Abstract

Tissue-specific inactivation of E-cadherin combined with tumor suppressor loss leads to invasive and metastatic cancers in mice. While epidermal E-cadherin loss in mice induces squamous cell carcinomas, inactivation of E-cadherin in the mammary gland leads to invasive lobular carcinoma. To further explore the carcinogenic consequences of cell-cell adhesion loss in these compartments, we developed a new conditional mouse model inactivating E-cadherin (Cdh1) and p53 (Trp53) simultaneously in cells expressing the leucine-rich repeat-containing G-protein coupled receptor 6 (Lgr6), a putative epithelial stem cell marker in the skin and alveolar progenitor marker in the mammary gland. Compound Lgr6-CreERT2;Cdh1F/F;Trp53F/F female mice containing either heterozygous or homozygous Cdh1F alleles were bred, and Lgr6-driven Cre expression was activated in pre-puberal mice using tamoxifen. We observed that 41% of the mice (16/39) developed mostly invasive squamous-type skin carcinomas, but also a non-lobular mammary tumor was formed. In contrast to previous K14cre or WAPcre E-cadherin and p53 compound models, no significant differences were detected in the tumor-free survival of Lgr6-CreERT2 heterozygous Cdh1F/F;Trp53F/F versus homozygous Cdh1F/F;Trp53F/F mice (778 versus 754 days, p=0.5). One Cdh1F homozygous mouse presented with lung metastasis that originated from a non-lobular and ERα negative invasive mammary gland carcinoma with squamous metaplasia. In total, 2/8 (25%) Cdh1F heterozygous and 3/12 (25%) Cdh1F homozygous mice developed metastases to lungs, liver, lymph nodes, or the gastro-intestinal tract. In conclusion, we show that inducible and conditional Lgr6-driven inactivation of E-cadherin and p53 in mice causes squamous cell carcinomas of the skin in approximately 40% of the mice and an occasional ductal-type mammary carcinoma after long latency periods.

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Keywords: E-cadherin, Lgr6, Squamous cell carcinoma, Skin, Breast cancer

Introduction

E-cadherin is the central component of the adherens junction (AJ), a structure that is crucial for epithelial integrity by controlling cell-cell adhesion through homotypic extra-cellular interactions [1]. In line with its central function, loss of E-cadherin expression has been causally linked to tumor development and progression of several cancers such as hereditary diffuse gastric cancer [2,3], invasive lobular breast cancer (ILC) [4–6] and recently, plasmacytoid bladder cancer [7]. Loss of E-cadherin in lobular breast cancer has been studied extensively, showing that mutational inactivation leads to tumor progression through the acquisition of anoikis resistance, mostly through constitutive activation of growth factor receptor signaling and p120-catenin (p120) dependent actomyosin contraction [8–13].
Mammary gland epithelium consists of an outer myoepithelial layer and an inner layer of luminal cells that can be further subdivided in a ductal and an alveolar lineage. Despite this modest heterogeneity, multiple breast cancer subtypes can be distinguished based on histology, suggesting that not the progenitor cell type, but specific genetic lesions define the breast cancer histo-morphological type. Indeed, mammary gland-specific conditional inactivation of E-cadherin leads to the development of lobular-type tumors in mice when combined with loss of p53 [5], PTEN [14], or activation of PI3K [15], regardless of whether a luminal whey acidic protein (WAP) Cre or myoepithelial cytokeratin 14 (K14) Cre driver is used. These models, however, do not express the estrogen receptor (ER), a common feature of human ILC [16]. In sum, these data may suggest that the genetic inactivation of E-cadherin drives the development of lobular breast cancer in the mouse mammary gland, and not the progenitor cell type [5,17].

Leucine-rich repeat-containing G-protein coupled receptor 6 (Lgr6) has been identified as a marker of stem cells of the lungs [18], alveolar taste buds [19] and skin [20,21], and associates with tumor development and progression in these organs [22,23]. In the mammary gland, Lgr6 marks
progenitor cells that contribute to alveolar expansion during pregnancy [17]. Moreover, Lgr6POS epithelial progenitor cells were reported to underpin the development of luminal ERPOS mammary carcinomas in mice upon inactivating Brca1 and Trp53 mutations in these cells [17].

