in vaginal carcinoma. Gynecol Oncol 2013; 129: 406–411. https://doi.org/10.1016/j.ygyno.2013.02.004 PMID: 23402906

13. Smith JS, Backes DM, Hoots BE, Kurman RJ, Pimenta JM. Human papillomavirus type-distribution in vulvar and vaginal cancers and their associated precursors. Obstet Gynecol 2009; 113: 917–924. https://doi.org/10.1097/AOG.0b013e31819bd6e0 PMID: 19305339

14. Hellman K, Lindquist D, Ranchem C, Wilander E, Andersson S. Human papillomavirus, p16(INK4A), and Ki-67 in relation to clinicopathological variables and survival in primary carcinoma of the vagina. Br J Cancer 2014; 110: 1561–1570. https://doi.org/10.1038/bjc.2014.32 PMID: 24526965

15. Brunner AH, Grimm C, Polterauer S, Hefer L, Stani J, Heinze G, et al. The prognostic role of human papillomavirus in patients with vaginal cancer. Int J Gynecol Cancer 2011; 21: 923–929. https://doi.org/10.1097/IGC.0b013e31821bc015 PMID: 21666483

16. Habermann JK, Hellman K, Freitag S, Heselmeyer-Haddad K, Heststrom AC, Shah K, et al. A recurrent gain of chromosome arm 3q in primary squamous carcinoma of the vagina. Cancer Genet Cytogenet 2004; 148: 7–13. PMID: 14967353

17. Hellman K, Johansson H, Andersson S, Pettersson F, Auer G. Prognostic significance of cell cycle- and invasion-related molecular markers and genomic instability in primary carcinoma of the vagina. Int J Gynecol Cancer 2013; 23: 41–51. https://doi.org/10.1097/IGC.0b013e31827670c4 PMID: 23154268

18. Lindquist D, Kvambrik S, Henriksson R, Hedman H. LRIG and cancer prognosis. Acta Oncol 2014; 53: 1135–1142. https://doi.org/10.3109/0284186X.2014.953258 PMID: 25180912

19. Simon C, Cedano-Prieto ME, Sweeney C. The LRIG family: enigmatic regulators of growth factor receptor signaling. Endocr Relat Cancer 2014; 21: R431–R443. https://doi.org/10.1530/ERC-14-0179 PMID: 25813430

20. Lindstrom AK, Ekman K, Stendahl U, Tot T, Henriksson R, Hedman H, et al. LRIG1 and squamous epithelial uterine cervical cancer: correlation to prognosis, other tumor markers, sex steroid hormones, and smoking. Int J Gynecol Cancer 2008; 18: 312–317. https://doi.org/10.1111/j.1525-1438.2007.01021.x PMID: 17624990

21. Muller S, Lindquist D, Kanter L, Flores-Staino C, Henriksson R, Hedman H, et al. Expression of LRIG1 and LRIG3 correlates with human papillomavirus status and patient survival in cervical adenocarcinoma. Int J Oncol 2013; 42: 247–252. https://doi.org/10.3892/ijo.2012.1702 PMID: 23165628

22. An Y, Zhao Z, Ou P, Wang G. Expression of LRIG1 is associated with good prognosis for human non-small cell lung cancer. Medicine 2015; 94: e2081. https://doi.org/10.1097/MD.0000000000002081 PMID: 26632716

23. Kvambrik S, Karlsson T, Edlund K, Botling J, Lindquist D, Jirstrom K, et al. LRIG1 is a prognostic biomarker in non-small cell lung cancer. Acta Oncol 2015; 54: 1113–1119. https://doi.org/10.3109/0284186X.2015.1021427 PMID: 25813475

24. Krig SR, Frietze S, Simon C, Miller JK, Fry WH, Rafidi H, et al. Lrig1 is an estrogen-regulated growth suppressor and correlates with longer relapse-free survival in ERalpha-positive breast cancer. Mol Cancer Res 2011; 9: 1406–1417. https://doi.org/10.1158/1541-7786.MCR-11-0227 PMID: 21821674

25. Lando M, Fjeldbo CS, Wilting SM, Snoek BC, Aarnes EK, Forsberg MF, et al. Interplay between promoter methylation and chromosomal loss in gene silencing at 3p11-p14 in cervical cancer. Epigenetics 2015; 10: 970–980. https://doi.org/10.1080/15592294.2015.1085140 PMID: 26291246

26. Rafidi H, Mercado F 3rd, Astudillo M, Fry WH, Saldana M, Carraway KL 3rd, et al. Leucine-rich repeat and immunoglobulin domain-containing protein-1 (Lrig1) negative regulatory action toward ErbB receptor tyrosine kinases is opposed by leucine-rich repeat and immunoglobulin domain-containing protein 3 (Lrig3). J Biol Chem 2013; 288: 21593–21606. https://doi.org/10.1074/jbc.M113.486050 PMID: 23723069

27. Hedman H, Lindstrom AK, Tot T, Stendahl U, Henriksson R, Hellberg D. LRIG2 in contrast to LRIG1 predicts poor survival in early-stage squamous cell carcinoma of the uterine cervix. Acta Oncol 2010; 49: 812–815. https://doi.org/10.3109/0284186X.2010.492789 PMID: 20553099
28. Guo D, Nilsson J, Haapasalo H, Raheem O, Bergenheim T, Hedman H, et al. Perinuclear leucine-rich repeats and immunoglobulin-like domain proteins (LRIG1-3) as prognostic indicators in astrocytic tumors. Acta Neuropathol 2006; 111: 238–246. https://doi.org/10.1007/s00401-006-0032-5 PMID: 16532360

29. Holmlund C, Nilsson J, Guo D, Starefeldt A, Golovleva I, Henriksson R, et al. Characterization and tissue-specific expression of human LRIG2. Gene 2004; 332: 35–43. https://doi.org/10.1016/j.gene.2004.02.002 PMID: 15145052

30. Lindquist D, Nasman A, Tarjan M, Henriksson R, ToT T, Dalianis T, et al. Expression of LRIG1 is associated with good prognosis and human papillomavirus status in oropharyngeal cancer. Br J Cancer 2014; 110: 1793–1800. https://doi.org/10.1038/bjc.2014.87 PMID: 24548859

31. Nilsson J, Starefeldt A, Henriksson R, Hedman H. LRIG1 protein in human cells and tissues. Cell Tissue Res 2003; 312: 65–71. PMID: 12684867

32. Lindstrom AK, Asplund A, Hellberg D. Correlation between LRIG1 and LRIG2 expressions and expression of 11 tumor markers, with special reference to tumor suppressors, in CIN and normal cervical epithelium. Gynecol Oncol 2011; 122: 372–376. https://doi.org/10.1016/j.ygyno.2011.04.049 PMID: 21632100

33. Lindstrom AK, Hellberg D. Immunohistochemical LRIG3 expression in cervical intraepithelial neoplasia and invasive squamous cell cervical cancer: association with expression of tumor markers, hormones, high-risk HPV-infection, smoking and patient outcome. Eur J Histochem 2014; 58: 2227. https://doi.org/10.4081/ejh.2014.2227 PMID: 24998916

34. Tanemura A, Nagasawa T, Inui S, Itami S. LRIG-1 provides a novel prognostic predictor in squamous cell carcinoma of the skin: immunohistochemical analysis for 38 cases. Dermatol Surg 2005; 31:423–430. PMID: 15871317

35. Wu X, Hedman H, Bergqvist M, Bergstrom S, Henriksson R, Gullbo J, et al. Expression of EGFR and LRIG proteins in oesophageal carcinoma with emphasis on patient survival and cellular chemosensitivity. Acta Oncol 2012; 51: 69–76. https://doi.org/10.3109/02841075.2011.562239 PMID: 21417672

36. Yi W, Holmlund C, Nilsson J, Inui S, Lei T, Itami S, et al. Paracrine regulation of growth factor signaling by shed leucine-rich repeats and immunoglobulin-like domains 1. Exper Cell Res 2011; 317: 504–512.