Given the reported retention of ER expression in Lgr6-CreERT2;Bra1POS;Trp53POS mice, we investigated the consequences of tamoxifen-induced inactivation of E-cadherin and p53 in Lgr6POS cells. Concomitant loss of these key tumor suppressors upon systemic administration of tamoxifen induced the formation of mostly invasive squamous skin carcinomas with a long-term latency. We observe development of a non-inflamed mammary tumor in 1 mouse that progressed towards metastatic disease.

Materials and methods

Generation of Lgr6-EGFP-Ires-creERT2;CdhiF;Trp53F female mice

Lgr6Cre;CdhiF;Trp53F mice were generated by crossing heterozygous female Lgr6-EGFP-Ires-creERT2 (Lgr6Cre) mice [20] with male CdhiF;Trp53F mice [5]. The resulting heterozygous Lgr6Cre;CdhiF;Trp53F offspring was backcrossed onto homozygous CdhiF;Trp53F mice and intercrossed to produce female Lgr6Cre;CdhiF;Trp53F (n=17) and Lgr6Cre;CdhiF;Trp53F (n=22) offspring. Eight-week-old female mice were injected with 100 µL intraperitoneal Tamoxifen (Sigma) (10 mg/mL dissolved in corn oil (Sigma)) three times with two-day intervals to activate Cre recombinase. Mice were monitored weekly and sacrificed when tumors reached a maximum tumor volume of 1,500 mm³ (mammary tumors), or 1,000 mm³ (skin tumors), when mice were moribund and displayed severe discomfort, or when mice reached an age of >800 days. Mice that presented multiple tumors were sacrificed when a cumulative tumor volume of 1,500 mm³ was reached. All animal experiments were performed in accordance with local, national, and European guidelines under permit AVID115002015623 issued by The Netherlands Food and Consumer Product Safety Authority (NVWA) of the Ministry of Agriculture, Nature and Food.

Genotyping

DNA was isolated from ear punches with DirectPCR Lysis Reagent (Ear) buffer (Viagen) containing 4% Proteinase K, and incubated overnight at 56°C. Proteinase K was inactivated the following day by heating the sample to 95°C. In post-experimental tissues, DNA was isolated using the Qiagen DNEasy blood and tissues kit (Qiagen). Detection of Cre, Trp53F, Trp53A, CdhiF, and CdhiF was performed as previously described [5].

Histology and immunohistochemistry

Tissues were fixed in 4% formaldehyde for 24 hrs. and paraffin embedded. Immunohistochemistry (IHC) and hematoxylin eosin (HE) staining were performed on 4 µm thick tissue sections as described previously [5]. For IHC, antigen retrieval was accomplished by boiling for 20 min in a Tris-EDTA pH 9.0 buffer or by proteinase K incubation (10 ug/mL) at 37°C, followed by an overnight primary antibody incubation at 4°C. Sections were then incubated for 30' with secondary ab followed by incubation with liquid permanent red (DAKO) when required. Hematoxylin was used as a counterstaining. Membranous E-cadherin staining intensity was scored as negative (0) or positive (1). All scoring was performed in a blinded fashion and was performed by at least two observers.

Antibodies

The following antibodies were used: mouse anti-E-cadherin (Clone D5; 1:200; BD Bioscience), rabbit anti-Keratin-14 (Poly19053; 1:10000; BioLegend), rat anti-Keratin 8 (TROMA-I; 1:100; Developmental Studies), rabbit anti-GFP (D5,1; 1:1000; Cell Signaling) and ERα (clone 33; 1:100; Invitrogen). The following secondary antibodies were used: rabbit anti-rat HRP (1:100; DAKO), Brightvision anti-rabbit-AP (Immunologic), Brightvision anti Mouse-AP (Immunologic).

Results

Inactivation of E-cadherin and p53 in Lgr6POS cells induces tumor formation

To study the oncogenic effect of tumor suppressor inactivation in Lgr6POS progenitor cells, we crossed Lgr6-EGFP-Ires-creERT2 (Lgr6Cre) mice [20] with conditional E-cadherin- and p53 (CdhiF;Trp53F) mice [5]. Heterozygous E-cadherin Lgr6Cre;CdhiF;Trp53F and homozygous E-cadherin Lgr6Cre;CdhiF;Trp53F mice (8-10 weeks old; n=39) were injected with tamoxifen to induce Cre recombinase-mediated inactivation of the conditional alleles in LRG6 expressing cells (Fig. 1A). Both heterozygous Lgr6Cre;CdhiF;Trp53F and homozygous Lgr6Cre;CdhiF;Trp53F mice developed tumors with a median latency of 778 and 732 days, respectively (Fig. 1B,C). We observed tumor development in 8 out of 17 (47%) Lgr6Cre;CdhiF;Trp53F and 12 out of 22 (55%) Lgr6Cre;CdhiF;Trp53F mice up to a period of 800 days, of which most were skin carcinomas (Table 1). Homozygous deletion of CdhiF did not accelerate development of cancer in Lgr6Cre;CdhiF;Trp53F compared to heterozygous Lgr6Cre;CdhiF;Trp53F mice (p=0.5). The genetic status of CdhiF and Trp53 was determined in all tumors that developed in the
LGR6-dependent conditional inactivation of E-cadherin and p53 leads to skin squamous cell carcinoma. A&B: H&E stained sections of skin squamous cell carcinomas (SCC) that developed in Lgr6Cre;CdhaWT;Trp53F/F (A) or Lgr6Cre;CdhaTP53 (B) female mice with expansive and invasive phenotypes. Insets in the left panels depict the zoomed image in the right panels. Scale bars, 100 μm.

**Fig. 2.** Conditional inactivation of E-cadherin and p53 in Lgr6POS cells induces skin squamous cell carcinoma. A&B: H&E stained sections of skin squamous cell carcinomas (SCC) that developed in Lgr6Cre;CdhaWT;Trp53F/F (A) or Lgr6Cre;CdhaTP53 (B) female mice with expansive and invasive phenotypes. Insets in the left panels depict the zoomed image in the right panels. Scale bars, 100 μm.

Lgr6Cre;CdhaWT;Trp53F/F and Lgr6Cre;CdhaTP53 (B) mice (Table S1). Homozygous loss of the conditional Trp53 alleles was detected in all skin and mammary tumors, whereas the conditional Cdha was retained in some tumors that developed in both Lgr6Cre;CdhaWT;Trp53F/F and Lgr6Cre;CdhaTP53 (B) mice. These findings suggest that, in contrast to previous studies using K14cre [5,16], homozygous loss of E-cadherin does not provide a selective advantage for Lgr6POS cancer stem cells in the skin (Table S1). We also observed the development of lymphomas in 4/22 (18%) Lgr6Cre;CdhaWT;Trp53F/F mice, but only one lymphoma showed switching (deletion) of the conditional p53 alleles (Table 1 and S1). In contrast

**Fig. 3.** E-cadherin and CK14 expression in SCCs of Lgr6Cre;CdhaWT;Trp53F/F and Lgr6Cre;CdhaTP53 (B) mice. A and B: Immunohistochemical analysis on SCC that developed in Lgr6Cre;CdhaWT;Trp53F/F (A) or Lgr6Cre;CdhaTP53 (B) female mice (B). Shown are E-cadherin (top panels) and CK14 protein expression (bottom panels). Insets in the left panels depict the zoomed image in the right panels. Scale bars, 100 μm.
In summary, these data indicate that homozygous deletion of Cdh1 and Tip53 in Lgr6^{POS} cells induces sporadic formation of non-lobular mammary tumors with metastatic potential.

**Discussion**

E-cadherin is a cell-cell adhesion molecule that controls tissue homeostasis and epithelial integrity. In the mouse mammary gland, early conditional inactivation of E-cadherin and p53 results in the formation of ILC [5,16]. Unfortunately, mouse lobular tumors and the resulting metastatic disease in these models do not express estrogen receptor (ER), a common feature of human ILC [24]. We therefore developed a compound conditional mouse model to enable somatic inactivation of E-cadherin and p53 in a candidate ER^{POS} luminal progenitor cell type. For this, we used an Lgr6-dependent and inducible Cre recombinase mouse model [20], based on published data that conditional concomitant inactivation of 

The alternative oncogenic drivers or inactivated tumor suppressors in both mouse models can possibly explain the differences in latency time. Although both Brca1 and Cdh1 are strongly associated with breast cancer when mutated, it may be that conditional deletion of E-cadherin, even in the context of concomitant p53 inactivation, may not be tolerated in Lgr6^{POS} mammary progenitor cells or provides a selective disadvantage in these cells. Additionally, although we confirmed loss of E-cadherin in our mammary tumor, this carcinoma did not express typical ILC characteristics. Notably, the mammary carcinoma did not express ERα, despite the finding that Lgr6^{POS} cells can function as tumor initiating cells of luminal and ER^{POS} mammary tumors [17]. Given that dual E-cadherin and p53 loss leads to ILC in mice using either CK14 or WAP-dependent Cre drivers [5,16], we initially reasoned that the tumor phenotype is mainly guided by the genetic lesion, not the progenitor or cancer stem cell type. However, the lack of ILC development in our model may render an interplay between cell of origin and mutational load as a more likely hypothesis. Because we detected only one mammary tumor in a cohort of 39 mice, and given that all WAPcre;Cdh1^{POS};Tip53^{POS} female mice develop tumors of which roughly 50% are diagnosed as ILC [16], we consider it more probable that the absence of ILC development is due to the low propensity of Lgr6^{POS} mammary cells to develop tumors following E-cadherin loss.

Somatic inactivation of Cdh1 and Tip53 using Lgr6Cre predominantly resulted in the formation of invasive SCC in mice. Development of skin SCC in the Lgr6Cre;Cdhl^{F/F};Tip53^{F/F} model is comparable with previous published results, where E-cadherin and p53 were stochastically inactivated using K14Cre [5]. Although both mouse models develop skin SCC, tumor-free survival lifespans are considerably longer in the current Lgr6-driven mouse model, and only 41% of the mice develop tumors. Additionally, to skin tumors, we observed sarcomas and lymphomas in both cohorts. Since these tumors did not have genetic deletion of the Cdh1 allele, it is likely to suggest they arose due to age. The relatively low penetration of tumor development in the current model may be due to the variance in Cre driver activation, or because the skin hosts a more abundant presence of CK14^{POS} versus Lgr6^{POS} stem/progenitor cells. Alternatively, the dissimilar localization of CK14^{POS} and Lgr6^{POS} in the hair follicle may underpin the observed differences. While Lgr6^{POS} cells are strictly located to the interfollicular...
epidermis (IFE), the central isthmus and sebaceous gland, CK14POS cells are located more broadly throughout the hair follicle [20]. Although our data clearly show that homozygous E-cadherin loss induces a more invasive phenotype, this did not lead to a significant difference in tumor development latency between Lgr6Cre;Cdh1F/F, Trp53F/F and Lgr6Cre;Cdh1F/F, Trp53F/F mice. Of note, Lgr6POS cells in the skin contribute to the epidermal lineage and can fully reconstitute hair follicles [20]. Given this essential homeostatic role of Lgr6 in the skin, we anticipate that simultaneous deletion of E-cadherin and p53 attenuates epidermal differentiation of Lgr6POS cells and as such hinders tumor formation. While deletion of E-cadherin and p53 in Lgr6POS cells specifically results in the formation of SCC, we observe that these carcinomas heterogeneously express CK14, but lack expression of Lgr6. The lack of Lgr6 expressing cells in the SCC samples may be a consequence of epidermal cell differentiation, where Lgr6 expressing stem/progenitor cells contribute to tumor onset but not to further progeny in current mutational model. This assumption is in line with data showing that loss of Lgr6 associates with increased proliferation and differentiation of the epidermal lineage [23].

In conclusion, we demonstrate that stochastic loss of E-cadherin and p53 in Lgr6POS cells induces the modest formation of SCC and incidental ductal-type mammary carcinomas in mice. In contrast to previously reported K14cre and WAPcre drivers, our work shows that Lgr6-dependent loss of E-cadherin and p53 does not lead to the development of lobular cancer in the mouse mammary gland. These findings either confirm the existence of multiple different progenitor cell types that underpin the formation of different mammary cancer types or suggest that E-cadherin loss is not tolerated in an Lgr6-driven alveolar progenitor cell type. Notwithstanding these findings, our mouse model represents a valuable tool to study the oncogenic contributions of Lgr6POS cells to the development of invasive skin carcinoma.
Author contributions

EJtS, TS, ERMB and PWBD designed the experiments. Mouse studies: LE. Histology: EJtS, LE, SK and PWBD. Immunohistochemistry: WH, EJtS, TS, and PWBD. ERMB interpreted results and provided input. EJtS, TS and PWBD wrote the manuscript. EJtS and TS contributed equally.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Competing Interests

The authors declare no competing interests.

CRediT authorship contribution statement

Eline J. ter Steege: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. Thijmen Sijnesael: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. Lotte Enserink: Investigation. Sjoerd Klarenbeek: Investigation. Wisse E. Haakma: Investigation. Elvira R.M. Bakker: Conceptualization, Supervision. Patrick W.B. Derksen: Conceptualization, Methodology, Supervision, Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neo.2022.100844.

References

[1] Meng W, Takeichi M. Adherens junction: molecular architecture and regulation. Cold Spring Harb Perspect Biol 2009;1:a002899.
[2] Guilford P, Hopkins J, Harraway J, McLeod M, McLeod N, Harawira P, Taite H, Scouler R, Miller A, Reeve AE. E-cadherin germline mutations in familial gastric cancer. Nature 1998;392:402–5.
[3] Shimada S, Mimata A, Sekine M, Mogushi K, Akiyama Y, Fukamachi H, Jonkers J, Tanaka H, Eishi Y, Yuasa Y. Synergistic tumour suppressor activity of E-cadherin and p53 in a conditional mouse model for metastatic diffuse-type gastric cancer. Gut 2012;61:344–53.
[4] Bers G, Cleton-Jansen AM, Nollet F, Leewt WJ, Vijver M, Cornelisse C, Roy F. E-cadherin is a tumour/invasion suppressor gene mutated in human lobular breast cancers. Embo J 1995;14:6107–15.
[5] Derksen PWB, Liu X, Sareid F, Gulden H van der, Zevenhoven J, Evers B, Beijnum JR van, Griffioen AW, Vink J, Krimpenfort P, Petere CLJ, Cardiff RD, et al. Somatic inactivation of E-cadherin and p53 in mice leads to metastatic lobular mammary carcinoma through induction of anoikis resistance and angiogenesis. Cancer Cell 2006;10:437–49.
[6] Moll R, Mitze M, Fri xen UH, Birchmeier W. Differential loss of E-cadherin expression in infiltrating ductal and lobular breast carcinomas. Am J Pathol 1993;143:1731–42.
[7] Al-Ahmadi HA, Iyer G, Lee BH, Scott SN, Mehra R, Bagrodia A, Jordan EJ, Gao SP, Ramirez R, Cha EK, Desai NB, Zabor EC, et al. Frequent somatic CDH1 loss-of-function mutations in plasmyctoid variant bladder cancer. Nat Genet 2016;48:356–8.
[8] Bajrami I, Marlow R, Ven M van de, Brough R, Pemberton HN, Frankum J, Song F, Rafii R, Konde A, Krastev DB, Menon M, Campbell J, et al. E-Cadherin/ROS1 Inhibitor Synthetic Lethality in Breast Cancer. Cancer Discov 2018;8:498–515.
[9] Hornsveld M, Tenhagen M, Ven RA van de, Smits AMM, Triest MH van, Amersfoort M van, Kloet DEA, Dansen TB, Burgarding BM, Derksen PWB. Restraining FOXO3-dependent transcriptional BMF activation underpins tumour growth and metastasis of E-cadherin-negative breast cancer. Cell Death Differ 2016;23:1483–92.
[10] Nagle AM, Levine KM, Tasdemir N, Scott JA, Burlbaugh K, Kehm JW, Katz TA, Boone DN, Jacobsen BM, Atkinson JM, Oesterreich S, Lee AV. Loss of E-cadherin enhances IGF1-IGF1R pathway activation and sensitizes breast cancers to anti-IGF1R/InsR inhibitors. Clin Cancer Res 2018;24:5167–77.
[11] Schackmann RCJ, Amersfoort M van, Hazhuis JHI, Vlugt EJ, Halim VA, Roodhart JML, Vermaat JS, Voest EE, Groep P van der, Drost PJF, Jonkers J, Derksen PWB. Cytosolic p120-catenin regulates growth of metastatic lobular carcinoma through Rock1-mediated anoikis resistance. J Clin Invest 2011;121:3176–88.
[12] Teo K, Gómez-Cuadrado L, Tenhagen M, Byron A, Rütze M, Amersfoort M van, Renes J, Strengman E, Mandoli A, Singh AA, Martens JH, Stunnenberg HG, et al. E-cadherin loss induces targetable autocrine activation of growth factor signalling in lobular breast cancer. Sci Rep-uk 2018;8:15454.
[13] Rütze MAK, Koornman T, Sijnesael T, Bassey-Achibong B, Ven R van de, Ensersink L, Visser D, Jakab S, Vici ano I, Bakker ERMD, Richard F, Tuut A, et al. Loss of E-cadherin leads to Id2-dependent inhibition of cell cycle progression in metastatic lobular breast cancer. Oncogene 2022;41:2932–44.
[14] Boedens MC, Nethe M, Klarenbeek S, Ruiter JR de, Schut E, Bonzanni N, Zeeman AL, Wientjens E, Burg E van der, Wessels L, Amerongen R van, Jonkers J. PTEN loss in E-cadherin-deficient mouse mammary epithelial cells rescues apoptosis and results in development of classical lobular invasive carcinoma. Cell Rep 2016;16:2087–101.
[15] Annunziato S, Kas SM, Nethe M, Yücel H, Bravo JD, Pritchard C, Ali RB, Gerwen B van, Siteur B, Drenth AP, Schut E, Ven M van de, et al. Modeling invasive lobular breast carcinoma by CRISPR/Cas9-mediated somatic genome editing of the mammary gland. Gene Dev 2016;30:1470–80.
[16] Derksen PWB, Braumuller TM, Burg E van der, Hornsveld M, Mesman E, Wesseling J, Krimpenfort P, Jonkers J. Mammary-specific inactivation of E-cadherin and p53 impairs functional gland development and leads to pleomorphic invasive lobular carcinoma in mice. Dev Model Mech 2011;4:547–58.
[17] Blaas L, Pucci F, Messal HA, Andersson AB, Ruiz EJ, Gerling M, Douagi I, Spencer-Dene B, Musch A, Mitter R, Bhaw L, Stone R, et al. Lgr6 labels a rare population of mammary gland progenitor cells that are able to originate luminal mammary tumours. Nat Cell Biol 2016;18:1346–56.
[18] Oeztuerk-Winder F, Guinot A, Ochalek A, Ventura J-J. Regulation of human lung alveolar multipotent cells by a novel p38α MAPK/miR-17-92 axis. Embo J 2012;31:3431–41.
[19] Ren W, Lewandowski BC, Watson J, Aihara E, Iwatsuki K, Bachmanov AA, Margolskee RF, Jiang P. Single Lgr5– or Lgr6-expressing taste stem/progenitor cells generate taste bud cells ex vivo. Proc National Acad Sci 2014;111:16401–16406.
[20] Snippert HJ, Hagegebahrt A, Kasper M, Jaks V, Es JH van, Barker N, Wetering M van de, Born M van den, Begthel H, Vries RG, Stange DE, Toftgard R, et al. Lgr6
LGR6-dependent conditional inactivation of E-cadherin and p53 leads to E.J. ter Steege et al. Neoplasia Vol. 35, No. xxx 2023

Marks Stem Cells in the Hair Follicle That Generate All Cell Lineages of the Skin. Science 2010;327:1385–9.

[21] Füllgrabe A, Joost S, Are A, Jacob T, Sivan U, Hasegbarrh A, Linnarson S, Simons BD, Clevers H, Tofgdär R, Kasper M. Dynamics of Lgr6+ Progenitor Cells in the Hair Follicle, Sebaceous Gland, and Interfollicular Epidermis. Stem Cell Rep 2015;5:843–55.

[22] Guinot A, Oeztuerk-Winder F, Ventura J-J. miR-17-92/p38α Dysregulation Enhances Wnt Signaling and Selects Lgr6+ Cancer Stem-like Cells during Lung Adenocarcinoma Progression. Cancer Res 2016;76:4012–22.

[23] Huang PY, Kandyba E, Jabouille A, Sjolund J, Kumar A, Halliwill K, McCreery M, DelRosario R, Kang HC, Wong CE, Seibler J, Beuger V, et al. Lgr6 is a stem cell marker in mouse skin squamous carcinomas. Nat Genet 2017;49:1624–32.

[24] Christgen M, Cserni G, Floris G, Marchio C, Djerroudi L, Kreipe H, Derksen PWB, Vincent-Salomon A. Lobular breast cancer: histomorphology and different concepts of a special spectrum of tumors. Cancers 2021;13:3695.